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Sequential induction of 5-lipoxygenase gene expression and activity in Mono Mac 6 cells by transforming growth factor β and 1,25-dihydroxyvitamin D_3

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ABSTRACT **5-Lipoxygenase (5-LO; EC 1.13.11.34) activity in the human monocytic cell line Mono Mac 6 was upregulated by combined treatment with transforming growth factor β1 (TGF-β) and 1,25-dihydroxyvitamin D_3 (VD_3). In undifferentiated cells, 5-LO enzyme activity was undetectable. After the addition of TGF-β plus VD_3, the activity of intact cells was 800 ng per 10^6 cells—500 times more than the assay detection limit. Also 5-LO protein and mRNA expression were induced >128-fold and 64-fold, respectively, as compared to undifferentiated cells. Both TGF-β and VD_3 were required for these prominent responses. Either agent alone gave small amounts of 5-LO protein and mRNA but very low 5-LO activities. After the addition of TGF-β and VD_3, the induction of 5-LO protein was obvious after 1 day, but the increase in activity was delayed and did not appear until the second day. Pretreatment of cells with TGF-β or VD_3 alone for 2 days led to 5-LO protein expression but very low enzyme activity. Addition of the lacking second inducer was required for full induction of 5-LO protein expression and for upregulation of enzyme activity. Partial purification of 5-LO from Mono Mac 6 cells and recombination with soluble cellular proteins from different sources indicated the presence of cytosolic factors that affect the activity of 5-LO.**

Leukotrienes are produced by granulocytes, mast cells, monocytes, and macrophages after stimulation, and the capacity for leukotriene biosynthesis of these cells is supposedly acquired during cell maturation. Arachidonate 5-lipoxygenase (5-LO; EC 1.13.11.34) is a key enzyme, which catalyzes two initial steps in leukotriene biosynthesis (1), and 5-LO mRNA, protein expression, and activity were shown to be upregulated after *in vitro* differentiation of the human promyeloic cell line HL-60 (2–5). Human monocytes and macrophages express 5-LO mRNA and protein (6, 7), and differentiation of monocytes to macrophages is accompanied by an upregulation of 5-LO activity and protein expression (8–10).

Transforming growth factor β (TGF-β) was recently found to upregulate cellular 5-LO activity in dimethyl sulfoxide (DMSO)-differentiated HL-60 cells (11). The most prominent effects of TGF-β were observed for the 5-LO activity of intact cells, whereas the amount of 5-LO protein was less affected. It was concluded that TGF-β induced or modified other components of importance for 5-LO activity in the intact HL-60 cell.

To identify factors that induce 5-LO activity during monocytic differentiation, we studied the effects of TGF-β and other differentiation inducers on the human monocytic cell line Mono Mac 6, which has characteristics of mature monocytes (12). Under standard cell culture conditions, Mono Mac 6 cells do not express detectable amounts of 5-LO protein or activity (6). Here, we describe the profound upregulation of 5-LO activity, protein, and mRNA in Mono Mac 6 cells by the combination of TGF-β and 1,25-dihydroxyvitamin D_3 (VD_3).

MATERIALS AND METHODS

Materials. HPLC solvents and DMSO were from Merck, and molecular biology reagents were from Sigma, GIBCO, or Promega. Fetal calf serum and human transferrin were obtained from Sigma; RPMI 1640 medium was from GIBCO. Insulin was a gift from Hoechst–Roussel. 5-LO antiserum was kindly provided by Armin Hatzelmann, Bayer (Leverkusen, F.R.G.). PCR primers were purchased from Scandinavian Gene Synthesis (Köping, Sweden) or Roth (Karlsruhe, F.R.G.). Human TGF-β1 was purified from outdated platelets as described (13), except that the Bio-Sil TSK CM-2-SW column (Bio-Rad) was replaced by a LiChrospher 1000 COO^- column (10 × 100 mm) (Merck). VD_3 was kindly provided by Ulf Fischer (Hoffmann–La Roche).

Cells. Mono Mac 6 cells were kindly provided by H. W. L. Ziegler-Heitbrock (Munich) and maintained in RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum, streptomycin at 100 μg/ml, penicillin at 100 units/ml, 1 mM sodium pyruvate, 1× nonessential amino acids, 1 mM oxalacetic acid, and bovine insulin at 10 μg/ml (12). Cultures were seeded at a density of 2×10^5 cells per ml.

Determination of 5-LO Activity. Cells were harvested after the indicated times and washed once in phosphate-buffered saline (PBS). When intact cells were assayed, the cells ($2–10 \times 10^6$) were finally taken up in 1 ml of PBS (with 1 mM Ca^{2+} and glucose at 1 g/liter), and the reaction was started by addition of arachidonic acid and ionophore A23187 in 10 μl of methanol (40 μM and 10 μM final concentrations, respectively). After 10 min at 37°C, the reaction was stopped with 1 ml of methanol and 30 μl of 1 M HCl; 200 ng of prostaglandin B_1 (internal standard) and 500 μl of PBS were added. The samples were centrifuged (800 × *g*, 10 min), and the supernatant was applied to C_{18} solid-phase extraction columns (100 mg, preconditioned with 1 ml of methanol and 1 ml of water). After washing with 1 ml of water and 1 ml of 25% (vol/vol) methanol, 5-LO metabolites were eluted with 300 μl of methanol. The eluate was diluted with 120 μl of water, and 100 μl of the diluted extract was analyzed by HPLC as described (14).

When cell homogenates were assayed, the cells were finally taken up in 1 ml of PBS (with glucose at 1 g/liter and without Ca^{2+} and Mg^{2+}) and cooled on ice. After addition of EDTA (1 mM), sonication (three times for 5 sec each), and addition of 1 mM ATP, the samples were preincubated for 30 sec at

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Abbreviations: TGF-β, transforming growth factor β; 5-LO, 5-lipoxygenase; RT, reverse transcription; DMSO, dimethyl sulfoxide; VD_3, 1,25-dihydroxyvitamin D_3; ATP-PT fraction, ATP-agarose column pass-through fraction.

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37°C, and the incubation was started by the addition of Ca^{2+} and arachidonic acid (2 mM and 40 μM final concentrations, respectively). 5-LO activity is expressed as nanograms of 5-LO products per 10^6 cells.

Western Blots. Cells (2×10^6) were lysed in 50 μl of 20 mM Tris·HCl, pH 8.0/2 mM EDTA/5% (wt/vol) SDS/10% (vol/vol) 2-mercaptoethanol and heated at 95°C for 5 min. Then, 50 μl of water was added, the mixture was heated for an additional 3 min, and the samples were centrifuged to remove precipitated material. Two microliters of glycerol/0.1% bromphenol blue (1:1, vol/vol) was added per 10-μl sample. SDS/PAGE and Western blot analyses were performed as described (15). Relative amounts of 5-LO protein on blots were determined by scanning of membranes with a Zeiss KM3 scanner at 620 nm.

Reverse Transcription (RT) and PCR Analysis. Total cellular RNA was isolated, reverse transcribed into cDNA, and amplified exactly as described (15). Twenty-four cycles were carried out for β-actin, and 26 cycles were performed for 5-LO determinations unless otherwise mentioned in the text. The following primers were used: β-actin, 800-bp primer set from the β-actin control amplimer panel (Clontech); 5-LO, 5′-ACCATTGAGCAGATCGTGGACACGC and 5′-GCAGTCCTGCTCTGTGTAGAATGGG. Competitive PCR was performed in the presence of the indicated amounts of plasmid pEMBL 5BS XL2, which gives an elongated PCR product with the 5-LO PCR primers (561 bp as compared to the normal 486 bp). The plasmid pEMBL 5BS XL2 was prepared from pEMBL 5BS (pEMBL9 linearized with *Eco*RI and ligated to the entire original 5-LO cDNA; ref. 16). The sequence between the *Not* I and the *Bcl* I sites in the 5-LO cDNA was removed, and instead a longer DNA fragment (from rat 12-lipoxygenase cDNA) was inserted to give pEMBL 5BS XL2.

Purification of 5-LO from Mono Mac 6 Cells. Mono Mac 6 5-LO was purified by ATP-agarose affinity chromatography (17, 18). Cells ($1.5–2.0 \times 10^8$) were homogenized in 8 ml of PBS buffer supplemented with 1 mM EDTA, aprotinin at 0.1 μg/ml, pepstatin at 1 μg/ml, and leupeptin at 1 μg/ml at 4°C. After centrifugation (100,000 × *g*, 4°C, 60 min), the supernatant was applied to a column of ATP-agarose (Sigma no. A2767; bed volume of 2 ml) equilibrated with PBS buffer containing 1 mM EDTA and 1 mM 2-mercaptoethanol (equilibration buffer). The column was first washed with equilibration buffer supplemented with catalase at 20 μg/ml (7 ml), followed by 0.05 M phosphate buffer, pH 7.4/0.5 M NaCl/1 mM EDTA/catalase at 20 μg/ml (10 ml) and 0.05 M phosphate buffer, pH 7.4/1 mM EDTA (10 ml). 5-LO enzyme was eluted with 0.05 M phosphate buffer, pH 7.4/1 mM EDTA/20 mM ATP (10 ml).

RESULTS

Effects of Differentiation Inducers on 5-LO Activity in Mono Mac 6 Cells. Mono Mac 6 cells were grown in the presence of TGF-β (1 ng/ml), VD_3 (50 nM), or DMSO (1.2%) or combinations of these compounds. After 4 days, 5-LO activity of intact cells and cell homogenates was determined. TGF-β, VD_3, or DMSO induced only low 5-LO activities in intact cells and cell homogenates (Fig. 1*A*). The combination of TGF-β and VD_3 strongly induced 5-LO activity in intact cells and cell homogenates (791 ± 7 and 524 ± 70 ng per 10^6 cells, respectively; $n = 3$). The detection limit of the HPLC assay was 1 ng per 10^6 cells, and in samples from undifferentiated cells 5-LO activity was undetectable; thus, the activity was upregulated at least 500-fold. This prominent upregulation of 5-LO activity required both VD_3 and TGF-β; either of these agents alone was almost ineffective. The combination of TGF-β and DMSO gave a modest increase in 5-LO activity of intact cells and homogenates (17 ± 4 ng and 40 ± 12 ng, respectively; $n = 3$). Combination of all-*trans*- or 9-*cis*-retinoic acid (0.3 μM) with TGF-β gave low 5-LO activities in homogenates (8.3 and 14.4 ng per 10^6 cells, respectively).

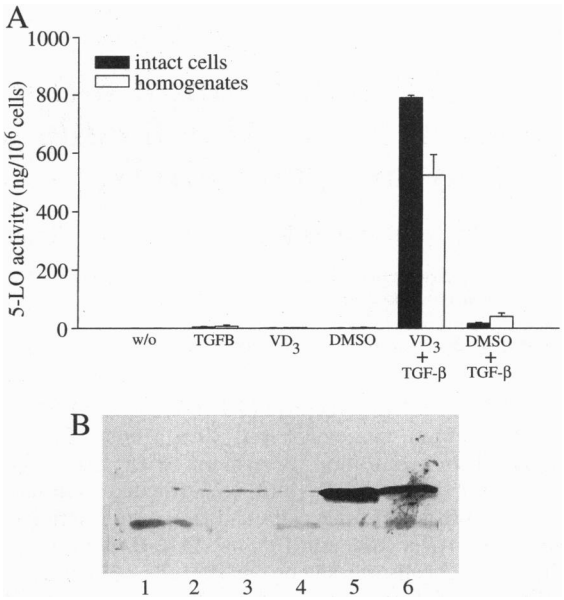

FIG. 1. Effects of differentiation inducers on 5-LO activity (*A*) and 5-LO protein amounts (*B*) of Mono Mac 6 cells. Cells were grown in the absence of additives (lane 1) or cultured in the presence of TGF-β (1 ng/ml) (lane 2), VD_3 (50 nM) (lane 3), DMSO (1.2%) (lane 4), TGF-β (1 ng/ml) plus VD_3 (50 nM) (lane 5), or TGF-β (1 ng/ml) plus DMSO (1.2%) (lane 6). (*A*) After 4 days, cells were harvested and intact cells (filled bars) and homogenates (open bars) were assayed for 5-LO activity. Values are expressed as mean + SE of three independent experiments. (*B*) The same samples (4×10^5 cells, solubilized in SDS/PAGE loading buffer) were analyzed for 5-LO protein by Western blot.

Effects of TGF-β, VD_3, and DMSO on 5-LO Protein Amount. Next, effects of TGF-β, VD_3, DMSO, and combinations of these inducers on 5-LO protein content was investigated. Cells were grown for 4 days in the presence of the indicated compounds. Then, the cells were harvested and aliquots (corresponding to 4×10^5 cells) were analyzed by Western blot. No 5-LO band was detected in samples from untreated Mono Mac 6 cells (Fig. 1*B*, lane 1). Weak signals of similar intensities were obtained from cells that were cultured in the presence of TGF-β (1 ng/ml), VD_3 (50 nM), or DMSO (1.2%) (Fig. 1*B*, lanes 2–4). As expected from 5-LO activity determinations, the combination of VD_3 and TGF-β led to a strong induction of 5-LO protein (lane 5). Serial dilution of the sample in lane 5 and visual inspection of the dilution series showed that it contained at least 128-fold more 5-LO protein than the detection limit. A fairly intense 5-LO band was also observed in samples from cells that were differentiated in the presence of DMSO and TGF-β (lane 6). Thus, a 3- to 5-fold difference in 5-LO protein concentration was found between samples 5 and 6, by Western blot analysis of serial dilutions of the samples and visual comparison of the bands.

Effects of Differentiation Inducers on 5-LO mRNA Levels. Total RNA was isolated from cells treated with the various differentiation inducers. RNA was reverse transcribed and analyzed by PCR. 5-LO mRNA was nearly undetectable in undifferentiated cells (Fig. 2, lane 1). Clear bands were observed in samples from cells that received TGF-β, VD_3, or DMSO (lanes 2–4). The combination of TGF-β with VD_3 led to the strongest induction of 5-LO mRNA (lane 5). Treatment of cells with TGF-β and DMSO gave a slightly stronger signal (lane 6) as compared to TGF-β or DMSO alone.

The differences in 5-LO mRNA expression were determined with competitive PCR (Fig. 3). The indicated amounts of internal standard (pEMBL 5BS XL2 plasmid) were added to

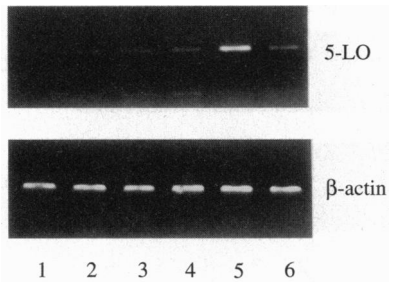

FIG. 2. Mono Mac 6 cells were treated as described in the legend to Fig. 1. Samples of total RNA from each cell culture were subjected to RT-PCR for analysis of 5-LO mRNA content. β-Actin mRNA served as constitutively expressed control.

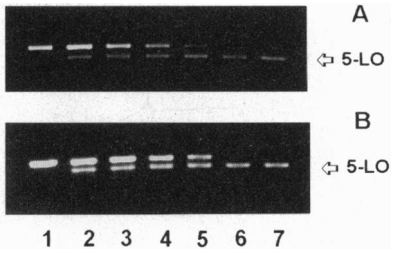

FIG. 3. Competitive PCR analysis of 5-LO mRNA of undifferentiated Mono Mac 6 cells (*A*) and of cells differentiated with TGF-β (1 ng/ml) plus VD_3 (50 nM) for 4 days (*B*). Total RNA was extracted and transcribed into cDNA. Competitive PCR was performed in the presence of the indicated amounts of internal standard plasmid pEMBL 5BS XL2. (*A*) Lane 1, plasmid only (49×10^{-21} mol); lanes 2–6, Mono Mac cDNA (100 ng) plus 49, 24, 12, 6.1, and 3.1×10^{-21} mol of standard, respectively; lane 7, cDNA only (100 ng). (*B*) Lane 1, plasmid only (6.2×10^{-18} mol); lanes 2–6, Mono Mac cDNA plus 6.2, 3.1, 1.6, 0.8, and 0.4×10^{-18} mol of standard, respectively; lane 7, cDNA only (100 ng). Thirty-two and 26 cycles were performed with samples of undifferentiated and differentiated cells, respectively.

cDNA samples corresponding to 100 ng of RNA from undifferentiated cells (*A*) and from cells differentiated with VD_3 and TGF-β for 4 days (*B*). For differentiated cells, PCR products of similar intensities were obtained when cDNA was multiplied for 26 cycles together with 0.8×10^{-18} mol of internal standard (Fig. 3*B*). For undifferentiated cells, PCR products of similar intensities were obtained when cDNA was amplified for 32 cycles together with 12×10^{-21} mol of internal standard (Fig. 3*A*). Thus, assuming that the RTs were equally efficient for the different samples, VD_3 plus TGF-β led to an about 64-fold induction of 5-LO mRNA expression as compared to undifferentiated cells. Also, the amount of 5-LO mRNA in cells treated with only TGF-β was determined by competitive PCR. There was 8-fold more 5-LO mRNA in the cells treated with both TGF-β and VD_3 as compared to the cells that received only TGF-β (Table 1). DMSO plus TGF-β led to a slight increase in 5-LO mRNA. As estimated from serial dilutions, there was about a 4- to 6-fold difference in 5-LO mRNA between cells treated with TGF-β plus VD_3 (Fig. 2, lane 5) and cells receiving TGF-β plus DMSO (lane 6), which correlates with the difference in protein amount (see above). Results for 5-LO activity, protein, and mRNA expression are summarized in Table 1.

The apparent size of the 5-LO mRNA species induced by TGF-β and VD_3 corresponded to that of the mRNA species detected in neutrophil samples, as determined by Northern blot (data not shown) (16). 5-LO mRNA expression in cells treated with TGF-β, VD_3, DMSO, or DMSO plus TGF-β was too low to be detected by Northern blot analysis.

Time Dependence of TGF-β Plus VD_3 Effects on Mono Mac 6 Cells. The time course of induction of 5-LO activity and 5-LO protein expression was investigated. TGF-β (1 ng/ml) and VD_3 (50 nM) were added, and cells were harvested after the indicated times (Fig. 4*A*). 5-LO activity of intact cells and cell homogenates was determined, and 5-LO protein expression was analyzed by Western blot analysis. The effects were very slow in onset. 5-LO protein appeared after 1 day, but 5-LO activity was not found until the second day. Maximal expression of 5-LO protein was reached after 2 days, while 3 days of exposure to VD_3 and TGF-β was required for complete upregulation of 5-LO activity. Thus, there was a delay in the upregulation of the activity as compared to the 5-LO protein.

Effects of Preincubation with TGF-β or VD_3 on 5-LO Induction. Mono Mac 6 cells were grown for 2 days in the presence of TGF-β at 1 ng/ml (Fig. 4*B*) or 50 nM VD_3 (Fig. 4*C*). Then VD_3 or TGF-β was added, and the cells were differentiated for the indicated times and analyzed for 5-LO activity and protein expression. As shown in Fig. 4 *B* and *C* (day 0), preincubation with TGF-β or VD_3 for 2 days induced some 5-LO protein expression but almost no activity. Addition of the second inducer (VD_3 or TGF-β, respectively) was required for induction of activity. Complete upregulation of 5-LO activity was observed after cultivation for 2 days in the presence of TGF-β and VD_3 (Fig. 4 *B* and *C*). Without preincubation, full upregulation of 5-LO activity required 3 days (Fig. 4*A*). Thus, induction of 5-LO activity by TGF-β plus VD_3 was faster when Mono Mac 6 cells had been preincubated with TGF-β or VD_3 for 2 days.

Partial Purification of 5-LO from Mono Mac 6 Cells: Effects on Enzyme Activity of Recombination with Cellular Proteins. The discrepancy in upregulation of 5-LO protein and activity found in the time course studies (Fig. 4) was further investigated. For example, treatment of Mono Mac 6 cells with TGF-β for only 2 days induced 5-LO protein expression but very little 5-LO activity (Fig. 4*B*). To investigate whether this lack of activity was due to inactive 5-LO protein or to an inhibitor present in Mono Mac cells cultured under these conditions, 5-LO was purified from cells that had been treated with TGF-β for 2 days and then assayed in the absence or presence of cellular proteins.

After harvest, cells were homogenized and found to have low 5-LO activity (total homogenate: 2.5 ± 0.6 ng per 10^6 cells, $n = 3$; 100,000 × *g* supernatant: 1.0 ± 0.2 ng per 10^6 cells, $n = 4$). A partial purification of 5-LO in supernatants was

Table 1. Effects of TGF-β and TGF-β plus VD_3 on 5-LO activity, protein, and mRNA expression

Parameter	Control	TGF-β	TGF-β + VD_3	Ratio of TGF-β + VD_3 to TGF-β
5-LO activity in homogenates, ng per 10^6 cells	ND*	7.3	524	71.6
5-LO protein,† peak area in mV·sec	ND	5.7	76.5	13.4
5-LO mRNA,‡ amol/100 ng of total RNA	0.012	0.098	0.8	8

ND, not detectable.
*Detection limit of 1 ng per 10^6 cells.
†Aliquots corresponding to 4×10^5 cells were analyzed by Western blot.
‡Determined as 5-LO-derived PCR product with competitive PCR.

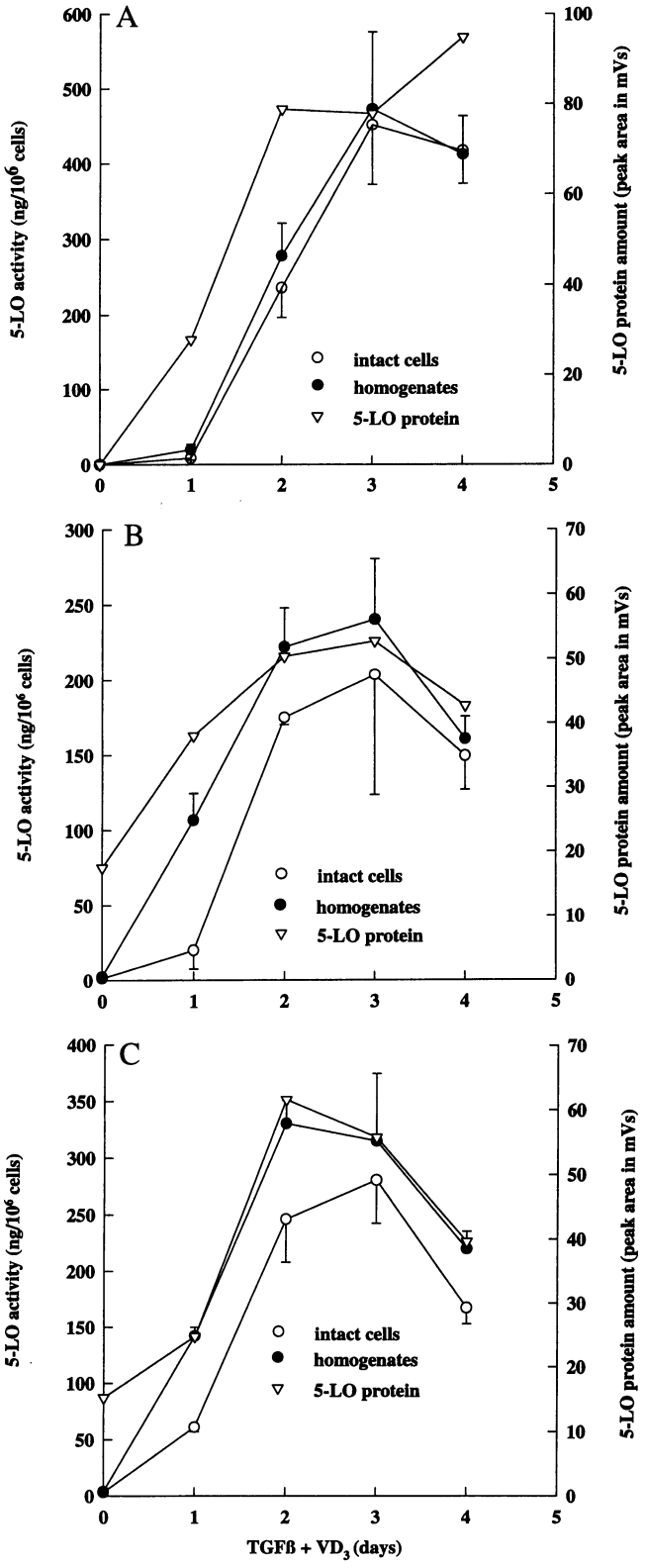

FIG. 4. Time courses of upregulation of 5-LO activity and protein in Mono Mac 6 cells treated with TGF-β (1 ng/ml) and VD_3 (50 nM). (*A*) VD_3 and TGF-β were added together at day 0. (*B*) Cells were pretreated with TGF-β for 2 days, and then VD_3 was added at day 0. (*C*) Cells were pretreated with VD_3 for 2 days, and then TGF-β was added at day 0. Cells were harvested and analyzed after the indicated times as described in *Materials and Methods*. 5-LO activity was determined for intact cells (○) and for homogenates (●). Values are expressed as mean ± SE of two or three independent experiments. 5-LO protein amount (▽) was determined by scanning of Western blots.

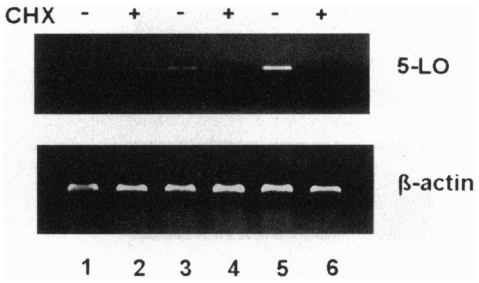

FIG. 5. Effect of cycloheximide (CHX) on 5-LO mRNA induction in Mono Mac 6 cells. Cells in culture were treated with TGF-β (1 ng/ml) plus VD_3 (50 nM) for 8 h (lanes 1 and 2), 12 h (lanes 3 and 4), and 16 h (lanes 5 and 6) in the absence or presence of cycloheximide (10 μM). After harvest, total RNA was isolated and analyzed for 5-LO mRNA by RT-PCR. Twenty-six cycles were performed. β-Actin mRNA served as constitutively expressed control.

achieved using an ATP-agarose column. This column chromatography thus resulted in a 5-LO fraction and a pass-through fraction (ATP-PT) containing most of the soluble cellular proteins. No 5-LO protein could be detected in the pass-through fraction by Western blot.

The activity of the semipurified 5-LO was determined in the presence of either phosphatidylcholine at 20 μg/ml and γ-globulin at 20 μg/ml or after addition of cellular protein in ATP-PT fractions. ATP-PT fractions were prepared from granulocytes (10^7 cells), the B-lymphocytic cell line BL41E95A (10^7 cells), or Mono Mac 6 cells (5×10^6 cells). The semipurified 5-LO corresponding to 10^6 cells gave 4.3 ± 1.2 ng of product ($n = 4$) when assayed in the presence of phosphatidylcholine and γ-globulin. Recombination with the original Mono Mac ATP-PT fraction resulted in a lower enzyme activity (1.6 ± 0.4 ng, $n = 3$). Additional assays of identical aliquots from the same enzyme preparations in the presence of ATP-PT fractions from Mono Mac 6 cells (TGF-β plus VD_3, 4 days), granulocytes, or lymphocytes gave 11.8 ± 2.5 ($n = 3$), 9.2 ± 3.3 ($n = 2$), and 9.0 ± 1.2 ng ($n = 4$) of products, respectively.

Effect of Cycloheximide on 5-LO mRNA Induction. To check whether protein synthesis is required for induction of 5-LO mRNA, cells receiving TGF-β (1 ng/ml) and VD_3 (50 nM) were cultured in the presence or absence of 10 μM cycloheximide (Fig. 5). After 8, 12, and 16 h, RNA was isolated and analyzed for 5-LO mRNA by RT-PCR. As shown in Fig. 5, induction of 5-LO mRNA by VD_3 and TGF-β is slow: 5-LO mRNA was clearly detectable first after 12 h and was further increased at 16 h. In cells treated with cycloheximide, 5-LO mRNA induction was nearly completely inhibited (Fig. 5). This suggests that protein synthesis is required for 5-LO mRNA induction by VD_3 and TGF-β.

DISCUSSION

The effects of differention inducers on 5-LO activity and protein and mRNA expression in Mono Mac 6 cells were investigated. Under standard culture conditions, this human monocytic cell line does not express detectable amounts of 5-LO activity or protein. Addition of VD_3, TGF-β, or DMSO led to small amounts of 5-LO protein and very low 5-LO activities, but the combination of TGF-β and VD_3 gave strong induction of both 5-LO activity and protein expression. However, the upregulation of activity was more pronounced than the induction of 5-LO mRNA and protein expression. Thus, there was a 72-fold increase in 5-LO activity by TGF-β plus VD_3 as compared to TGF-β alone, corresponding to 8- and 13-fold differences in 5-LO mRNA and protein, respectively (Table 1). This indicates that the combination of TGF-β and VD_3 not only causes a more pronounced upregulation of 5-LO

protein expression but also apparently leads to either a modification of the 5-LO protein that increases its specific activity or induces or modifies other cellular components of importance for 5-LO activity.

Differences between activity and protein expression were also observed when time courses of induction of 5-LO activity and protein expression were investigated (Fig. 4). In comparison to the upregulation of 5-LO protein expression, appearance of activity was delayed. Moreover, pretreatment of cells with TGF-β or VD_3 alone for 2 days induced 5-LO protein but almost no activity; addition of the second inducer was required for upregulation of the activity (Fig. 4 *B* and *C*).

To clarify whether the observed differencies in activity were due to modifications of the 5-LO protein or to other cellular components, 5-LO was partially purified from cells with low activity (grown with TGF-β for 2 days) by ATP-agarose column chromatography. This preparation of 5-LO was then recombined with ATP-PT fractions containing most of the soluble cellular proteins, and 5-LO activities were determined. 5-LO activity was inhibited when this enzyme preparation was recombined with the original ATP-PT fraction. On the other hand, recombination with ATP-PT fractions prepared from Mono Mac 6 cells treated with TGF-β plus VD_3, granulocytes, or the B-lymphocytic cell line BL41E95A resulted in increased enzyme activity. Apparently, 5-LO in Mono Mac 6 cells grown for 2 days in the presence of TGF-β is inhibited by cytosolic components. In addition, these cells could lack an activator that may be present in ATP-PT fractions from Mono Mac cells grown for 4 days in the presence of TGF-β and VD_3, granulocytes, or lymphocytes.

Synergistic effects of TGF-β and VD_3 on leukemic cell lines have been described (19, 20). A number of differentiation-related parameters were affected, and it was concluded that TGF-β plus VD_3 together stimulate the terminal differentiation of monocytic cells. It appears reasonable that the effects on 5-LO are part of such a differentiation process. Synergistic effects of these agents have also been seen for other cell types. One interesting example was the 40- to 70-fold upregulation of alkaline phosphatase activity in MG-63 osteosarcoma cells, combined with 5- to 6-fold upregulation of mRNA and protein (21). We previously found an upregulatory effect of TGF-β, primarily on 5-LO activity, in DMSO-differentiated HL-60 cells (11). In those studies, the presence of a cellular lipid fraction was required to have the best effect of TGF-β. It is conceivable that this lipid fraction contained VD_3.

The mechanism for stimulation of expression of 5-LO by TGF-β plus VD_3 in Mono Mac 6 cells should involve upregulation of transcription or effects on mRNA stability. Both alternatives are compatible with the effect of cycloheximide on 5-LO mRNA (Fig. 5), which indicated that protein biosynthesis (transcription factors or mRNA stabilizing proteins) was required for the increased expression.

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