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Transforming growth factor β upregulates 5-lipoxygenase activity during myeloid cell maturation

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ABSTRACT **Transforming growth factor β (TGFβ) increased the arachidonate 5-lipoxygenase (5-LO; EC 1.13. 11.34) activity in HL-60 cells induced to granulocytic differentiation by dimethyl sulfoxide. The presence of a factor in human serum that caused a similar increase was recently demonstrated. Several observations indicate that the serum factor consists of isoforms of TGFβ. Heat-treated serum and TGFβ both resulted in ≈10-fold increased 5-LO activity of HL-60 cells, antiserum to TGFβ neutralized the 5-LO-increasing activity in serum, and physical properties of the serum factor (lipophilic nature, alkaline pI, stability to heat and acid) coincided with those of TGFβ. The pattern of activity of native and heat-treated sera is compatible with activation of a latent form of TGFβ in serum. This activity was specific for TGFβ, since none of several other cytokines could increase 5-LO activity in differentiating HL-60 cells. However, granulocyte/macrophage-colony-stimulating factor (GM-CSF) and tumor necrosis factor α enhanced the effect of TGFβ. The most prominent effects of TGFβ, whether alone or together with GM-CSF, were observed for 5-LO activity in intact cells (10-fold or 30-fold induction, respectively). 5-LO protein levels were less affected (up to 2- or 5-fold, respectively, as judged from Western blots). There was no appreciable effect of TGFβ, or a combination of TGFβ and GM-CSF, on 5-LO mRNA expression.**

Leukotrienes are mediators of inflammatory responses that can be formed in granulocytes, monocytes/macrophages, and mast cells after stimulation. Arachidonate 5-lipoxygenase (5-LO) catalyzes the first two steps of leukotriene biosynthesis from arachidonic acid (1). In intact cells, 5-LO requires additional components for full activity; one of them has been isolated and termed FLAP (2, 3). 5-LO mRNA, protein, and activity have been shown to be increased during myeloid cell maturation (4–7). We found that a heat-stable but protease-sensitive component in human serum induced an increased 5-LO activity in maturing HL-60 promyelocytic leukemia cells and that this increase was not primarily due to alterations in 5-LO or FLAP expression (7, 8).

Transforming growth factor β (TGFβ) is a peptide that has numerous regulatory actions on a great variety of cell types (for review see refs. 9 and 10). TGFβ was first identified by its ability to cause anchorage-independent growth of normal rat kidney fibroblasts (11). It is a multifunctional protein that can, depending on the target cell, either stimulate or inhibit cell proliferation and differentiation. TGFβ is most abundant in platelets (12), bone (13), and placenta (14).

Here we describe the partial purification and characterization of the 5-LO-stimulatory activity in serum, and its tentative identification as TGFβ.

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MATERIALS AND METHODS

HPLC solvents were from Rathburn Chemicals (Walkerburn, Scotland) and molecular biology reagents were from Pharmacia. Human serum was from Karolinska Hospital (Stockholm) and insulin and transferrin from Sigma. Platelet-derived growth factor (PDGF, A/A) was from Boehringer Mannheim. Granulocyte/macrophage-colony-stimulating factor (GM-CSF) and recombinant human TGFβ1 were from Amersham. Porcine TGFβ2 and anti-TGFβ1/2 neutralizing antibody were from R&D Systems (Minneapolis). Tumor necrosis factor α (TNFα) and connective-tissue activating peptide III (CTAP-III) were gifts from W. Fiers (State University, Gent, Belgium) and A. Walz (Theodor Kocher Institut, University of Bern, Bern, Switzerland). TNFα and GM-CSF were diluted and stored in phosphate-buffered saline with 0.1% bovine serum albumin. Protein was determined by the method of Lowry *et al.* (15).

Cells and 5-LO Assay. HL-60 cells were grown one passage (3 or 4 days) in serum-free medium before experiments (7, 16). Fractions or compounds tested for effect on 5-LO activity were added together with dimethyl sulfoxide (DMSO) and assayed after 4 days (7, 17). 5-LO activity is expressed as ng of products per 10^6 cells.

Reverse transcription–PCR Analysis. Total cellular RNA was isolated, reverse-transcribed into cDNA, and amplified as described (7). Twenty-four cycles were carried out for β-actin and 30 cycles for 5-LO and FLAP determinations. The identity of the 5-LO PCR product (486 bp) was confirmed by sequencing with *Tth* DNA polymerase (using the Autocycle sequencing kit and an automated laser fluorescence DNA sequencer; Pharmacia). The identity of the FLAP PCR product (352 bp) was confirmed by digestion with *Bst*EII, which gave the expected DNA fragments (263 and 89 bp).

Western Blots. Cells (5×10^6) 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 (7).

Heat Treatment of Human Serum. Human serum was diluted 1:1 with RPMI 1640 medium, heated in a water bath (30 min for volumes up to 50 ml, 45 min for larger volumes) at 80°C, and chilled on ice. After centrifugation (10,000 × *g*, 4°C, 90 min) the supernatant was separated from the pellet, and used as heat-treated human serum (HTHS).

Abbreviations: 5-LO; 5-lipoxygenase; FLAP, 5-lipoxygenase-activating protein; DMSO, dimethyl sulfoxide; TNF, tumor necrosis factor; GM-CSF, granulocyte/macrophage-colony-stimulating factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; CTAP, connective-tissue activating peptide; HTHS, heat-treated human serum; IL, interleukin.

Delipidation of HTHS. HTHS (25 ml) was added dropwise to 75 ml of methanol (on ice), followed by 150 ml of diethyl ether. The mixture was kept on ice for 15 min and then centrifuged (2000 × g, 4°C, 10 min). The pellet was washed with 150 ml of diethyl ether, kept on ice for 15 min, centrifuged again, and dried under nitrogen. The dry protein was dissolved in 25 ml of 0.05 M phosphate buffer, pH 7.2/methanol, 80:20 (buffer I). The lipid fraction was evaporated and reconstituted to the original volume (25 ml) with 0.05 M phosphate buffer (pH 7.4).

Cation-Exchange Separation of the Protein Fraction. An aliquot of the protein fraction from the delipidation step (20 ml) was applied to an S-Sepharose fast flow (Pharmacia) column (bed volume, 6 ml) equilibrated with buffer I. The pass-through fraction is referred to as the SSPT fraction. After the column was washed with 20 ml of buffer I, adsorbed material was eluted with 10 ml buffer I containing 0.5 M NaCl (SSF fraction).

Reverse-Phase Separation. A PepRPC column (5 × 50 mm) was connected to an FPLC system (Pharmacia) with UV detection at 220 nm. Mobile phase A was 0.1% trifluoroacetic acid in water, and mobile phase B was 0.1% trifluoroacetic acid in acetonitrile. The column was equilibrated with A/B, 80:20 (vol/vol) at a flow rate of 1 ml/min. The SSF fraction from cation-exchange chromatography (10 ml) was supplemented with 2 M urea and 0.5% trifluoroacetic acid prior to injection. After elution of nonadsorbed material, a linear gradient from 30% to 50% B (20 min) was started and 1-ml fractions were collected.

RESULTS

Characteristics and Purification of the Serum Factor. Previous studies (7, 8) showed that human serum contains a heat-stable protein which increases 5-LO activity in differentiating HL-60 cells. When HTHS was subjected to trichloroacetic acid precipitation the activity was recovered in the protein pellet, indicating that the factor was also remarkably stable to acid.

Hydrophobic interaction chromatography (phenyl-Sepharose; Pharmacia) indicated that the factor was lipophilic, since elution of activity required 10–30% acetonitrile in water. This indicated that lipid could be bound to (and possibly part of) the active factor. HTHS was delipidated by extraction with methanol/diethyl ether. The resulting protein and lipid fractions (reconstituted to the original volume) contained protein at 4.8–10.3 mg/ml and 0.26–0.66 mg/ml, respectively. Protein or lipid fraction (3%, vol/vol) was added together with 1.5% dimethyl sulfoxide to HL-60 cells in serum-free medium. After 4 days, cellular 5-LO activity had increased from 30 ng per 10^6 cells to 80 ng and 34 ng per 10^6 cells (n = 3), respectively (Fig. 1). Cells receiving HTHS (3%) obtained a 5-LO activity of 85 ng per 10^6 cells. Thus, almost all activity was recovered in the protein fraction. When the lipid fraction was added back to the protein fraction prior to administration to cells, an enhanced 5-LO activity was obtained (130 ng per 10^6 cells) (Fig. 1). Therefore, the lipid fraction (1%, vol/vol) was added routinely in subsequent experiments, when effects of protein fractions or cytokines were determined.

The factor in HTHS could not be purified by ion-exchange chromatography, probably because of adsorption to lipids. However, after delipidation the 5-LO activity-increasing protein was successfully purified by cation-exchange chromatography. The bulk of the proteins (typically 90–98%) were eluted in the pass-through (SSPT) fraction, but the activity was mainly found in the fraction eluted with 0.5 M NaCl (SSF fraction). Typically, HL-60 cells treated with the SSF fraction showed 5- to 30-fold more 5-LO activity than cells treated with a comparable aliquot of the SSPT fraction.

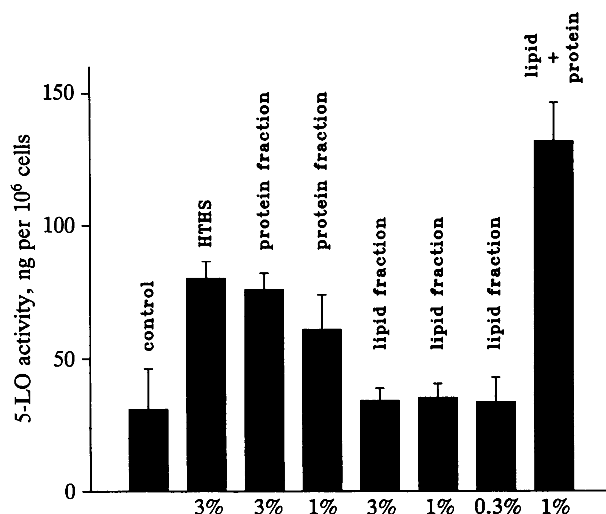

FIG. 1. Effects of lipid and protein fractions obtained from HTHS on 5-LO activity in HL-60 cells. The indicated concentrations of lipid and protein fractions, prepared as described under *Materials and Methods*, were added to cells together with 1.5% DMSO. After 4 days, the cells were harvested and assayed for 5-LO activity. Values are expressed as mean + SE of three separate experiments. Control cells received only DMSO.

The factor in the SSF fraction was further analyzed by reverse-phase chromatography (PepRPC, Pharmacia). After elution with a 30–50% acetonitrile gradient, no activity was found in any discrete fraction. However, activity was recovered when several fractions were pooled (indicated in Fig. 2). This led to the conclusion that either several components (separated on the column) were required for activity or the active material was heterogeneous.

Release of 5-LO-Increasing Activity from Platelets. Other possible sources for the factor were investigated. Serum-free conditioned media from several cells in culture were tried: lipopolysaccharide-stimulated human monocytes, phytohemagglutinin-stimulated peripheral lymphocytes, fibroblasts, HepG2 hepatoma cells, endothelial cells, and thrombin-stimulated platelets. Only the medium from the thrombin-stimulated platelets showed significant activity. Thus, it is possible that the serum factor was released from clotting platelets during the preparation of serum; consequently it should probably not be present in plasma. This was confirmed when heat-treated protein fractions (final concentration, 2%) from different plasma and serum preparations were tested for their effect on 5-LO activity in HL-60 cells (added

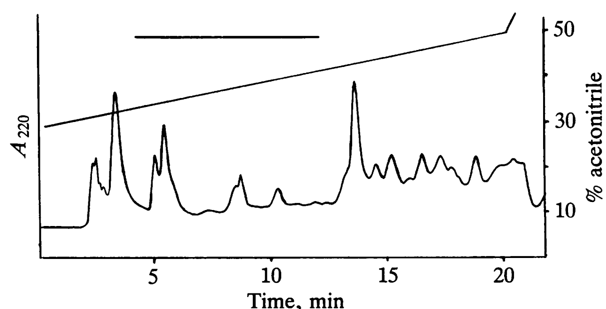

FIG. 2. Reverse-phase separation of proteins in the SSF fraction (from the preceding cation-exchange chromatography). Urea and trifluoroacetic acid (final concentrations 2 M and 0.5%, respectively) were added to the SSF fraction, and 10 ml was loaded onto the PepRPC column (connected to an FPLC system). After the absorbance at 220 nm had returned to baseline, elution with a 30–50% acetonitrile gradient was started. Horizontal line marks the fractions that were combined to recover 5-LO-upregulating activity.

together with 1% lipid fraction). All serum samples were able to induce 5-LO activity, but none of the plasma samples (n = 5).

5-LO-Increasing Activity of TGFβ. Platelets have been reported to release PDGF, CTAP-III, and TGFβ upon stimulation (18). PDGF (A/A, 20 ng/ml), CTAP-III (1 μg/ml), TGFβ1 (2 ng/ml), or the serum factor (1% by volume of the SSF fraction) were added to HL-60 cells together with DMSO and 1% lipid fraction. After 4 days 5-LO activities were determined. TGFβ1 and the serum factor gave similar increases in 5-LO activity [from 0.2 ± 0.2 ng/ml to 37 ± 8 and 31 ± 6 ng/ml (n = 4), respectively], whereas PDGF and CTAP-III were inactive. When anti-TGFβ antibodies (5 μg/ml) were added to the delipidated protein fraction from HTHS, the 5-LO-increasing activity of this protein fraction (2%, by volume) was completely blocked (data not shown).

Besides the cytokines tested previously (8), no 5-LO-increasing activity was found for bovine endothelial cell growth factor, bovine basic fibroblast growth factor, epidermal growth factor, recombinant human interleukin (IL) 8, neutrophil activating peptide 2, or recombinant human IL-1β (data not shown).

Effect of Heat and Acid Treatment or TGFβ Supplementation of human serum. Previous results suggested that human serum contains a 5-LO-upregulatory factor which is activated by heat treatment (7, 8). Serum contains TGFβ in an inactive form, covalently bound to α_2-macroglobulin (19). Interestingly, TGFβ added to native serum is not converted into its latent form, which allows for very sensitive detection of TGFβ in the presence of serum and α_2-macroglobulin (19, 20). Thus, we compared the effect of heat (and acid) treatment of serum, to that of addition of active TGFβ to serum. Serum was diluted 1:1 with RPMI 1640 medium and heated at 80°C for 30 min. Precipitated material was removed by centrifugation to obtain HTHS. HTHS was acidified with 1 M HCl (final concentration, 0.1 M), heated for 10 min at 80°C in a water bath, chilled on ice, and neutralized with 1 M NaOH. HTHS and acid-treated HTHS (final concentration, 3%) were added to HL-60 cells, together with 1.5% DMSO. Control cultures received DMSO and native serum (3%). After 4 days, cells were harvested and assayed for 5-LO activity. Heat treatment, and especially combined heat and acid treatment of serum, led to significant induction of cellular 5-LO activity [from 3.0 to 5.4 and 32 ng per 10^6 cells (n = 3), respectively]. Native serum with addition of TGFβ1 (2 ng/ml) also strongly induced 5-LO activity (from 3.0 to 46 ng per 10^6 cells, n = 3), demonstrating that the 5-LO-inducing activity of added TGFβ1 is not inactivated by serum. Thus, serum treated with heat and acid [which has been shown to activate latent TGFβ (21)] and serum with TGFβ added induced similar 5-LO activities in HL-60 cells.

Comparison of Effects of TGFβ1 and TGFβ2. HL-60 cells maintained in 10% fetal bovine serum were transferred directly to RPMI 1640 medium containing 3% human serum and then were induced to differentiate with 1.5% DMSO in the presence of the indicated concentrations of TGFβ1 and TGFβ2 (Fig. 3). After 4 days, cells were harvested and cellular 5-LO activity was determined. There was a dose-dependent increase of 5-LO activity by TGFβ1, TGFβ2, and a combination of both proteins. TGFβ2 induced 5-LO activity at lower concentrations than TGFβ1 and led to higher 5-LO activities (Fig. 3). However, the difference in potency was rather variable: TGFβ2 induced 1.2- to 10-fold higher cellular 5-LO activities than TGFβ1 in different experiments (Figs. 3 and 5 and data not shown). Interestingly, coincubation of TGFβ1 and TGFβ2 led to lower activity than TGFβ2 alone. This effect was more pronounced at higher concentrations. When the two factors were present at 1 ng/ml each, the 5-LO activity of the HL-60 cells became comparable to that obtained with TGFβ1 alone (Fig. 3). This demonstrates

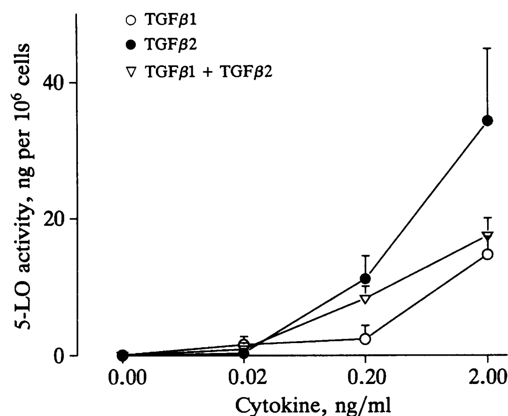

FIG. 3. Comparison of 5-LO-upregulatory activity of TGFβ1 and TGFβ2. HL-60 cells were induced to differentiate with 1.5% DMSO in the presence of 3% human serum and the indicated concentrations of TGFβ1, TGFβ2, or a combination (1:1) of both proteins. After 4 days, cells were harvested and 5-LO activity was determined. Results are expressed as mean + SE of three independent experiments.

that there was no synergy between the two forms of TGFβ and that the response of the cells to TGFβ2 could be inhibited by TGFβ1.

Modulation of TGFβ Effects by TNFα and GM-CSF. The 5-LO-increasing activity of the serum factor is enhanced by TNFα or GM-CSF (8). To test whether also the effect of TGFβ is modulated in a similar way, HL-60 cells were treated with 1.5% DMSO (plus 1% lipid fraction) for 4 days in the presence or absence of the indicated concentrations of TGFβ, GM-CSF, or TNFα (Fig. 4). Control cultures receiving only lipid fraction or lipid fraction plus GM-CSF or TNFα at 1 ng/ml showed low 5-LO activities. Addition of TGFβ1 (0.2 or 2 ng/ml) gave increased 5-LO activities which were enhanced 2- to 3-fold by GM-CSF or TNFα (Fig. 4). The higher dose of TGFβ1 (together with TNFα or GM-CSF) gave 25- to 40-fold increments of 5-LO activity compared with cells receiving only DMSO (Fig. 4).

Effects of TGFβ and GM-CSF on 5-LO Activities of Intact Cells, Cell Homogenates, 5-LO Protein, FLAP, and 5-LO mRNA. The 5-LO-increasing activity of serum was most prominent when intact cells were assayed for 5-LO activity, whereas 5-LO activities in cell homogenates and 5-LO protein levels were less affected. Also, serum had no or only minor effects on 5-LO and FLAP mRNA levels (7, 8).

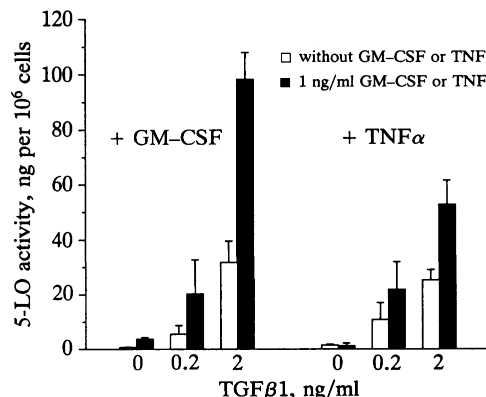

FIG. 4. Modulation of 5-LO-upregulatory effect of TGFβ1 by TNFα and GM-CSF. TGFβ1 without (open bars) or with (filled bars) GM-CSF or TNFα was added to HL-60 cells together with 1.5% DMSO and 1% lipid fraction. After 4 days, cells were harvested and 5-LO activity was determined. Values are expressed as mean + SE of three independent experiments.

The effects of TGFβ, GM-CSF, or combinations of the two growth factors on these parameters were determined. HL-60 cells grown for 4 days in serum-free medium were induced to differentiate with 1.5% DMSO (plus 1% lipid fraction) in the presence of various cytokines at 1 ng/ml (Fig. 5). Cells were harvested after 4 days, and aliquots were analyzed for 5-LO activity, 5-LO protein, and FLAP and 5-LO mRNAs. The activities of intact cells increased about 10-fold after addition of TGFβ1 or TGFβ2 and about 30-fold after addition of TGFβ plus GM-CSF (Fig. 5*A*). However, the activities of the corresponding cell homogenates increased only 3- and 6-fold, respectively, since the homogenates from cells not receiving TGFβ had higher activities than the corresponding intact cells. Western blot analysis showed an ≈2-fold increase in 5-LO protein after addition of TGFβ, whereas TGFβ plus GM-CSF gave an ≈5-fold increase (Fig. 5*B*). Addition of GM-CSF only (together with DMSO) did not result in an increase in 5-LO protein. No significant changes in 5-LO or FLAP mRNA expression were observed (Fig. 5*C*). Thus, TGFβ has a more prominent effect on 5-LO activity than on the amount of 5-LO protein in intact cells.

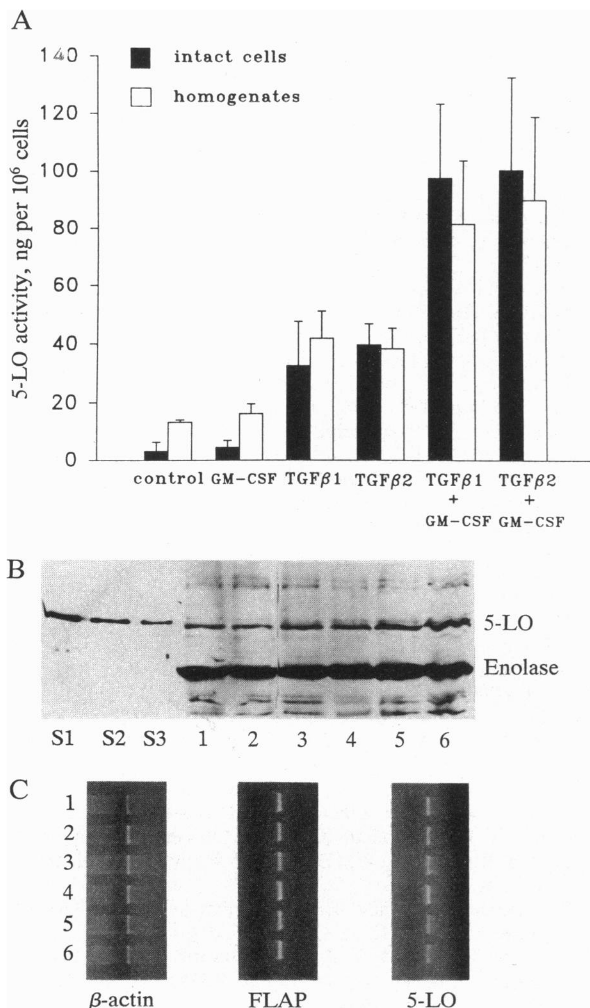

FIG. 5. Effect of TGFβ and GM-CSF on 5-LO activities in intact cells and cell homogenates, on 5-LO protein, and on 5-LO and FLAP mRNA. (*A*) HL-60 cells were treated with 1.5% DMSO, 1% lipid fraction, and the indicated cytokines at 1 ng/ml. After 4 days cells were harvested, and intact cells (filled bars) as well as cell homogenates (open bars) were assayed for 5-LO activity (mean + SE of three independent experiments). (*B*) Samples of protein (corresponding to 10^6 cells solubilized in loading buffer) were analyzed for 5-LO protein by Western blot. Standards (recombinant 5-LO, 95% pure) are indicated by S1 (50 ng), S2 (25 ng), and S3 (12 ng). Lanes 1–6 show same samples as in *A* (lane 1, control cells; lane 6, cells treated with TGFβ2 plus GM-CSF). The 5-LO antiserum also contained antibodies to enolase, hence the intense enolase bands which serve as a constitutively expressed control. (*C*) Samples of total RNA from each cell culture were subjected to reverse transcription–PCR for analysis of FLAP and 5-LO mRNA. Samples are as in *A* and *B* (lane 1, control cells; lane 6, cells treated with TGFβ2 plus GM-CSF). β-Actin mRNA served as constitutively expressed control.

DISCUSSION

Human serum contains a factor that increases 5-LO activity in differentiating HL-60 cells. Characterization of the serum factor revealed that it is a heat- and acid-stable lipophilic protein that binds to cation-exchange resin at pH 7.4. Human platelets were identified as a possible source for the serum factor.

TGFβ, one of the cytokines released from platelets, also increased the 5-LO activity in HL-60 cells. In assays of intact cells the activity increased about 10-fold (similar to the effect of serum) and the increase in cellular 5-LO activity became quite similar, when TGFβ or HTHS was added to the cells. Approximately 2-fold differences in 5-LO protein expression were found. It should be noted that the 5-LO activities obtained in different experiments have been variable. Previously, HL-60 cells treated with serum gave activities above 100 ng of 5-LO products per 10^6 cells (7); more recently the activities of intact cells from corresponding experiments (using serum or TGFβ) have been 30–40 ng per 10^6 cells. The activities of cell homogenates, however, have been more constant. The reason for these discrepancies is not understood but may be related to changes of the cell line over time.

TGFβ is released from platelets in a latent form (22, 23), consisting of the mature TGFβ dimer plus two TGFβ precursors disulfide-linked to an additional 130-kDa protein. In serum, the latent complex consists of TGFβ covalently bound to α_2-macroglobulin (19). The precise mechanisms for activation of latent TGFβ *in vivo* are not known. *In vitro*, latent TGFβ can be activated by heating or by treatment with acid, alkali, or chaotropic agents (21). In this respect, our findings regarding effects of native and heat-treated sera, in comparison to the effects of TGFβ, are of interest. HL-60 cells which received native serum together with DMSO did not obtain a higher 5-LO activity than cells receiving only DMSO, indicating that these cells could not activate latent TGFβ present in serum. However, when cells were treated with DMSO in serum-free medium for 4 days before the addition of human serum, serum was able to increase 5-LO activity to the level found for normal human granulocytes (7). Thus, HL-60 cells that are induced to differentiate with DMSO in serum-free medium for 4 days seem to acquire the capability to activate latent TGFβ in serum or to respond to extremely low levels of free TGFβ. Heat- and acid-treated serum gave the same effect as exogenous TGFβ (see *Results* and ref. 8); i.e., it increased 5-LO activity when added together with DMSO, compatible with an *in vitro* activation of latent TGFβ during such treatment of serum.

TGFβ can act in synergy with or antagonize the effects of other cytokines. For example, TGFβ inhibits IL-2-stimulated proliferation of T lymphocytes (24) and IL-3-induced colony formation of early hematopoietic progenitor cells (25). On the other hand, TGFβ stimulates GM-CSF-induced granulopoiesis of more mature progenitor cells (26) and acts together with TNFα to induce differentiation of HL-60 and U-937 cells (27). We found previously that the 5-LO-upregulating effect of heat-treated serum was modulated by GM-CSF and TNFα (8). Thus, it was not unexpected that both GM-CSF and TNFα were able also to increase the 5-LO-upregulating effect of TGFβ. Both cytokines were virtually inactive when added without TGFβ (Fig. 4).

Three isoforms of mammalian TGFβ have been cloned, TGFβ1, -2, and -3. The three isoforms display similar activity and potency in many assays, but marked differences have been noted. For example, TGFβ1 but not TGFβ2 can be a potent inhibitor of hematopoietic cell proliferation (28). TGFβ1 has a 10-fold higher affinity for the overall population of type I or II receptors (29, 30), but also a subset of these receptors has been detected with high affinity for TGFβ2 (29). TGFβ1 can almost completely displace TGFβ2 from type I and II receptors but not vice versa. TGFβ2 was more active than TGFβ1 in upregulating the 5-LO activity in HL-60 cells, but the difference in activity between the two factors was highly variable in different experiments. No synergy between the two forms was found, and at higher concentrations a combination of both factors (1 ng/ml each) led to activities which were comparable with the activity of TGFβ1 alone. This indicates that TGFβ1 inhibits the response of HL-60 cells to TGFβ2, possibly by competing with TGFβ2 for receptor binding.

Several findings thus indicate that the 5-LO-upregulatory activity present in human serum is identical to TGFβ. An $\approx$10-fold increase of 5-LO activity in intact HL-60 cells (while the amount of 5-LO protein increased 2-fold to 3-fold) was obtained both with heat-treated serum and with TGFβ. Physical properties of the serum factor (lipophilic nature, alkaline pI, stability to heat and acid) coincided with those of TGFβ. Serum treated with antiserum to TGFβ was no longer effective. The pattern of activity of native and heat-treated sera is compatible with activation of a latent form of TGFβ present in serum. The effects of the serum factor and TGFβ were similarly modulated by TNFα and GM-CSF. Plasma lacked the 5-LO-upregulatory activity. Thus, it appears reasonable that the factor is released from platelets (which contain TGFβ) during the preparation of serum. These considerations make it feasible that the 5-LO-upregulatory factor in human serum consists of isoforms of TGFβ. As suggested before for the serum factor (7, 8), it appears that TGFβ upregulates 5-LO activity primarily via induction or modification of other cellular components that are important for the 5-LO activity in the intact cell.

These results were obtained using the leukemic HL-60 cell line. One might speculate that the 5-LO activity of normal human granulocytes is upregulated by TGFβ during normal differentiation in the bone marrow, where TGFβ is present in high concentrations.

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