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Effect of TGFβ on liver genes expression

Antagonistic effect of TGFβ on IL-6-stimulated genes in Hep 3B cells

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The effect of transforming growth factor beta (TGFβ) on the expression of a group of liver genes has been investigated in the hepatoma cell line Hep 3B. TGFβ induces a decrease of the basal level of apolipoprotein A-II (ApoA-II), retinol binding protein (RBP) and α-fetoprotein (αFp). Furthermore, TGFβ efficiently antagonizes the IL-6-induction of hemopexin (Hpx) and haptoglobin (Hp) and α1-acid glycoprotein (AGP). These effects of TGFβ are apparently mediated by post-transcriptional mechanism(s). These findings, together with previously reported data on the inhibitory effect of TGFβ on acute phase genes (e.g. ApoA-I and albumin), suggest a role for TGFβ in the regulation of expression of liver genes.

Transforming growth factor β; Interleukin-6; Liver gene expression; Cytokine

1. INTRODUCTION

Transforming growth factor beta (TGFβ) is a multifunctional polypeptide that can modulate growth and differentiation of many cell types. It also influences various processes in vivo, including early embryo development, angiogenesis and wound healing [1]. Most of the effects exerted by TGFβ appear to be consequent to the regulation of the expression of specific genes [2,3]. We have lately studied the effect of TGFβ on expression of liver genes. Expression of several liver genes is altered following induction of the acute phase response. This results in an increase in the synthesis of several plasma proteins like hemopexin, haptoglobin, C-reactive protein, serum amyloid A, the α, β, and γ chains of fibrinogen, and in a decrease in the levels of other proteins such as albumin, ApoAI and transferrin. Many studies have demonstrated that human hepatoma cell lines can reproduce the changes in gene expression characteristic of the hepatic acute phase response when stimulated with monocyte conditioned medium (MoCM) or with purified cytokines [4–6].

Several cytokines appear in fact to be involved in the control of gene expression in the liver: in particular, IL-6 upmodulates the expression of genes coding for positive acute phase reactants [4–7]. On the contrary in Hep 3B cells TGFβ down-regulates the mRNA levels of genes negatively modulated during liver acute phase reaction without stimulating the expression of positive AP genes [2]. TGFβ also inhibits the expression of the coagulation factor VII and of both basal and IL-6-stimulated expression of the mRNA of the α, β, and γ chains of fibrinogen [3]. An inhibitory effect of TGFβ on some positive acute phase proteins has also been described [8,9]. Here we extend our analysis to other liver genes. The data we report indicate that TGFβ decreases the expression of ApoA-II, RBP and αFp and efficiently counteracts IL-6-stimulated expression of hemopexin, haptoglobin and AGP in Hep 3B cells. Both run-on analysis and CAT assays indicate that the effects of TGFβ on the expression of these genes are mostly exerted at the post-transcriptional level.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Hep 3B cells were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS). Monocyte conditioned medium (MoCM) was obtained from peripheral blood mononuclear cells (PBMC) as described [2]. Recombinant IL-6 was from Boehringer-Mannheim and TGFβ-1 from R & D, Minneapolis.

2.2. *RNA preparation and Northern blot analysis*

RNA was extracted by guanidium thiocyanate lysis and ultracentrifugation on CsCl density gradient. Total RNA (20 μg) was separated by electrophoresis on 1.3% denaturing agarose–formaldehyde gels and transferred onto nylon filters (GeneScreen Plus, Du Pont-New England Nuclear). Filters were hybridized with ^{32}P-labelled probes and autoradiographed as described [2].

2.3. *Transfection experiments*

DNA transfections were performed with the calcium phosphate precipitation technique. After 16 h cells were trypsinized, plated on separate dishes and treated with different stimulants. Chloroamphenicol acetyltransferase assay were performed as described [4,7]. A RSV-

luciferase plasmid was included in the transfections as control and the CAT activity was normalized by comparison with the luciferase activity of the corresponding sample. The plasmids containing the 5' regulatory sequence of hemopexin (Hpx-175 and Hpx-2000) and of haptoblobin (Hp-186) have been previously described [4,7].

2.4. *Run-on experiments*

For transcriptional analysis, 4×10^7 cells were washed with cold PBS, scraped and pelleted. Nuclei were prepared and transcription carried out in the presence of [^{32}P]UTP as described [2,3]. cDNAs bound to nitrocellulose filters were hybridized with 1×10^6 cpm/ml of radiolabelled nuclear transcripts.

3. RESULTS

3.1. *Effect of TGFβ on expression of apolipoprotein A-II, retinol binding protein and alpha fetoprotein*

Hep 3B cells were treated with MoCM or purified porcine TGFβ for 48 h. As shown in Fig. 1 treatment with MoCM results in a slight decrease of ApoA-II mRNA compared to untreated cells while incubation with TGFβ-1, from 0.2–5 ng/ml, produced a pronounced dose-dependent decrease of the ApoA-II mRNA. The expression of the mRNA of two other genes α-fetoproteina (αFp) and retinol binding protein (RBP), whose mRNAs are constitutively expressed in

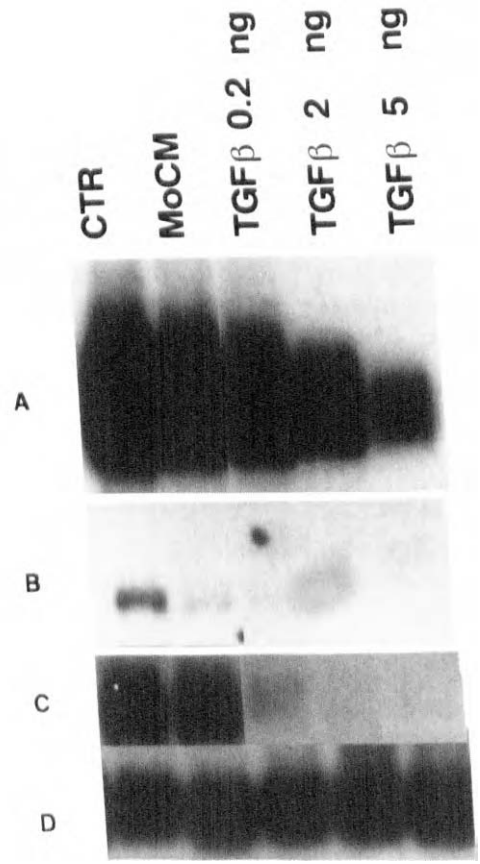

Fig. 1. Effect of TGFβ on (A) ApoA-II, (B) αfetoprotein, (C) retinol binding protein and (D) γ-actin. Hep 3B cells were cultured for 48 h with either 15% MoCM and the indicated amount of TGFβ. RNA was extracted and hybridized with the specific probes as in Materials and Methods.

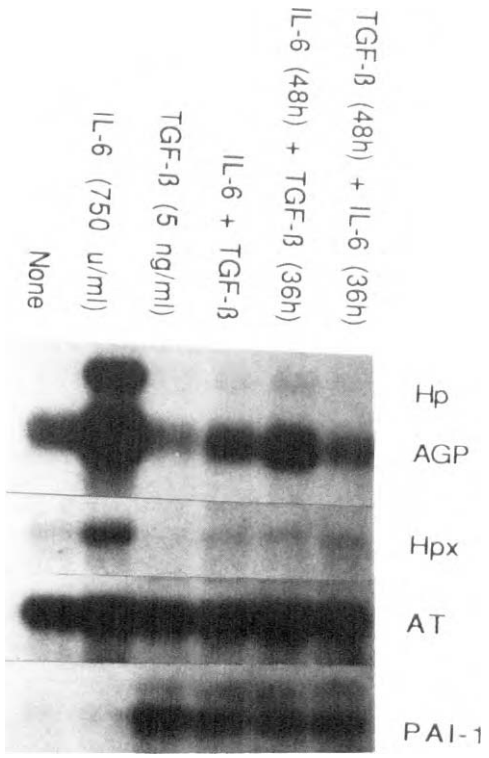

Fig. 2. Northern blot analysis of RNA from Hep 3B cells, either untreated or after addition of IL-6 (750 U/ml), IL-6 (750 U/ml) + TGFβ (5 ng/ml) for 48 h, or pre-treated for 12 h with IL-6 (750 U/ml) or TGFβ (5 ng/ml) and then with the other cytokine. Hp, haptoblobin; AGP, α1-acid glycoprotein; Hpx, hemopexin; At, α1-antitrypsin; PAI-I, plasminogen activator inhibitor type I.

Hep 3B cells and not part of the liver acute phase reaction was also studied. αFp mRNA accumulation was also sensitive to both MoCM and TGFβ, which at 2 ng/ml completely abrogated αFp mRNA expression in Hep3B. The expression of RBP mRNA was not significantly affected by MoCM but was down-regulated by TGFβ. The same RNA preparations were hybridized with a γ-actin cDNA as internal control.

3.2. *Antagonistic effect of TGFβ on IL-6-induced genes in Hep 3B cells*

We extended our analysis to genes whose expression is stimulated by IL-6 in Hep 3B cells. Addition of MoCM to most hepatoma cell lines results in at least a 10-fold increase in the expression of the positively regulated acute phase genes like haptoglobin, AGP and hemopexin, and a similar effect is observed after treatment with IL-6 [4]. Incubation of Hep 3B cells with IL-6 resulted in an induction of the mRNA for haptoglobin, while treatment with TGFβ at 5 ng/ml had no effect on expression of this gene (Fig. 2). Combined treatment with TGFβ and IL-6 resulted in almost complete abrogation of induction of the haptoglobin mRNA. Similar results were observed when expression of hemopexin was analyzed.

Unlike hemopexin and haptoglobin mRNA, which

are not detectable in unstimulated cells, AGP mRNA is expressed in untreated Hep 3B cells, yet its levels can be further increased by addition of IL-6. Treatment with TGFβ results in both a decrease in the basal levels and in a reduction of IL-6-induced expression of AGP. No effect on α1-antitrypsin was detected in either control Hep 3B cells or in cells treated with TGFβ, IL-6 or a combination of them.

Expression of the plasminogen activator inhibitor gene type I (PAI-I) is known to be regulated by TGFβ in many cell types. We therefore tested whether contemporary treatment with IL-6 would affect TGFβ induction of this gene. As shown in panel E, IL-6 did not affect induction by TGFβ of PAI-I. It has been reported [9] that pre-treatment of PCL/PRF/5 cells with TGFβ followed by incubation with IL-6 increased the synthesis of C-reactive protein (CRP), whereas pre-treatment with IL-6 followed by TGFβ or co-treatment with the two cytokines both resulted in a dramatic decrease of CRP production. In our experiments as shown in Fig. 2, pre-treatment of Hep 3B cells with either cytokine and addition of the other 12 h later (lanes 5–6) did not show significant differences compared with co-treatment with both molecules (lane 4) for all the mRNAs analyzed.

3.3. *TGFβ acts mainly at the post-transcriptional level in regulating liver gene expression in Hep 3B cells*

To analyze the effect of TGFβ on the transcription of the AGP and RBP genes the neo-synthesis of RNA was measured in isolated nuclei. The rate of transcription of the AGP gene but not of RBP was stimulated by MoCM (Fig. 3). Treatment with TGFβ did not affect transcription of AGP and RBP although TGFβ significantly diminished the accumulation of these mRNA as evaluated by Northern analysis (Fig. 1). As previously reported albumin transcription was reduced by around 2-fold following TGFβ treatment.

In preliminary experiments we observed that analysis of chloramphenicol acetyltransferase (CAT) activity, in cells transfected with plasmids containing the promoter region of hemopexin and haptoglobin fused to the coding region of the bacterial CAT gene, was a better assay for transcriptional regulation by IL-6 than run-on assays for genes whose regulatory regions have been extensively characterized, such as hemopexin and haptoglobin [4,7]. To evaluate whether TGFβ exerted an inhibitory effect at the transcriptional or post-transcriptional (e.g. stability of the mRNA) level, plasmids containing a segment of the 5′ non-coding regulatory sequence of the haptoglobin and the hemopexin cloned in front of the CAT gene were transfected in Hep 3B cells. Cells were then treated with IL-6, TGFβ, or a combination of both for 48 h, protein extracts prepared and CAT activity determined (Table I). A strong IL-6 dependent stimulation of CAT activity from these constructs was observed. However no major difference was observed in CAT activity in cells treated with IL-6 and IL-6/TGFβ. This suggests that the reduction in mRNA levels induced by TGFβ, but not those induced by IL-6, are not mediated at the transcriptional level, but rather post-transcriptionally.

Table I

Induction rates of transfected Hpx- and Hp-CAT plasmids in Hep 3B cells

Template	Control	IL-6	TGFβ	IL-6+TGFβ
Hpx-175	1	8	2	6
HPX-2000	1	20	4	14
Hp-186	1	5.5	0.9	4.2

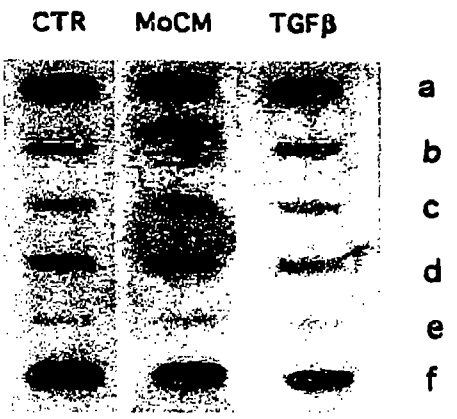

Fig. 3. Nuclear run-on assay performed on control, MoCM- and TGFβ-treated Hep 3B cells. Plasmids were fixed to nitrocellulose filters and hybridized with 1 × 10⁶ cpm/ml of radiolabelled nuclear transcripts. pBR322 was used as background control. (a) γ-actin; (b) AGP; (c) RBP; (d) α1-antitrypsin; (e) pBR322; (f) albumin.

4. DISCUSSION

Human plasma protein synthesis in liver cell cultures is regulated by several cytokines including IL-1-β, IL-6, and TNF-α at the gene level [2,4–6]. We have previously reported that TGFβ down-regulates the mRNA level of genes which are negatively modulated during the acute phase reaction (e.g. albumin and ApoA-I) [2]. This effect of TGFβ contrasts with the ability of IL-6 to stimulate expression of several positively regulated acute phase genes, suggesting that TGFβ and IL-6 may have opposite effects in the physiological regulation of these two groups of genes. TGFβ and IL-6 can also affect the expression of a set of proteins involved in coagulation [3]. In this context TGFβ efficiently counteracts the IL-6-induced increase of fibrinogen mRNA and down-regulates the basal expression of fibrinogen and factor VII mRNA in Hep 3B cells [3].

The data presented here help to better define the role of TGFβ in the regulation of liver gene expression. TGFβ appears to abolish expression of several genes

expressed in basal conditions in Hep 3B cells (like AGP and RBP) and also inhibits IL-6-induced expression of fibrinogen, haptoglobin, hemopexin and AGP. On the other hand IL-6 does not interfere with the expression of PAI-1 gene induced by TGFβ. In accordance with our earlier findings on ApoA-I and albumin gene transcription most of the effects of TGFβ in down- regulating the expression of the above reported genes appears, by either transfection experiments or run-on analysis, to be mediated mostly at the post-transcriptional level.

These results, together with previous findings, show that TGFβ can regulate the expression of a number of liver specific genes. The ability to down-regulate both genes constantly expressed in liver as well as genes which are stimulated by IL-6 in consistent with the proposed role of TGFβ in the inflammatory and immunological responses to injury, infection and wound repair [10].

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