Transforming growth factor-β and epidermal growth factor modulate basal and interleukin-6-induced amino acid uptake and acute phase protein synthesis in cultured rat hepatocytes

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Rat hepatocytes cultured for 2 days with interleukin-6 show increased synthesis of acute phase proteins and enhanced accumulation of ^{14}C-labelled α-aminoisobutyric acid. Transforming growth factor-β_1 (0.1–10 ng/ml) inhibits whereas epidermal growth factor (1–100 ng/ml) enhances both basal and interleukin-6-induced amino acid uptake by rat hepatocytes with only a slight alteration of acute phase protein synthesis.

Acute phase protein; α-Aminoisobutyric acid; Interleukin-6; Transforming growth factor-β; Epidermal growth factor; Hepatocyte

1. INTRODUCTION

Interleukin-6, also known as interferon-B_2 and B-cell stimulatory factor-2, is the principal cytokine responsible for the acute phase response of rat liver cells [1,2]. Epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) are regulatory polypeptides controlling not only cell proliferation and differentiation but also induction of certain proteins in tissue culture [3–6]. The liver parenchymal cells are known to be an important target for EGF action [7–9] and recently Mackiewicz and co-workers [10] and Morrone and co-workers [11] reported that TGF-β, regulates synthesis of some acute phase plasma proteins in human hepatoma cells Hep G2 and Hep 3B. Here we compare effects of EGF and TGF-β on AIB uptake and acute phase protein synthesis in cultured rat hepatocytes stimulated with IL-6.

2. MATERIALS AND METHODS

Adult rat hepatocytes were isolated by collagenase technique and cultured for 2 days as monolayers on collagen-coated 35-mm plastic dishes (10^6 cells/dish) in Williams E medium containing 5% foetal calf serum and 1 μM each of insulin and dexamethasone [2,12]. The media alone, or containing IL-6, TGF-β and EGF as indicated, were collected daily and used for measuring concentrations of five plasma proteins by electroimmunoassay. The cell monolayer was further incubated for 20 min with [^{14}C]AIB (aminoisobutyric acid, α-[1-^{14}C] from New England Nuclear) and then amino acid uptake was measured [2]. Human recombinant IL-6 (sp. act. of 5.9×10^7 units/mg in 7TD1 mouse-mouse hybridoma cell proliferation assay) was provided by one of us (W.F.) [2]. TGF-β_1 purified from human platelets was kindly supplied by Dr M.B. Sporn (National Institutes of Health, Bethesda, MA) while EGF (from mouse maxillary gland) was bought from Serva, Heidelberg. Effects of TGF-β and EGF on hepatocyte proliferation were evaluated by measuring incorporation of [^{3}H]thymidine [6-^{3}H]TdR, Praha, Czechoslovakia) and determination of total cellular protein according to Carr et al. [13].

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3. RESULTS AND DISCUSSION

In agreement with earlier findings [2] we observed that rat hepatocytes cultured for 2 days with IL-6 show significantly augmented rate of uptake of [^{14}C]AIB (Table I). The accumulation of AIB was proportional to IL-6 concentration, decreased in the presence of TGF-β but increased in the cells cultured in the presence of EGF (Fig. 1). These effects were already noted in 24 h cultures but were more pronounced after 48 h exposure of cells to IL-6 and growth factors. TGF-β was active in the range of 0.1–10 ng/ml of medium while EGF at 1–100 ng/ml, both in the presence and absence of IL-6 (Table I). Opposite effects of EGF and TGF-β on AIB uptake were also manifested when their mixtures were added to hepatocyte cultures (data not shown). On the other hand, Morin et al. [14] and Auberger et al. [15] demonstrated previously that 1 nM EGF (approx. 6 ng/ml) did not stimulate AIB transport in cultured rat hepatocytes exposed to EGF for a short time (up to 3 h) while Moule and McGivan [8] found a transient stimulation of alanine transport at 40 min after exposure of rat hepatocytes to 10 nM EGF.

The effect of IL-6 on synthesis of acute phase proteins in cultured hepatocytes is well documented [2] and has been confirmed in the present experiments (Table

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Table I

Modulation of AIB uptake in rat hepatocytes by various concentrations of TGF-β and EGF in the absence or presence of interleukin-6

Growth factor added		AIB uptake (pmol/10^6 cells/min)	
		Without IL-6	With IL-6 (5 ng/ml)
None		10.8 ± 2.2	16.7 ± 2.3[a]
TGF-β	0.1	10.3 ± 1.2	15.5 ± 1.9
(ng/ml)	1.0	8.4 ± 1.7[a]	12.0 ± 2.1[b]
	10.0	6.1 ± 1.6[a]	8.8 ± 1.8[b]
EGF	1	12.5 ± 2.0	17.5 ± 2.1
(ng/ml)	10	16.8 ± 1.8[a]	22.3 ± 2.0[b]
	100	19.7 ± 1.9[a]	23.6 ± 2.3[b]

Rat hepatocytes were cultured for 2 days with the indicated amounts of growth factors. The results are the means ± SD of 4–8 experiments and were calculated from specific activities of [^{14}C]AIB taken up the cells during 20 min incubation.

[a] Value statistically different ($P<0.01$) in comparison to hepatocytes cultured without IL-6 and growth factors

[b] Value statistically different ($P<0.01$) in comparison to hepatocytes cultured with IL-6 but without growth factors

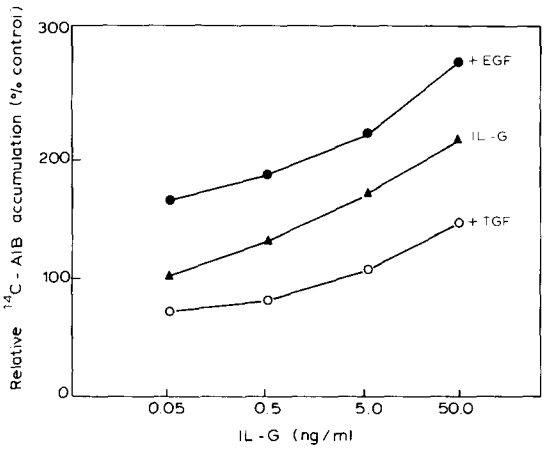

Fig. 1. Modulation of IL-6-induced [^{14}C]AIB uptake by TGF and EGF. Rat hepatocytes were cultured for 2 days with indicated amounts of IL-6 alone (Δ—Δ), IL-6 and 1 ng of TGF (○—○), or IL-6 and 10 ng/ml of EGF (●—●). The results derive from a typical experiment. For statistical evaluation, see Table I. The value of 100% represents [^{14}C]AIB radioactivity accumulated in the cells during 20 min of incubation of control cultures (i.e. without IL-6, TGF or EGF).

II). On the other hand, exposure of rat hepatocytes to the tested growth factors for periods up to 48 h only slightly affected protein production in distinction to the results reported by Mackiewicz et al. [10] and Morrone et al. [11] for human hepatoma cells. In our experiments EGF increased synthesis and secretion of all tested proteins, and especially of albumin, while TGF-β showed an opposite trend, with the exception of α_2-macroglobulin. These effects became more pronounced in the presence of IL-6 (5–50 ng/ml) and thus inhibition of albumin synthesis by IL-6 was totally reversed by 10 ng of EGF (Table II). When the results were expressed as the ratio of synthesis of α_2-macroglobulin to albumin as recommended in our

Table II

Effects of IL-6, TGF-β and EGF on synthesis of five plasma proteins by rat hepatocytes

Cytokine added		Protein produced				
		ALB	AI3	A2M	CPI	FBG
None (control)	μg	26.7 ± 3.9	11.1 ± 0.1	2.9 ± 0.5	1.9 ± 0.3	6.5 ± 1.1
	%	100	100	100	100	100
IL-6 (5 ng/ml)	%	74	95	176	158	186
(50 ng/ml)	%	65	89	254	215	238
TGF-β (1 ng/ml)	%	95	91	102	96	89
(10 ng/ml)	%	90	96	105	99	85
EGF (1 ng/ml)	%	103	98	114	98	104
(10 ng/ml)	%	120	105	125	105	116
(100 ng/ml)	%	131	106	136	106	125
IL-6 + TGF 1 ng	%	72	88	175	142	142
IL-6 + TGF 10 ng	%	66	81	186	152	135
IL-6 + EGF 1 ng	%	85	92	181	165	192
IL-6 + EGF 10 ng	%	109	99	205	158	206
IL-6 + EGF 100 ng	%	118	103	189	166	209

Cells were cultured for 2 days in 1 ml of Williams E medium with addition of either 0.25 ml medium alone (control) or with indicated amounts of cytokines in 0.25 ml of medium. In the case of cytokine mixtures, concentration of IL-6 was always 5 ng/ml. Proteins: albumin (ALB), α_1-inhibitor3 (AI3), α_2-macroglobulin (A2M), α_1-cysteine proteinase inhibitor (CPI) and fibrinogen (FBG) were determined by electroimmunoassay. The results of control cultures ($n=7$) are reported as the mean protein production (μg protein ± SD/ 24 h × 10^6 cells) assumed as 100%. The results obtained with IL-6, TGF-β and EGF ar3e the means of 3 experiments.

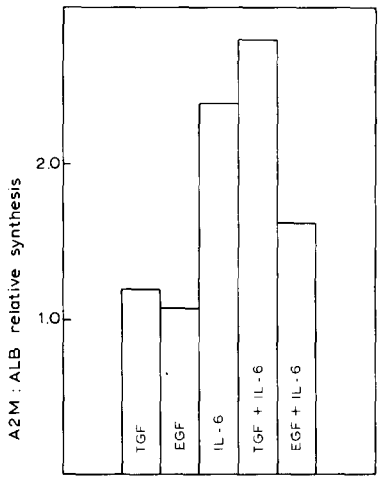

Fig. 2. Effects of TGF-β, EGF and IL-6 on the relative synthesis of α_2-macroglobulin (A2M) in comparison with albumin (ALB) by cultured rat hepatocytes. Concentrations of TGF were 10 ng/ml, EGF-100 ng/ml and IL-6-5 ng/ml. For further detail see Table II.

earlier study [12] TGF-β increased and EGF decreased the acute phase response (Fig. 2). Similar results were obtained with cysteine proteinase inhibitor but not with fibrinogen, the synthesis of which was significantly suppressed by TGF-β.

Effects of TGF-β and EGF on acute phase protein synthesis may be related to cell proliferation thus we measured [^{3}H]TdR incorporation into hepatocyte DNA (Fig. 3). It appears that DNA synthesis gradually increases during hepatocyte culture, is slightly inhibited by TGF-β and strongly enhanced by EGF, especially on the second and third day in agreement with the observations of Carr et al. [13] and Wollenberg et al. [9]. We do not know, however, whether EGF-induced DNA synthesis in hepatocytes is related to enhanced plasma protein production and amino acid uptake since during the first 48 h of culture the total cellular protein remained constant both in control and in growth factor-treated cultures. On the other hand, participation of TGF-β in the process of hepatic fibrosis has been postulated in a recent study of Czaja et al. [16].

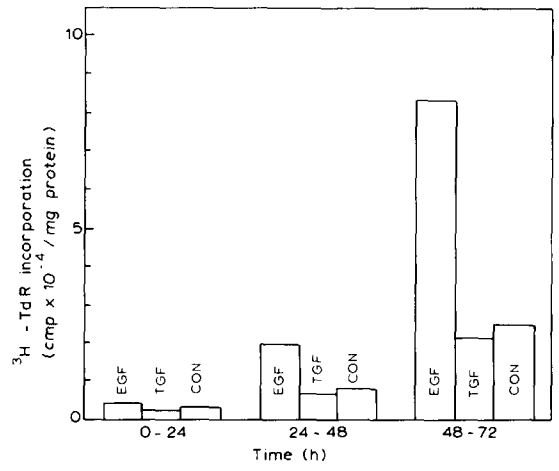

Fig. 3. Incorporation of [^{3}H]thymidine (^{3}H-TdR) into hepatocyte DNA during consecutive 3 days of culture without (control) or with 10 ng of TGF or EGF. The results are the means of duplicate cultures in two independent experiments.

Taken together, it appears that in cultured rat hepatocytes AIB uptake is regulated differently than acute phase protein synthesis thus confirming our earlier findings on the effect of glucagon and phorbol myristate acetate [17]. Moreover, our results suggest that although the rat hepatocyte acute phase response is primarily regulated by IL-6 some peptide growth factors, such as TGF-β or EGF, may be involved in its fine tuning.

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