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The synthesis of human α-2-HS glycoprotein is down-regulated by cytokines in hepatoma HepG2 cells

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The regulation of the synthesis of α-2-HS glycoprotein (AHSG) by inflammatory mediators from activated monocytes was studied on the human hepatoma cell line HepG2 and compared to that of albumin. Monocyte-conditioned medium, recombinant human interleukin-6 (rhIL6) and interleukin-1β (rhIL1β) all down-regulated the synthesis of AHSG. This decrease was found both at the protein and the mRNA level. The most efficient mediator was the monocyte-conditioned medium, when rhIL1β was found to be less efficient than rhIL6. The combination of rhIL6 and rhIL1β resulted in an additive down-regulation of the AHSG mRNA levels. Similar results were obtained with albumin. These data indicate that AHSG is a negative acute-phase protein whose synthesis is regulated by cytokines in a manner similar to that of albumin.

Acute-phase protein; Glycoprotein α-2-HS; Albumin; Interleukin-6; Interleukin-1

1. INTRODUCTION

α-2-HS glycoprotein (AHSG) is a glycoprotein with a molecular mass of 50 kDa, which is found in normal human plasma at values around 60 mg/dl. No exact function has yet been determined for this protein, although it has been found to be concentrated in bone matrix during mineralization [1]. In addition, recent data indicate that it has the ability to regulate the flux of calcium in vitro from bone [2]. It has also the capacity to act as an opsonin [3] and to promote endocytosis [4]. Furthermore, its plasma levels decrease significantly during an inflammatory response [5].

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Abbreviations: BSF-2, B-cell differentiation factor; IFN-β2, interferon-β2; FCS, fetal calf serum; CM, conditioned medium; ITG, guanidine isothiocyanate

In response to inflammation, the levels of some plasma proteins synthesized by the liver increase (positive acute-phase proteins) whereas others decrease (negative acute-phase proteins) [6]. The levels of each protein return to their basal values following the end of the acute-phase of the inflammatory response. The local reaction includes the release of inflammatory mediators secreted by human monocytes [7,8]. Hepatocyte-stimulating factor (HSF) [9,10], interleukin-1 (IL1) [11] and tumor necrosis factor (TNFα) [12] are considered as being the major mediators of the acute-phase response. Recently, HSF and interleukin(IL)-6 (BSF2, IFNβ2, 26 kDa protein and interleukin HP-1) were found to be functionally and immunologically identical [13,14]. In the rat hepatoma cell line Fao, acute-phase proteins are regulated differently by the mediators IL6, IL1β and TNFα, indicating that the acute-phase response is more complex than previously described [15].

In the present study, we have analyzed the production of α-2-HS (AHSG) by human hepatoma

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(HepG2) cells in the presence of CM from LPS-activated monocytes as well as using the two following cytokines: rhIL1β and rhIL6. Our results indicate that AHSG is a negative acute-phase protein whose synthesis is down-regulated by cytokines.

2. MATERIALS AND METHODS

2.1. *Hepatoma cell cultures*

HepG2 cells were grown in RPMI medium containing 10% FCS, 50 U/ml penicillin, 50 μg/ml streptomycin, 1 μM insulin, and were passaged by trypsinisation once a week. When the cells reached 80 to 100% confluency, equal volumes of CM from stimulated monocytes (see below) and fresh RPMI medium plus FCS were added and cells were incubated for 24 or 48 h. Cells cultivated with fresh medium only were used as controls. The culture media were collected after either 24 or 48 h, and analyzed by rocket immuno-electrophoresis to determine the levels of AHSG and albumin produced. HepG2 cells were similarly exposed to cytokines (see below) for 48 h at different concentrations.

2.2. *Preparation of the conditioned medium*

Human peripheral blood monocytes were separated by buoyant density centrifugation, purified by adherence and stimulated with lipopolysaccharide (LPS) (10 μg/ml) (*E. coli* 055:B5, Sigma) for 24 h. The supernatant was dialyzed against phosphate buffered saline and sterilized by filtration through a 0.22 μm membrane (Millipore).

2.3. *Recombinant cytokines*

Purified human rIL1β (1000 U/ml) was purchased from Genzyme (TEBU SA, France). Purified rBSF2 (lot 703) (1×10^5 U/19.3 μg per ml) was generously provided by T. Hirano and T. Kishimoto (Institute of Molecular and Cellular Biology, Osaka, Japan) [16].

2.4. *cDNA probes*

Human albumin cDNA (pHSA59B3) was kindly provided by Dr M. Frain (Lab. Enzymologie, CNRS, Gif-sur-Yvette, France) and α-2-HS cDNA was obtained as described [17].

2.5. *Analysis of secreted proteins*

Concentrations of the proteins were determined by electro-immunoassay [18] using monospecific antisera to human plasma proteins. The results were expressed as μg/ml of protein for 24 or 48 h.

2.6. *Northern analysis of mRNA levels*

Cells (5×10^6 per flask) were lysed in ITG. Total cellular RNA was extracted, separated by electrophoresis in 1.2% agarose gels containing formaldehyde and transferred to nylon membrane (Amersham) [19]. The RNA was fixed by UV illumination. The blots were then prehybridized and hybridized overnight with ^{32}P-labelled cDNA probes. The cDNAs were radiolabelled by oligonucleotide priming [20]. Autoradiography of Northern blots was analyzed by densitometric scanning.

3. RESULTS AND DISCUSSION

The human hepatoma (HepG2) cell line was used to quantitate AHSG and albumin secretion and mRNA levels, before and after treatment with CM of monocytes and purified cytokines, in order to evaluate the influence of these mediators on the synthesis of these two proteins. Estimation of the

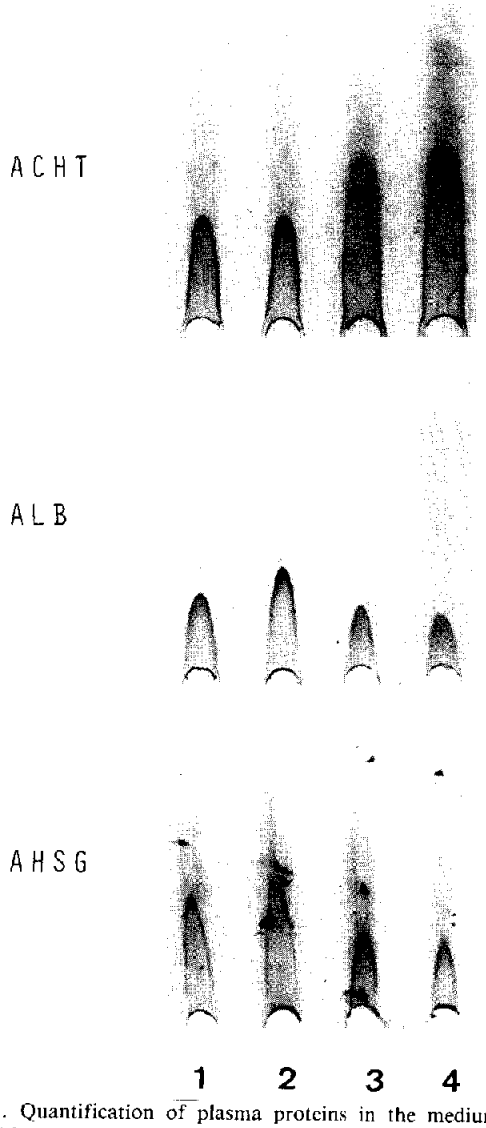

Fig.1. Quantification of plasma proteins in the medium of HepG2 cells by rocket immuno-electrophoresis: effect of the conditioned medium from LPS-activated monocytes (CM) on the secretion of α-1-antichymotrypsin (ACHT), albumin (ALB) and α-2-HS (AHSG). Cells were cultured for 48 h in presence of normal culture medium (1,2) and with CM (v/v) (3,4). Wells were loaded with 50 μl of the cell culture media.

Table 1

Levels of proteins secreted by HepG2 cells treated with conditioned media from LPS-activated monocytes (CM)

	Control		CM		CM/control	
Time (h):	24	48	24	48	24	48
ACHT	8.00 ± 4.49 (n = 5)	13.05 ± 5.70 (n = 6)	14.46 ± 5.90 (n = 5)	22.30 ± 6.56 (n = 6)	1.81	1.72
Albumin	4.96 ± 2.50 (n = 5)	4.21 ± 0.95 (n = 6)	2.54 ± 1.16 (n = 5)	2.33 ± 0.26 (n = 6)	0.51	0.55
AHSG	4.06 ± 2.15 (n = 5)	6.23 ± 3.78 (n = 6)	2.80 ± 1.58 (n = 6)	4.69 ± 3.56 (n = 6)	0.68	0.75

Protein values were expressed as microgram protein secreted in 24 h or 48 h/ml, and were measured by rocket immuno-electrophoresis using purified protein standards for calibration

secretion of AHSG, albumin and α-1-antichymotrypsin (ACHT) was made by rocket immunoelectrophoresis (fig.1). ACHT was used as a positive control to characterize the CM activities since the synthesis of ACHT in HepG2 cells is greatly enhanced by CM of monocytes [21]. As shown in fig.1 and table 1, the addition of CM induced a 1.81-fold increase in ACHT secretion, after 48 h stimulation, whereas that of albumin decreased by about fifty percent, and that of AHSG by about thirty percent. Further experiments were performed using rhIL1β (30 U/ml) and rhIL6 (50 U/ml). As shown in table 2, IL1 and IL6 increased the synthesis of ACHT, but IL6 was a more potent inducer than IL1. In contrast, the secretion of albumin was decreased both by IL1 and IL6, although the most important decrease was observed when using CM. Similarly, a moderate decrease in the amount of AHSG secretion was observed following exposure to recombinant cytokines for 48 h.

The amount of specific mRNAs for albumin and AHSG was measured by hybridization of cDNA probes to total cellular RNA extracted after 48 h exposure of HepG2 to cytokines. Fig.2 shows the results of the Northern analysis of total cellular α-2-HS RNA. Densitometric scanning of these samples indicated that AHSG mRNA at 48 h dramatically decreased in treated cells versus controls, showing a 25% decrease for IL1, a 50% decrease for IL6, a 75% decrease when these two cytokines were associated, and a decrease over 90% for the conditioned medium. An important decrease of 75% in albumin mRNA was observed with CM, whereas that obtained with IL6 was 70% and was 50% with IL1 (not shown).

Table 2

Amounts of ACHT, albumin and AHSG secreted by HepG2 cells in response to rhIL1, rhIL6 and conditioned medium (CM) of monocytes over a 48 h period (μg/ml)

Treatment	ACHT		Albumin		AHSG	
	Level	Fold increase	Level	Fold increase	Level	Fold increase
Control	21.0		4.20		16.4	
rhIL1β (30 U/ml)	30.0	1.43	3.40	0.80	13.0	0.79
rhIL6 (50 U/ml)	42.0	2.00	3.20	0.76	12.6	0.77
rhIL1β + rhIL6	44.0	2.09	3.08	0.73	11.6	0.70
CM	41.6	1.98	2.30	0.54	11.6	0.70

Values represent the mean of triplicate cultures and were measured by rocket immuno-electrophoresis

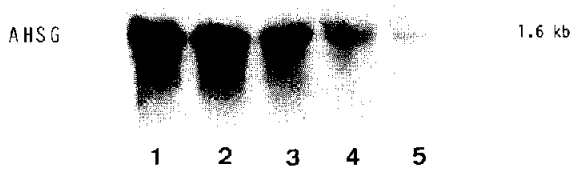

Fig.2. Northern analysis with human α-2-HS (AHSG) cDNA probe of total cellular RNA (30 μg) from HepG2 cells treated over a period of 48 h with different recombinant cytokines and conditioned medium. (1) Control; (2) rhIL1 (30 U/ml); (3) rhIL6 (50 U/ml); (4) rhIL1 (30 U/ml) + rhIL6 (50 U/ml); (5) conditioned medium from LPS-activated human monocytes.

The major finding in our study is that, in vitro, α-2-HS synthesis in human hepatoma HepG2 cells is decreased following exposure to cytokines and that this effect is due to at least two of them, namely IL6 and IL1. We have found only a moderate decrease of AHSG and albumin secretion in culture media following exposure to CM, whereas the corresponding amounts of mRNA are strongly decreased. Using the human hepatoma cells Hep3B, Darlington et al. [22] found similarly that the levels of albumin mRNA were not correlated with the amount of the secreted protein. In agreement with this finding, we could postulate that α-2-HS protein accumulation in the culture medium is due to the pool of mRNA synthesized by the hepatoma cells before the addition of monocyte-conditioned medium.

Our results have shown that the synthesis of AHSG, in human hepatoma HepG2 cells, is sensitive to the action of rhIL1 and rhIL6, but that the latter is a more potent inducer than rhIL1. In agreement with our observations, Andus et al. [23] have described an additive down-regulation of albumin synthesis in rat hepatocyte primary cultures using a combination of rhIL6 and rhIL1β. In our experiments, rhIL6 appears to be the major inducer of the decrease of AHSG synthesis in vitro. We have shown previously that the plasma concentration of AHSG is decreased during the course of an acute inflammation [5], but it was not known at the time if this was due to a decrease in synthesis or an increase in catabolism. Our present results demonstrate that AHSG belongs indeed to the group of negative acute-phase proteins and that its synthesis is regulated in a fashion similar to that of human serum albumin.

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