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Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes

José V. Castell, Maria J. Gómez-Lechón*, Martina David, Toshio Hirano+, Tadamitsu Kishimoto+ and Peter C. Heinrich

*Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG, *Centro de Investigación, Hospital La Fe, INSALUD, Avda de Campanar, 21, E-46009 Valencia, Spain* and *+Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University, 1–3, Yamada-oka, Suita, Osaka 565, Japan*

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Recombinant human IL-6 (rhIL-6) is a potent inducer of the synthesis of acute phase proteins in adult human hepatocytes. A wide spectrum of acute phase proteins is regulated by this mediator. After labeling of rhIL-6 stimulated human hepatocytes with [^{35}S]methionine acute phase protein synthesis was measured by immunoprecipitation. Serum amyloid A, C-reactive protein, haptoglobin, α_1-antichymotrypsin and fibrinogen were strongly induced (26-, 23-, 8.6-, 4.6- and 3.8-fold increases, respectively). Moderate increases were found for α_1-antitrypsin (2.7-fold) and α_1-acid glycoprotein (2.7-fold). RhIL-6 had no effect on α_2-macroglobulin, whereas fibronectin, albumin and transferrin decreased to 64, 56 and 55% of controls. In the cases of serum amyloid A, haptoglobin, α_1-antichymotrypsin, α_1-antitrypsin and α_1-acid glycoprotein, dexamethasone enhanced the action of rhIL-6. We conclude that rhIL-6 controls the acute phase response in human liver cells.

Acute-phase protein; Interleukin-6; Hepatocyte stimulating factor; B-cell stimulatory factor 2; (Human hepatocyte)

1. INTRODUCTION

The course of an inflammatory response occurring in a variety of pathological conditions is characterized by changes in serum levels of a group of proteins, the acute phase proteins [1,2]. In man C-reactive protein, serum amyloid A, fibrinogen, haptoglobin, α_1-antitrypsin, α_1-acid glycoprotein are positive acute phase reactants, while α_2-macroglobulin remains essentially unchanged. Serum transferrin and albumin decrease during the acute phase response [1–3]. It is presently believed that the major site of synthesis for the acute phase proteins are the parenchymal cells of the liver.

In rat hepatocyte primary cultures and in various cell lines it has been shown that the acute phase protein expression can be induced by mediators synthesized and secreted by mononuclear phagocytes [4–13]. Recent work strongly suggests that interleukin-6 also known as B-cell stimulatory factor-2, hybridoma plasmacytoma growth factor, 26 kDa protein or interferon-β2 [14] functions as an important mediator in the acute phase response in rat and human hepatoma cells [15–17]. RhIL-6 also stimulated acute phase protein synthesis in the rat in vivo [18].

Thus far, however, there is no direct evidence for IL-6 as an acute phase mediator in human hepatocytes. Here we show for the first time that rhIL-6 is a potent regulator of acute phase protein synthesis in adult human liver cells.

Correspondence address: P.C. Heinrich, Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG

Abbreviations: BSF-2, B-cell stimulatory factor 2; HSF, hepatocyte-stimulating factor; IL-6, interleukin-6; rhIL-6, recombinant human interleukin-6

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2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[^{35}S]Methionine (1000 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, England). Collagenase (>300 units/mg) was from Boehringer (Mannheim, FRG). Protein A-Sepharose CL-4B was obtained from Pharmacia (Freiburg, FRG). Antibodies to human plasma proteins were from Dakopatts (Hamburg, FRG), recombinant human BSF-2/IL-6 (5×10^6 units/mg protein) was produced in *E. coli* as a fusion protein, processed and purified as described in [19].

2.2. *Preparation of human hepatocyte primary cultures*

Human hepatocytes were obtained after perfusion with collagenase of a small surgical biopsy (1.5 g) of a healthy individual. Cell viability was >95%. Hepatocytes (1.5×10^5 cells/well) were seeded on 24-well dishes (Falcon, no.3047) previously coated with fibronectin (1.5 μg/cm^2) and cultured in Ham's F-12 medium supplemented with 0.2% bovine serum albumin, 10^{-8} M insulin and 2% newborn calf serum. 1 h later 90% of the cells were attached and medium was changed. After 24 h cells were shifted to Ham's F-12 medium containing 0.2% bovine serum albumin, 10^{-8} M insulin and 10^{-8} M dexamethasone.

2.3. *Synthesis of acute phase proteins*

After 48 h in culture hepatocytes were stimulated with 100 units/ml of rhIL-6 with or without 10^{-7} M dexamethasone for 20 h. Medium was changed and hepatocytes were labeled for 4 h with 25 μCi of [^{35}S]methionine per ml methionine-free culture medium. The various acute phase proteins were immunoprecipitated from 0.35 ml of the hepatocyte culture medium, subjected to SDS-polyacrylamide gel electrophoresis and fluorography as described in previous publications [20,21].

3. RESULTS

The aim of the present study was to investigate, whether rhIL-6 was capable of inducing acute phase protein synthesis in human hepatocyte primary cultures. Adult human hepatocytes were incubated with 100 units per ml rhIL-6 for 20 h with or without supplementation of 10^{-7} M dexamethasone. This rhIL-6 concentration as well as the time for stimulation was chosen in analogy to experiments, which we had carried out with the human hepatoma cell lines HepG2 and Hep3B2. After labeling hepatocytes with [^{35}S]methionine for 3 h, 12 plasma proteins were immunoprecipitated from the media and analyzed by SDS-poly-

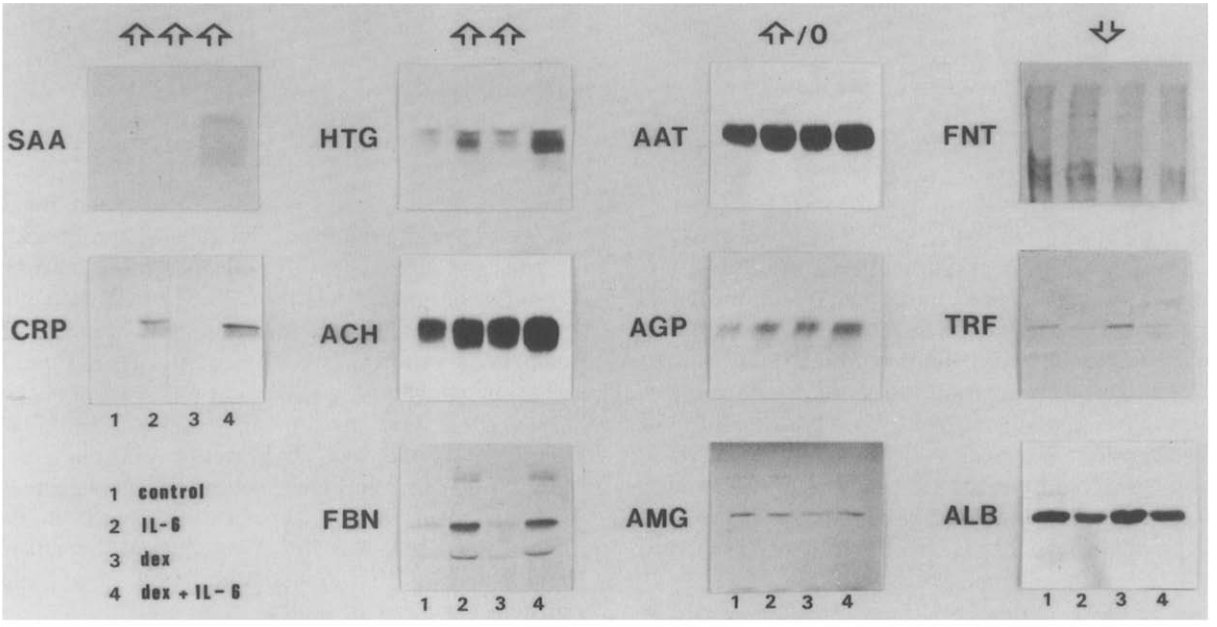

Fig.1. Induction of acute phase protein synthesis in human hepatocyte primary cultures. Adult human hepatocytes cultured as detailed in section 2 were incubated with 100 U/ml of rhIL-6 (lane 2), 10^{-7} M dexamethasone (lane 3), and 100 U/ml of rhIL-6 plus 10^{-7} M dexamethasone (lane 4) for 20 h. Non-treated hepatocytes were used as a control (lane 1). Culture medium was replaced by methionine-free RPMI 1640 medium containing 25 μCi L-[^{35}S]methionine per ml. After a labeling period of 4 h the acute phase proteins indicated in the figure were immunoprecipitated with specific antibodies and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. SAA, serum amyloid A; CRP, C-reactive protein; HTG, haptoglobin; ACH, α_1-antichymotrypsin; FBN, fibrinogen; AAT, α_1-antitrypsin; AGP, α_1-acid glycoprotein; AMG, α_2-macroglobulin; FNT, fibronectin; TRF, transferrin; ALB, albumin.

acrylamide gel electrophoresis and subsequent fluorography. Essentially for all acute phase proteins the immunoprecipitations led to single radioactively labeled bands. In the cases of fibrinogen and fibronectin the antisera had been raised against the holoproteins. Since fibrinogen is composed of 6 ($\alpha_2\beta_2\gamma_2$) subunits and fibronectin of 2 different subunits, the expected polypeptides were detected. It can be seen in fig.1 that the response to rhIL-6 is quite different for the various proteins studied. Serum amyloid A and C-reactive protein are very strongly induced, and a strong stimulation was also found for haptoglobin, α_1-antichymotrypsin and fibrinogen. Only moderate increases were observed for α_1-antitrypsin and α_1-acid glycoprotein. α_2-Macroglobulin, which is no acute phase protein in humans, did not change. In the cases of fibronectin, transferrin and albumin decreases were observed. No newly synthesized serum amyloid P could be detected under our experimental conditions.

In order to quantify the effects of rhIL-6 on the synthesis of the different acute phase proteins seen in fig.1, the individual protein bands were excised from the gel and their radioactivities were determined (table 1). RhIL-6 alone stimulated the synthesis and secretion of secretory proteins 1.4-fold, whereas rhIL-6 in combination with 10^{-7} M dexamethasone led to a 1.7-fold increase. In contrast, rhIL-6 had no effect on cellular protein synthesis.

From the analysis of the data of the various acute phase proteins listed in table 1, a subdivision into four groups is possible. Two proteins, serum amyloid A and C-reactive protein are very strongly induced in human hepatocytes (>20-fold). Haptoglobin, α_1-antichymotrypsin and fibrinogen fall into a second group characterized by changes between 3- and 10-fold. The third group comprises protein exhibiting changes between 1- and 3-fold namely α_1-antitrypsin, α_1-acid glycoprotein and α_2-macroglobulin. Finally, the negative acute phase proteins fibronectin, transferrin and albumin form a fourth group.

It is interesting that all the acute phase proteins studied could be induced by rhIL-6 alone. In the cases of serum amyloid A, haptoglobin, α_1-antichymotrypsin, α_1-antitrypsin and α_1-acid glycoprotein, dexamethasone further increased the effect of rhIL-6.

Table 1

Synthesis of acute phase proteins by rhIL-6 stimulated human hepatocytes in culture

Proteins	Radioactivity (cpm/μg cellular protein)			
	Control	RhIL-6	Dex	RhIL-6+ Dex
Total protein (cells)	4810	3780	5460	4650
		0.9	*1.3*	*1.1*
Total protein (medium)	309	441	408	525
		1.4	*1.3*	*1.7*
	(cpm/mg cellular protein)			
Serum amyloid A	105	490	465	2840
		4.9	*4.6*	*26*
C-reactive protein	90	1998	155	2030
		22.1	*1.7*	*22.5*
Haptoglobin	700	2545	1410	6610
		1.3	*1.8*	*8.6*
α_1-Antichymotrypsin	7695	15910	16695	35170
		2.0	*2.1*	*4.6*
Fibrinogen	1010	3775	980	3875
		3.7	*1.0*	*3.8*
α_1-Antitrypsin	12290	22885	20245	33020
		1.9	*1.6*	*2.7*
α_1-Acid glycoprotein	585	975	1120	1580
		1.6	*1.9*	*2.7*
α_2-Macroglobulin	245	225	225	240
		1.0	*1.0*	*1.0*
Fibronectin	690	335	710	445
		0.48	*1.0*	*0.64*
Transferrin	625	180	760	340
		0.3	*1.2*	*0.55*
Albumin	8970	4280	13485	5150
		0.47	*1.5*	*0.56*

Radioactivity of total cellular and medium protein after trichloroacetic acid precipitation is expressed as cpm/μg cellular protein. The immunoprecipitated acute phase proteins were separated by SDS-polyacrylamide gel electrophoresis as described in the legend of fig.1 and their radioactivity was expressed as cpm/mg cellular protein. Each number represents the average of 6 independently treated culture plates of human hepatocytes. Values in italics represent relative increases over their respective controls

4. DISCUSSION

Most studies on human acute phase protein induction have been carried out with the well dif-

ferentiated human hepatoma cell lines HepG2 and Hep3B2 [12,13]. Upon stimulation these cells express only some acute phase proteins, such as fibrinogen, haptoglobin, α_1-antichymotrypsin, albumin (Castell et al., unpublished). However, they do not synthesize the major human acute phase proteins, C-reactive protein and serum amyloid A [12,22]. The experiments presented in this paper clearly show that rhIL-6 induces in cultured human hepatocytes essentially the same spectrum of acute phase proteins, in particular C-reactive protein and serum amyloid A, as that found in man during inflammatory states [1–3].

Whereas IL-6 has recently been shown to be the most important acute phase protein inducer in the rat in vivo [18], no such direct evidence exists for man. Only indirect evidence, namely a correlation between serum levels of IL-6 and the acute phase C-reactive protein has been reported thus far [23]. Our results obtained with cultured human hepatocytes strongly suggest IL-6 to be the major inflammatory mediator in humans.

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