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Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes

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The three monokines interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) modulate acute phase plasma protein synthesis in adult human hepatocytes. Only IL-6 stimulates the synthesis of the full spectrum of acute phase proteins as seen in inflammatory states in humans, i.e. synthesis and secretion of C-reactive protein, serum amyloid A, fibrinogen, α_1-antitrypsin, α_1-antichymotrypsin and haptoglobin are increased while albumin, transferrin and fibronectin are decreased. IL-1β as well as TNFα, although having a moderate effect on the positive acute phase proteins and inhibiting the synthesis of fibrinogen, albumin and transferrin, fail to induce serum amyloid A and C-reactive protein. These data suggest that IL-6 plays the key role in the regulation of acute phase protein synthesis in human hepatocytes.

Acute phase protein; Interleukin-6; Interleukin-1β; Tumor necrosis factor α; (Human hepatocyte)

1. INTRODUCTION

Injuries such as bacterial or parasitic infections, physical and chemical traumata, malignant tumors and immunological disorders lead to a highly complex reaction of the organism, the so-called acute phase response, characterized by fever, leukocytosis, a negative nitrogen balance and an increase in the synthesis of hepatic acute phase proteins [1]. During recent years several investigators have shown that among other cells monocytes release hormone-like cytokines that act on hepatocytes regulating the synthesis of acute phase proteins. IL-6 [2,3], IL-1β [4] and TNFα [5,6] are presently considered as the most important mediators of acute phase protein synthesis in hepatocytes. Thus far, all studies in this regard have been carried out with rodent hepatocytes or hepatoma cell lines, but since the acute phase protein pattern in these biological models is different from the pattern observed in inflammatory states in man [1], the relevance of the observations made with those systems can be questioned.

In a previous paper we have demonstrated that rhIL-6 regulates the synthesis of acute phase proteins in human hepatocytes [7]. In the present communication we compare the action of the cytokines IL-6, IL-1β and TNFα on human hepatocytes and show that they are all active. However, only IL-6 induced essentially the same spectrum of acute phase proteins as that found in humans during inflammatory states. On the other hand, IL-1β and TNFα, formerly believed to be major acute phase protein inducers, led only to a weak response.

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Abbreviations: rhIL-1β, recombinant human interleukin-1β; rhIL-6, recombinant human interleukin-6; rhTNFα, recombinant human tumor necrosis factor α

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2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[^{35}S]Methionine (>37 TBq/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). RhIL-6 (5×10^6 U/mg protein) produced in *E. coli* as a fusion protein, processed and purified as described in [8,9] was a generous gift from Drs T. Hirano and T. Kishimoto (Osaka, Japan). RhIL-1β (batch Roc035497/14; LAF assay: 3×10^7 U/mg) was kindly supplied by Dr A. Shaw (Geneva, Switzerland). RhTNFα (lot no.K9129A1/70121, 6×10^7 U/mg) was a gift from Dr V. Schwendemann, Knoll AG (Ludwigshafen, FRG).

2.2. *Preparation of human hepatocyte primary cultures*

Human hepatocytes were obtained after perfusion with collagenase of small surgical biopsies (1.5–3 g) of healthy individuals. The cell suspension was centrifuged to separate hepatocytes from other cell types. Cell viability was about 95% and consisted for 99% of hepatocytes. 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. *Stimulation and labeling of human hepatocytes and immunoprecipitation of acute phase proteins*

After 48 h in culture, hepatocytes were stimulated either with 100 U/ml of rhIL-6 or 300 U/ml rhIL-1β, or 500 U/ml rhTNFα in the presence of 10^{-7} M dexamethasone for 20 h. Medium was changed and hepatocytes were labeled for 4 h with 0.9 MBq of [^{35}S]methionine per ml methionine-free culture medium. For the dose-response experiment, cultures were stimulated for 20 h and then labeled for 24 h with 1.6 MBq of [^{35}S]methionine per ml methionine-free culture medium, containing the cytokines at the indicated concentrations. The various acute phase proteins were immunoprecipitated from 0.35 ml of the hepatocyte culture medium, subjected to SDS-polyacrylamide gel electrophoresis and fluorography, and the radioactive bands were excised and quantified [10,11].

3. RESULTS AND DISCUSSION

Human hepatocytes obtained from liver biopsies were cultured in chemically-defined medium, stimulated for 20 h with rhIL-6, or rhIL-1β or rhTNFα, and the newly synthesized and secreted proteins were analyzed by SDS-polyacrylamide gel electrophoresis after immunoprecipitation with specific antisera. The results of the experiments depicted in fig.1 clearly show that rhIL-6

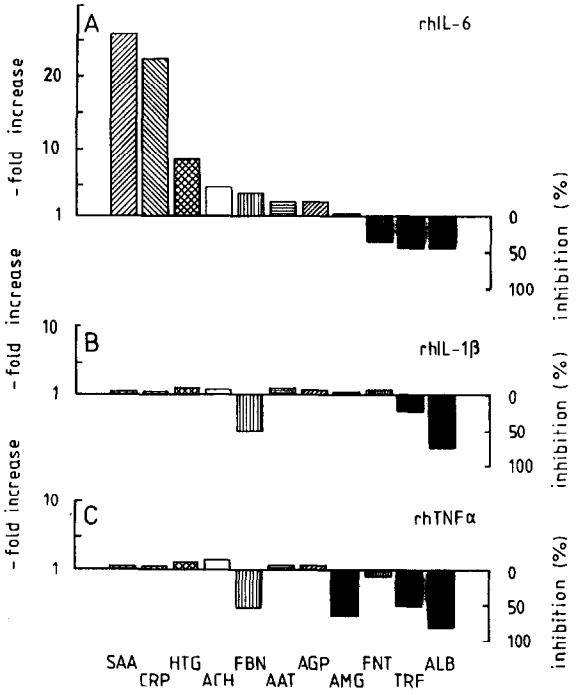

Fig.1. Acute phase response of adult human hepatocytes stimulated by rhIL-6, rhIL-1β and rhTNFα. Human hepatocytes cultured for 48 h were stimulated either with 100 U/ml rhIL-6, or 300 U/ml IL-1β or 500 U/ml TNFα for 20 h, and then labeled with [^{35}S]methionine as described. Acute phase proteins were immunoprecipitated with specific antibodies and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Data are expressed as relative increases over their respective controls. Each number represents the average of 6 independently treated plates. 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.

stimulates the synthesis of serum amyloid A, C-reactive protein, 26- and 22.5-fold. Haptoglobin, α_1-antichymotrypsin and fibrinogen increased 9-, 5- and 4-fold, respectively. At the same time albumin, transferrin and fibronectin production decreased considerably (fig.1A). In contrast to rhIL-6, rhIL-1β and rhTNFα exhibited only weak stimulatory effects on the synthesis of the positive acute phase proteins (fig.1B,C). Moreover, neither rhIL-1β nor TNFα were able to stimulate C-reactive protein or serum amyloid A, the most representative acute phase proteins in man. The synthesis of the negative acute phase protein albumin, however, was dramatically decreased (80% inhibition) by both monokines. Fibronectin

and α_2-macroglobulin were found to be essentially regulated by only one of the 3 mediators (fig.1). RhIL-6 impaired fibronectin synthesis and only rhTNFα decreased α_2-macroglobulin production. It is also remarkable that the levels of the positive acute phase protein fibrinogen, which increased after rhIL-6, decreased in rhIL-1β and rhTNFα stimulated human hepatocytes. These findings are shown in greater detail in fig.2, where the dose-dependent effect of the three mediators on the synthesis of fibrinogen is demonstrated.

When identical experiments were carried out with the human hepatoma cell lines HepG2 or Hep3B2, fewer acute phase proteins responded to the 3 monokines. In particular, neither serum amyloid A nor C-reactive protein synthesis could be induced by rhIL-6 (not shown), although as in human hepatocytes, it was the most potent inducer of acute phase proteins.

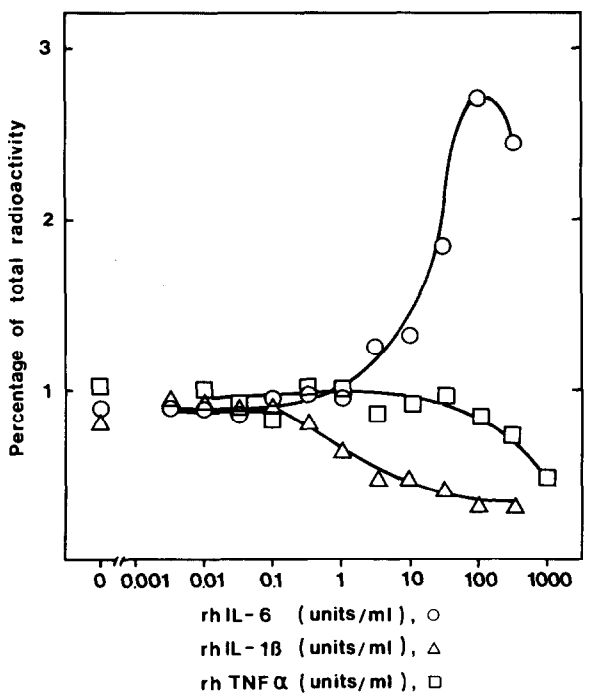

Fig.2. Dependence of fibrinogen synthesis on the concentration of rhIL-6, rhIL-1β and rhTNFα in cultured human hepatocytes. Human hepatocytes cultured for 48 h were incubated with rhIL-6 (○), rhIL-1β (△) and rhTNFα (□) for 20 h at the concentrations indicated in the figure, radioactively labeled, and fibrinogen was immunoprecipitated from the culture media as described. Data are expressed as percentage of total trichloroacetic acid-precipitable radioactivity. Each number represents the average of 3 independently treated culture plates of human hepatocytes.

In spite of the fact that TNFα and in particular IL-1 [4–6] have been repeatedly claimed to be the major regulators of acute phase protein synthesis, it follows from the data presented that IL-6 is the most important mediator of acute phase protein synthesis in human hepatocytes. Nevertheless, IL-1 and TNFα may be involved in the overall acute phase response of hepatocytes by acting on a subset of acute phase proteins, and by stimulating other cell types (i.e. fibroblasts) to synthesize IL-6 [12,13].

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