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THE EFFECT OF INTERLEUKIN-1, INTERLEUKIN-6 AND ITS INTERRELATIONSHIP ON THE SYNTHESIS OF SERUM AMYLOID A AND C-REACTIVE PROTEIN IN PRIMARY CULTURES OF ADULT HUMAN HEPATOCYTES

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During the acute phase response, synthesis of C-reactive protein and serum amyloid A is increased . To investigate whether the enhanced synthesis of these proteins are due to stimulatory effect of inflammatory mediators such as interleukin-1 (IL-1) and interleukin-6 (IL-6) produced by macrophages and monocytes, primary cultures of adult human hepatocytes were exposed to recombinant (r)IL-1, rIL-6 or rIL-1 and monospecific anti rIL-6 antibodies in the presence of 1 μM dexamethasone. The findings indicate that rIL-1 and rIL-6 both stimulate the liver synthesis of C-reactive protein and serum amyloid A, however monospecific anti rIL-6 antibodies reduce the stimulatory effect of rIL-1 on the synthesis of these proteins. These findings suggest that IL-6 plays a key role in the stimulation of synthesis of serum amyloid A and C-reactive protein by the human liver cells.

In response to tissue injury or infection, a coordinated sequence of systemic and metabolic changes, collectively known as the acute phase response, occurs. Adaptation of liver cells to the acute phase reaction is characterized by changes in synthesis of liver derived plasma proteins which lead to changes in plasma concentrations. These proteins, known as the acute phase proteins, are synthesized exclusively in the liver and are mostly glycosylated (1). Many studies indicate that the changes of plasma protein synthesis in the liver during the acute phase response are induced by mediators such as interleukin-1,

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interleukin-6 (synonym: hepatocyte stimulating factor, hybridoma growth factor) and tumor necrosis factor produced by monocytes and macrophages (2-12). However, these studies were mostly performed *in vivo* in rats or *in vitro* using rat hepatocytes or human hepatoma cells which do not produce serum amyloid A and C-reactive protein (9,13). In this study, we examine the effect of interleukin-1, interleukin-6 and its inter-relationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes.

Materials and Methods

Preparation of primary cultures of human hepatocytes: Post-mortem human liver tissues were obtained from kidney donors aged from 16 to 51 years. Hepatocytes were isolated by a two-step perfusion technique modified from that described for the isolation of rodent hepatocytes, as described previously (14) with the following modification: EGTA was omitted in the Ca^{2+}/Mg^{2+}-free Hepes buffer. The liver was perfused with 200 ml of a Hepes buffer, pH 7.6 containing collagenase (0.05% w/v) and 10 mM $CaCl_2$ and subsequently with 200 ml of a Hepes buffer, pH 7.6 containing collagenase (0.1% w/v) and 20 mM $CaCl_2$ for 20-40 min in a recirculation system.

Deep-freeze storage, thawing of hepatocytes and cell cultures were performed as described previously (14,15). Cells were seeded at a density of 175×10^3 hepatocytes per cm^2. The culture plastic was precoated with extracellular matrix prepared as described previously (15).

Experimental design: To study the effect of interleukins on protein synthesis, a modified experimental model (11) originally described by Koj et al (4) was used. After isolation and attachment (day 1), cells were cultured in Williams medium E containing 10% fetal calf serum for 1 day in the presence of 50 nM dexamethasone (day 2). On day 3, test substances were added in a refreshed medium containing 1 µM dexamethasone. At day 4, medium was renewed with the same additive and cells were incubated for the next 24 h, after which cells were washed with phosphate-buffered saline and harvested for DNA determination. Collected medium was frozen (-20°C) until analysed.

DNA determination: Cells were suspended in water and sonicated. After centrifugation, DNA in the supernatant was measured by a fluorometric technique as described by Kapuscinski and Skoczylas (16).

Serum amyloid A and C-reactive protein assays: Concentrations of serum amyloid A and C-reactive protein were determined by Sandwich ELISA using monoclonal murine anti-serum amyloid A antibodies and polyclonal rabbit anti-C-reactive protein antibodies as described previously (17).

The amount of albumin secreted into the medium was determined by radial immunodiffusion technique according to Mancini et al (18) as described previously (14).

Materials: Williams medium E, RPMI 1640 and fetal calf serum were purchased from Flow laboratories, Irvine, Scotland, Collagenase type I, oyster glycogen type III, bovine serum albumin and 4-(2 hydroxyethyl)-1-peperazine ethanesulfonic acid (Hepes) were purchased from Sigma, St. Louis, USA. Dexamethasone disodium phosphate (Oradexon) was obtained from Organon Oss, The Netherlands. Porcine insulin was from NOVO Industri, Kopenhagen, Denmark. Tissue culture plastics were from NUNC,

Roskilde, Denmark. All solutions used in the hepatocyte isolation and culturing were filtered through a 0.22 μm Schleicher and Schuell filter (Keene, USA) and stored frozen.
Recombinant murine interleukin 1-1α(rIL-1) (specific activity 3 $x10^4$ U/μg) was obtained from Dr. I. Otterness, Pfizer Croton USA and recombinant interleukin-6 (rIL-6) (specific activity 10^6 U/μg) was obtained as described previously (19). Polyclonal rabbit anti rIL-6 antibodies were developed by immunization of rabbits with rIL-6. These antibodies inhibit rIL-6 but not rIL-1 in thymocyte proliferation assay as originally described by Gery et al (20) and inhibit rIL-6 in interleukin6 bio-assay as described previously (21).

Results and Discussion

As shown in figure 1, in the presence of 50 nM dexamethasone without interleukins, the secretion and synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes were initially high but they were decreased after 2-3 days of cultures, while the production of albumin was still increasing in this period.

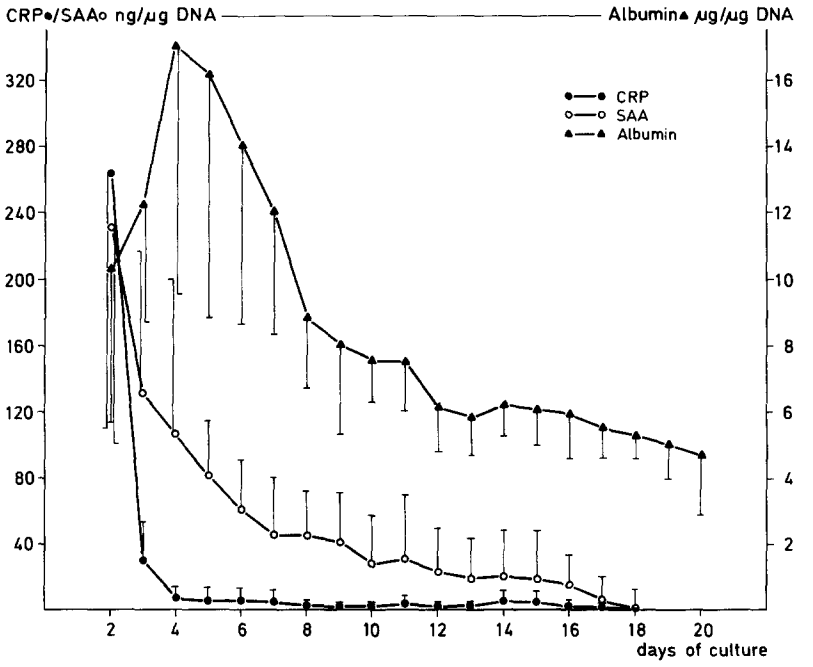

Figure 1
The secretion of albumin, serum amyloid A and C-reactive protein in medium containing 50 nM dexamethasone of adult human hepatocytes in primary cultures on plastic precoated with extracellular matrix prepared from human liver tissue as described in materials and methods. Each point on the curves is the average value ±SD of at least 5 experiments.

Table 1
The influence of recombinant interleukin-1 (100 U/ml), interleukin-6 (2000 U/ml) or recombinant interleukin-1 (100 U/ml) and monospecific anti-rIL-6 antiserum (dilution 1:100) in the presence of 1 μM dexamethosone on the secretion of C-reactive protein and serum amyloid A in primary cultures of adult human hepatocytes

	control	rIL-6	rIL-6	rIL-1+ rIL-6	rIL-1+ anti-IL-6
C-reactive protein					
day 2	100	320	220	680	200
day 3	100	5000	6000	13000	150
Serum amyloid A					
day 2	100	125	160	240	110
day 3	100	200	180	220	150

Results are expressed as % of control and are the average of five experiments.

After four days, the secretion of serum amyloid A into the medium was minimal. There was also a significant decrease in secretion of C-reactive protein and albumin. Addition of dexamethasone to one μM into the medium from the beginning of the cultures did not result in a significant change of the secretion of these proteins by the primary cultures of human hepatocytes (data not shown). However, when recombinant interleukin-1 (100 U/ml) or recombinant interleukin-6 (2000 U/ml) was added into the culture medium in the presence of 1 μM dexamethasone, the secretion of serum amyloid A and C-reactive protein was dramatically enhanced as shown in table 1. Although secretion of serum amyloid A and C-reactive protein and the magnitude of increased secretion by the stimulation of interleukin-1 or interleukin-6 varied considerably between hepatocytes from different donors, the pattern of secretion of these proteins and the stimulatory effects of interleukin-1 or interleukin-6 were highly reproducible for individuals donors. On day 3, the secretion of C-reactive protein in the presence of rIL-1 or rIL-6 was 6 to 100 folds increased, while the serum amyloid A secretion was 1.5 to 2.0 times higher as compared to the control values. The stimulatory effect of rIL-6 in a dose ranging from 1000 U/ml to 4000 U/ml showed no significant differences. However, when rIL-1 (100 U/ml)

was added simultaneously with rIL-6 (2000 U/ml) into the culture medium, an additive effect of IL-1- and IL-6-enhanced secretion of these two proteins was obtained (table 1).

To examine whether the rIL-1 exerts its stimulatory effect on the synthesis and secretion of serum amyloid A and C-reactive protein through a production of IL-6 in these primary cultures of human hepatocytes, experiments were performed using monospecific rabbit polyclonal anti-rIL-6 antibodies. In the presence of this anti serum in a dilution of 1:100 in the culture medium, the stimulatory effect of rIL-1 (100 U/ml) on the secretion of C-reactive protein was almost completely abolished, while the stimulatory effect on the serum amyloid A secretion was partially reduced (table 1). These findings indicate that IL-1 and Il-6 both stimulate the liver synthesis of serum amyloid A and C-reactive protein in an additive manner. In addition IL-1 exerts its action on the enhanced synthesis of C-reactive protein and serum amyloid A at least in part via IL-6. Nevertheless, the production of IL-6 in these primary cultures of human hepatocytes in the presence of IL-1 and the mechanism to explain the additive effect of IL-1 and IL-6 on the enhanced synthesis of C-reactive protein and serum amyloid A have still to be determined. From the present study, however, we can conclude that IL-6 has a key role in the enhanced secretion of C-reactive protein and serum amyloid A by the human liver cells.

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