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Interleukin 1β modulates hepatic synthesis of α1-acid glycoprotein in the fetal rat

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The ability of the fetal rat to respond to interleukin 1β (IL1β) by expressing α1-acid glycoprotein (AGP) was investigated. Eight and 20 h after injection of 7 ng IL1β into 19-day fetuses, liver AGP mRNA increased by a factor of 66 and 82 respectively, while serum AGP levels increased by a factor of 3 and 5. Similar treatment of the mothers altered in the fetuses neither AGP serum levels nor the amount of liver AGP mRNA. The induction of AGP gene expression in the fetal liver in response to IL1β was similar to that observed in the adult liver. These results demonstrate that at day 19 the fetal rat liver has acquired a mature acute-phase system.

Interleukin 1β; α1-Acid glycoprotein; Orosomucoid; Fetal rat

1. INTRODUCTION

A single injection of terpentine to the fetal rat elicits an inflammatory response involving a number of positive and negative acute-phase proteins [1]. This suggests that the activation mechanism of cells leading to the release of inflammatory mediators [2] and to the induction of the liver acute-phase response system in the fetal rat is at least partly mature.

Interleukin 1 (IL1), tumor necrosis factor α (TNFα) and interleukin 6 (IL6) are considered to be the major mediators of the acute-phase response in adult rats [3]. As the liver response is strongly dependent on (a) the hepatic cell type and (b) the acute-phase protein [4], we sought to determine whether the fetal liver is equally responsive to cytokines and with the same spectrum as the adult liver.

The pattern of protein concentrations in fetal rat blood is quite different from that in adult: α2-macroglobulin and α1-fetoprotein levels are very high in the former whereas they are nearly undetectable in the latter. Conversely, α1-acid glycoprotein (AGP, or orosomucoid) and albumin show lower levels in fetuses than in adults [1].

In this report, we show that IL1β acts as an acute-phase modulator in the rat fetus by increasing liver AGP mRNA and serum AGP levels.

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2. MATERIALS AND METHODS

2.1. *Treatment*

Sprague-Dawley rats (Charles River France, strain CD) were housed at 21–24°C under a 14 h–10 h light-dark cycle with laboratory chow (UAR) and tap water ad libitum. Human recombinant IL1β (a gift from Dr Boussaut, Rhône-Poulenc, France) had a specific activity of 5×10^7 units/mg and contained 0.91 ng/mg of endotoxin. IL1β was dissolved in sterile RPMI 1640 containing 5% fetal calf serum. Five-week-old male or female rats received an intravenous injection of either 0.2 or 1 μg IL1β in 500 μl or the solvent alone (control animals). Pregnant rats were injected at day 19 of pregnancy, the day after mating being taken as day 0. In another experiment, following laparatomy of mothers under ether anesthesia, the 19-day-old fetuses received a single dorsal s.c. injection of either IL1β (7 or 35 ng in 10 μl) or the solvent alone (10 μl). The whole operation was performed in less than 15 min. Eight or 20 h after injection, blood was collected after decapitation of adults and fetuses and centrifuged at 4000 rpm at 4°C. Pooled sera from 2 or 3 fetuses and serum from the corresponding mothers were stocked at −30°C until assay.

For RNA extraction, liver specimens (500 mg) from pregnant rats and fetuses were immediately frozen in sterile tubes maintained for a few minutes in a dry-ice/ethanol bath and then stored at −80°C until use.

2.2. *AGP assay and statistical assessment*

Serum AGP levels were determined using a sensitive enzyme-linked immunosorbent assay (ELISA) developed in our laboratory [5]. Values are given as mean ± SEM. Data were analyzed using the Mann and Whitney *U*-test.

2.3. *Isolation of RNA and Northern blot analysis*

Total cellular RNA was extracted from frozen pieces of rat liver by the guanidine isothiocyanate/cesium chloride method [6] using a Beckman SW 55 rotor for 16 h at 30000 rpm. Heat-denatured RNA samples were run as previously described [7] in 1.2% agarose gels containing 2.2 M formaldehyde for 16 h at 2 V/cm. Blotting of RNA onto Biodyne A nylon membranes (Pall Ultrafine Filtration Corporation) was done by Northern capillary transfer for about 36 h. After

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Table I

Effect of IL1β injection to fetuses on serum levels of α1-acid glycoprotein

Time after injection	Control fetuses	Treated fetuses		
		RPMI	IL1β (7 ng)	IL1β (35 ng)
8 h	4.75 ± 0.35	4.5 ± 0.7	14.4 ± 3.5*	–
20 h	7.22 ± 1.1	9.2 ± 1.5	45.1 ± 1.8*	42.7 ± 1.8*

Fetuses were treated at day 19 of fetal life. Serum concentrations are given as mg/l. Means (± SEM) of assays of 3–9 pools of 2–3 sera. * $P < 0.05$

Table II

Effect of IL1β injection to pregnant rats on serum levels of α1-acid glycoprotein in mothers and fetuses

Treatment	Pregnant rats	Fetuses
RPMI	66 ± 21	7.22 ± 1.1
IL1β (0.2 μg)	257 ± 179*	6.5 ± 2.5
IL1β (1 μg)	518 ± 254*	6.2 ± 0.6

Pregnant rats were treated at day 19 of gestation. Serum was collected 20 h after treatment. Serum AGP concentrations are given as mg/l. Means (± SEM) of 3–9 assays. * $P < 0.05$

transfer, the filters were air-dried and baked at 80°C for 2 h, then prehybridized for at least 3 h at 42°C in 50% formamide, 5 × SSC, 5 × Denhardt solution, 0.1% SDS, 50 mM sodium phosphate, pH 6.5, and 250 μg/ml denatured herring sperm DNA. A recombinant plasmid containing a 740 base-pair insert encoding rat AGP was used to probe Northern blots. The RR1 strain of *Escherichia coli* containing the recombinant plasmid was obtained from J.M. Taylor [8]. Plasmid DNA and the AGP gene insert were labelled by nick-translation to a specific activity of 1.6×10^8 cpm/μg DNA using [α-^{32}P]dCTP. Hybridization proceeded for 24–48 h at 42°C under the same conditions as for prehybridization. Filters were washed under high stringency several times at room temperature in large volumes of 2 × SSC and 0.1% SDS, and for about 1 h at 50°C in four changes of 0.1 × SSC and 0.1% SDS. The wet filters, enclosed in Saran film were exposed to hyperfilm MP (Amersham) at −80°C using Lightning Plus intensifying screens (Dupont Cronex).

2.4. *Dot-blot analysis*

AGP mRNA was quantified by dot-blot analysis using a Bio-dot apparatus (Bio-Rad). Using serial half-dilutions from 20 μg to 313 ng total cellular RNA was spotted onto Hybond-N$^+$ nylon membranes which were processed as recommended by the membrane supplier (Amersham), and in the same conditions as for Northern blots. Autoradiographies were scanned by means of photometric densitometry (Sebia).

3. RESULTS

As shown in Table I, the injection of 7 ng IL1β into fetuses induced 3- and 5-fold increases in serum AGP levels after 8 h and 20 h, respectively. Higher doses of IL1β (35 ng) did not lead to further increases. When IL1β was administered to only half the fetuses of a single pregnant rat, no increase in serum AGP levels was observed in the untreated fetuses (treated: 42.7 ± 1.8 mg/l, untreated: 7.7 ± 1.25 mg/l). In addition, maternal serum AGP levels were not altered by fetal treatment (results not shown). Inversely, the injection of IL1β (0.2 μg or 1 μg) into mothers did not affect

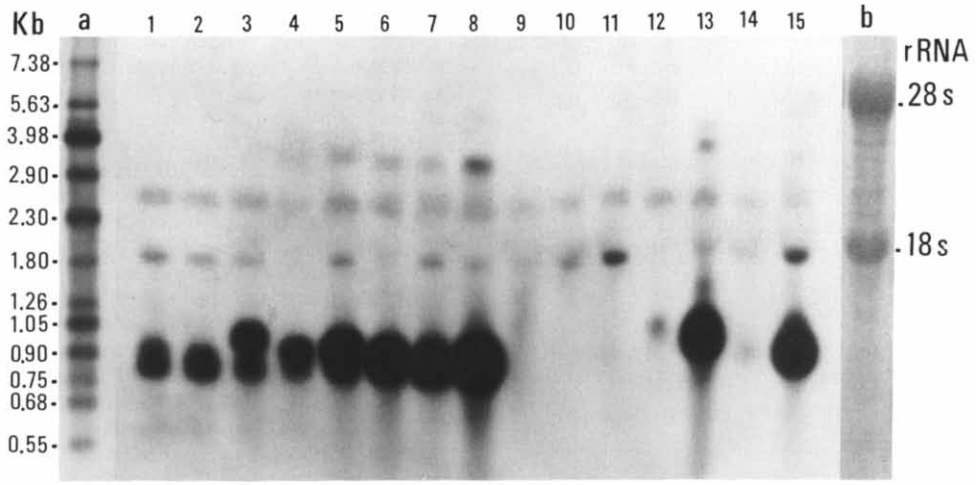

Fig. 1. Northern blot analysis of AGP mRNA from control and IL1β treated pregnant rats, and from their fetuses directly treated or not. Owing to the very low levels of serum AGP in fetuses (Table I), 40 μg total liver RNA were loaded per lane (9–15) instead of 20 μg for the RNA of the mothers (lanes 1–8). After a single injection of RPMI to control mothers, their liver mRNAs were analyzed at 3 h (lane 2), 20 h (lane 1), and liver mRNA from their fetuses at 8 h (lane 9). After a single injection of IL1β (0.2 μg) to the mothers, their liver mRNAs were analyzed at 3 h (lane 3), 8 h (lane 4) and 20 h (lane 6). With a higher dose of IL1β (1 μg) liver mRNAs were analyzed at 8 h (lane 5), 20 h (lanes 7, 8), and the liver mRNA from their fetuses at 20 h (lanes 10, 11). Only two bands of premessenger mRNA could be detected for the fetuses which had not directly received IL1β (lanes 9–11). After a direct injection of RPMI to control fetuses, their liver mRNAs were analyzed at 8 h (lane 12) and 20 h (lane 14). Lanes 13 and 15 correspond to liver mRNA from fetuses 8 h and 20 h respectively after a direct treatment with 7 ng IL1β. Only trace amounts of mature AGP mRNA could be detected in control fetuses (lanes 12, 14) in comparison with fetuses which had received IL1β (lanes 13, 15). The size of mature and premessenger mRNAs were estimated with 'Raoul I' (lane a) molecular weight marker (from Appligène, France) which hybridizes with pBR322 plasmid DNA, and also with 28S and 18S rRNA after methylene blue staining of the Northern blot (lane b).

AGP levels in fetal serum, despite a 4- or 8-fold increase in maternal values (Table II). The injection of 1 μg IL1β to adult male and adult non-pregnant female rats resulted in a 6- to 8-fold increase in serum AGP levels (not shown).

Rat liver AGP mRNA was evidenced specifically by hybridization of total cellular RNA with the AGP cDNA probe. Fig. 1 shows the results of the Northern blot analysis of 15 RNA samples from control (slots 1,2) and treated pregnant rats (slots 3–8) and from their fetuses directly treated (slots 13, 15) or untreated (slots 9–11) with IL1β. When IL1β (7 ng) was directly injected to the fetuses, mature AGP mRNA was strongly expressed 8 h and 20 h after injection (slots 13, 15) compared to only trace amounts in solvent-treated controls (slots 12, 14). In contrast, IL1β administered to the mothers did not induce any effect in their fetuses since no hybridization signal was observed (slots 10, 11). However, it is interesting to note that 2 or 3 bands of premessenger RNAs (size = 1850, 2650 and 3500 bases) were detected in both control and treated pregnant rats as well as in their fetuses, even when no mature mRNAs are expressed (slots 9–11).

Densitometric scanning of the RNA samples analyzed by dot-blot (Fig. 2) showed that fetal liver AGP mRNA was increased 66- and 82-fold, respectively, 8 h and 20 h after direct treatment of the fetuses with 7 ng IL1β. Maternal AGP liver mRNAs were increased 20- to 60-fold 20 h after treatment with 0.2 μg and 1 μg IL1β, respectively. As seen on the Northern blot (Fig. 1, slots 10, 11), the trace amounts of hybrids detected on dot-blots (Fig. 2, lines 10, 11) for the fetuses which were not directly treated with IL1β, correspond to high molecular weight premessenger RNAs (size = 1850 and 2650 bases).

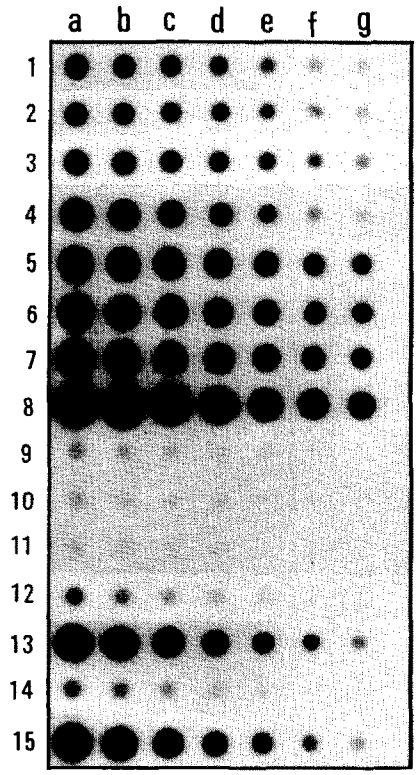

Fig. 2. Autoradiography of dot hybridization of AGP cDNA to the mRNA contained in the 15 RNA samples analyzed in Fig. 1. Columns a–g correspond to serial half-dilutions from 20 μg (a) to 0.313 μg (g). Mean integration values of dots giving proportional densities, estimated for 1 μg of total cellular RNA, were as follows: 116 (1), 140 (2), 233 (3), 146 (4), 1960 (5), 2544 (6), 2724 (7), 7048 (8), 1.3 (9), 1 (10), 0.6 (11), 4 (12), 450 (13), 6 (14), 435 (15).

4. DISCUSSION

In this work, hepatic stimulation by IL1β was studied without concomitant treatment with dexamethasone which is known to synergize the effect of cytokines [4]. In order to obtain a maximal effect of IL1β, experiments were performed at day 19 of gestation when the amount of corticosterone in fetal blood is highest [9], and when there is a simultaneous appearance of hepatocyte glucocorticoid receptor activity [10]. The amounts of IL1β administered to the fetus were chosen as a function of fetal weight, compared to doses normally administered to adult rats.

The major finding of this study is that AGP gene expression is induced in the fetal rat liver in response to IL1β treatment. The maximal increase in fetal liver AGP mRNA (82-fold) was of the same order of magnitude as that observed after the injection of an optimal dose of IL1β into adult rats (60-fold). Our results are in agreement with the finding that terpentine injection leads to a 45- to 90-fold increase in adult rat liver AGP mRNA [8,11] but contrast with the results of De Jong et al. [12], who observed only a 2.9-fold increase in cytoplasmic AGP mRNA in adult rat liver following the injection of IL1β. The 5-fold increase observed here in fetal serum AGP levels following IL1β treatment is similar to the 4-fold increase obtained 48 h after the injection of terpentine to 18-day fetuses [1].

Our finding strongly suggests that in the fetal rat, IL1β is the major inducer of AGP gene expression. Indeed it has been clearly shown that in adult rat primary hepatocyte cultures and in rat hepatoma cells, IL1 is a stronger inducer of AGP mRNA than are IL6 and TNFα [4]. However, it cannot be excluded that IL1β might have an additional effect on AGP synthesis through the induction of TNFα and IL6 [13]. Taken together, these results indicate that at day 19, the fetal rat liver is able to respond to IL1β in a similar way to the adult liver, and is insensitive to an inflammatory stimulus given to the mother.

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