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Absence of expression of interleukin-6 (IL-6) mRNA in regenerating rat liver

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The serum level of IL-6 and expression of IL-6 mRNA in hepatocytes from regenerating liver were investigated in the rat. The IL-6 level in the serum was not significantly different from that of a control group of rats submitted to an acute experimental inflammation. IL-6 mRNA expression did not occur in the liver of hepatectomized rats as judged from Northern blotting experiments using an IL-6 riboprobe. These results suggest that if IL-6 is implicated in hepatic regeneration, this cytokine is not produced by the regenerating liver and must be delivered exogenously to the liver to modulate hepatic regeneration.

Interleukin-6; Hepatic regeneration; Rat

1. INTRODUCTION

Hepatic regeneration is a common phenomenon in mammals which leads to the restoration of the hepatic parenchyma [1,2] following agressions such as carbon tetrachloride intoxication [3] or hepatectomy [4,5]. After two-third hepatectomy in rats, hepatocytes undergo mitosis within 24 to 36 hours and complete regeneration is obtained within 2 or 3 weeks [1]. Several mediators are implicated in this response (for review, see [6]) including EGF and TGF α [7–9] which increase the mitotic activity, or TGF β [10–12] which inhibits and stops it at the end of the regeneration. The interactions between these mediators during hepatic regeneration remain uncertain [6].

Interleukin-6 (IL-6) is a multifunctional cytokine which acts as a mediator of cell differentiation, of lymphocyte function, and as a factor in the regulation of the hematopoietic system (for review, see [13]). IL-6 has been demonstrated to be the major mediator in the acute phase response [14], and it acts by a receptor which has been found on hepatocytes and hepatoma cells [15,16]. Recently, it was suggested that IL-6 delivered exogenously to the liver could induce a tissue regeneration [17]. This data led us to study IL-6 expression in the regenerating liver in the rat.

The aim of the present study was to determine the IL-6 serum concentration and the expression of its mRNA in the liver following hepatectomy in rats, in order to assess whether IL-6 could be produced by the liver following partial hepatectomy.

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2. MATERIALS AND METHODS

2.1. *Animals*

Adult male Sprague–Dawley, weighing 250–300 g, were purchased from Charles-River (St Aubin les Elbeuf, France). They were maintained on a 12 h light, 12 h dark cycle with a standard laboratory chow and water available ad libitum.

2.2. *Surgical procedures*

Hepatectomies were performed under light ether anesthesia between 09.00 h and 12.00 h. 30% hepatectomy consisted of resection of the median and left lateral lobes of the liver. 80% hepatectomy consisted of resection of the median, left lateral, and right lateral lobes of the liver. Three control groups were realized simultaneously and consisted of laparotomy with mobilization of the liver and/or abscess by subcutaneous injection of 4 ml/kg turpentine oil.

Blood samples were collected from the tail tip at 0 h, 2 h, 6 h, day 1, day 2, day 3, day 4, day 5, day 6, day 7, day 8, day 9, day 10, day 11, day 14, day 17, day 22 and day 28.

After sacrifice of the animals at 2 h, 4 h, 6 h, 12 h, 24 h, day 6, day 10, day 14, day 28, the remaining liver (segments VI and VII) was collected, immediately frozen in liquid nitrogen, and next stored at −80°C until used.

2.3. *Serum IL-6 determination*

IL-6 in serum samples was assayed by a hybridoma growth stimulation assay using the mouse hybridoma 7TD1 cell line obtained through the courtesy of Dr J. Van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium). Cell numbers were evaluated colorimetrically after 4 days as previously described [18]. A titre of 1 U/ml was assigned to material which produced half maximal growth of 7TD1 cells. Samples were analyzed in duplicate, and the result expressed as the mean. The detection limit of the assay was about 10 U/ml. Statistical analysis was performed by Mann–Whitney test, P values < 0.05 were considered statistically significant.

2.4. *Preparation of peritoneal activated macrophages*

Rat peritoneal macrophages were obtained after 2 successive washes of the peritoneal cavity with 10 ml of MEM containing 10% bovine fetal serum and 1% glutamine. The cell suspension was filtered through sterile gauze and centrifuged at 150 × *g* for 4 min. Two sets of experiments were carried out. The cell number was adjusted to 1.5–2 × 10^6 cells/ml culture medium. Cells were plated in 75 cm^2 flasks and LPS (10 μg/ml) was added for 4 h in the first set, and for 24 h in the second set. Cell cultures were incubated in a humid atmosphere at 37°C in 5% CO_2, 95% air.

2.5. *Cellular RNA preparation*

Adherent rat peritoneal macrophages in the tissue culture disk were lysed directly with a guanidinium solution and RNA isolation was obtained by a single extraction with an acid guanidinium thiocyanate–phenol–chloroform mixture as described [19]. Rat livers were homogenized and total RNAs were obtained using the same one-step method. RNA concentrations were determined by measuring the absorbance at 260 nm. The integrity of RNA preparations was controlled by agarose electrophoresis and visualization of the 18S and 28S ribosomal RNA bands after ethidium bromide staining.

2.6. *Northern blot hybridization*

Samples of total RNAs (25 μg) were denatured for 15 min at 55°C in 1 × MOPS buffer pH 7.0, formaldehyde 2.2 M, 50% deionized formamide and then loaded in a 1.2% agarose gel in 1 × MOPS buffer, formaldehyde 2.2 M and separated by electrophoresis at 25–50 V for 14–20 h. Transfer of RNAs onto Hybond N+ nylon membranes (Amersham) was for 3 h in 50 mM NaOH. A RNA probe derived from a mouse IL-6 cDNA insert carried in a pGEM 4 vector (a generous gift from Dr. J. Doly, UPR 37, Villejuif, France) was used. The RNA probe was synthesized in vitro using T7 RNA polymerase, 1 μg of linearized plasmid DNA and 80 μCi [α-^{32}P]UTP (3000 Ci/mmol, Amersham). For hybridization 1.7 × 10^6 cpm/ml riboprobe was used. The blots were hybridized at 60°C for 20 h. The filters were washed in 100 ml 1 × SSC containing 0.1% SDS for 20 min at 65°C and then five times in 500 ml 0.1 × SSC containing 0.1% SDS for 20 min at 65°C. The filters were exposed to a Hyperfilm-MP (Amersham) at −80°C with an intensifying screen for 3 days.

3. RESULTS AND DISCUSSION

At 0 h serum IL-6 was below the level of detection in our assay but it rose within 2 h and reached a maximum at 4–6 h in the controls and in the two groups of hepatectomy (30% and 80%). Thereafter, the serum IL-6 level declined and was barely detectable at 72 h in all the groups. The maximal IL-6 level obtained 6 h after hepatectomy was not significantly different (*P* values between 0.1 and 0.45, Mann–Whitney test) from the maximum value obtained in controls (Figs. 1 and 2). Furthermore, the serum IL-6 levels were not different in the two groups of rats following 30% or 80% hepatectomy, although the importance of hepatic resection has been proved to modify hepatic regeneration [4].

Fig. 3 shows the lack of IL-6 mRNA in the livers of rats after 30% (lanes 1–5) or 80% (lanes 8–12) hepatectomy. For comparison, lanes 6 and 7 indicate the presence of the two IL-6 mRNA species of 1.3 kb and 2.4 kb [20] in RNA from rat peritoneal macrophages stimulated for 4 h (lane 6) and for 24 h (lane 7) with LPS.

These data establish that following hepatectomy in rats, IL-6 is released into the blood although the liver does not produce IL-6. In a previous study [21], we measured the production of IL-6 in rats suffering from an acute biliary sepsis and we showed that the IL-6 level was higher in the caval vein than in the suprahepatic veins. These previous results already suggested that liver is probably not involved in the synthesis of IL-6 during inflammation.

In the present work, it is noteworthy that serum IL-6 levels in hepatectomized rats and in the control groups

Figure 1

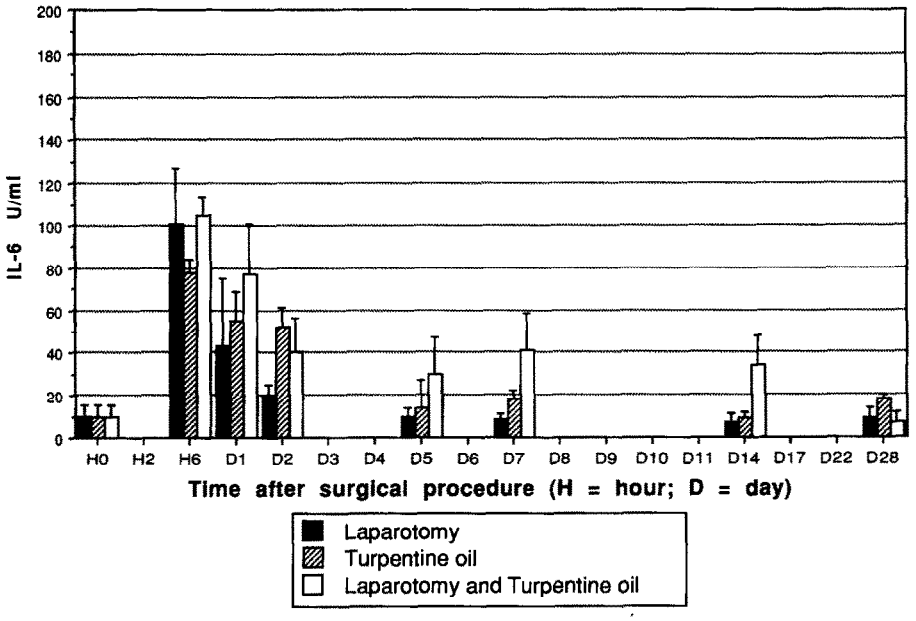

Fig. 1. Effect of subcutaneous injection of turpentine (□), laparotomy (■), turpentine and laparotomy (□), on the blood level of IL-6 in three different groups of rats (3 to 5 rats in each group). Blood samples were collected from the tail tip and serum IL-6 was measured in duplicate at three different dilutions using the 7TD1 hybridoma growth stimulation assay. Results are average values from 3 to 5 rats per time in each group.

Figure 2

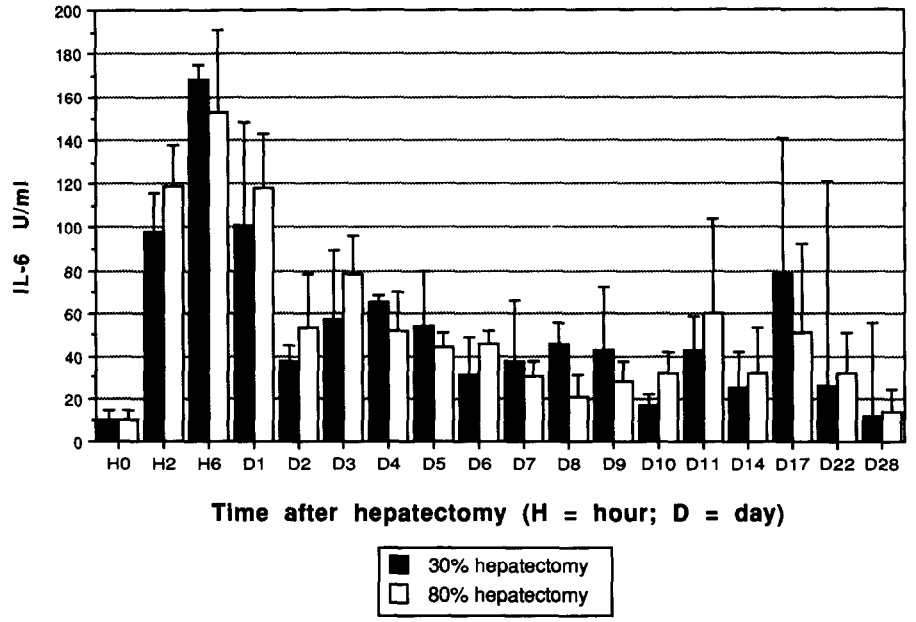

Fig. 2. Kinetics of production of IL-6 in the serum of rats following 30% (■), or 80% (□) hepatectomy. Blood samples and determination of IL-6 were obtained as indicated in Fig. 1.

are similar. Maximum IL-6 levels are higher in rats after hepatectomy although the difference is not statistically significant. In human, Cruickshank et al. [22] have shown that the serum IL-6 level after surgery is correlated with the duration and severity of the surgical procedure. The increase of serum IL-6 observed in our present study probably resulted from the surgical stress which was higher in the groups of hepatectomized rats. This suggests that the liver is not implicated in IL-6 synthesis during hepatic regeneration. The absence of expression of IL-6 mRNA in the regenerating liver is in keeping with this conclusion. Contradictory data have been published about IL-6 mRNA production in the liver. Tovey et al. [23] have shown that IL-6 mRNA was detected in the liver of normal individuals. These authors considered that IL-6 could be an autocrine regulator of hepatocyte growth. It has been reported that IL-6 secreted by certain rat hepatoma cell lines exerted an autocrine effect in vitro and induced the transcription of endogenous acute phase genes [24]. In contrast and despite the use of a sensitive IL-6 riboprobe or using the polymerase chain reaction, Gauldie et al. [25] and Northemann et al. [26] did not find any IL-6 mRNA expression in normal liver, nor in the liver of rats undergoing an acute-phase response following turpentine injection, suggesting that Kupffer cells were not especially activated during acute-phase response. However these authors did observe IL-6 mRNA expression in cultures of hepatocytes in absence of corticosteroid, which constitutes non physiologic conditions.

Although the liver does not produce IL-6 during hepatic regeneration, our study has shown an elevated level of IL-6 in rat serums following hepatectomy. As Koga et al. [17] have observed that a treatment of rats by IL-6 could induce a proliferative response in hepatocytes, it seems then reasonable to consider that an increased IL-6 production may interfere with hepatocyte proliferation. A delivery of IL-6 of exogenous origin would enhance hepatocyte mitosis. Another cytokine, the leukemia inhibitory factor (LIF) close to IL-6 in its

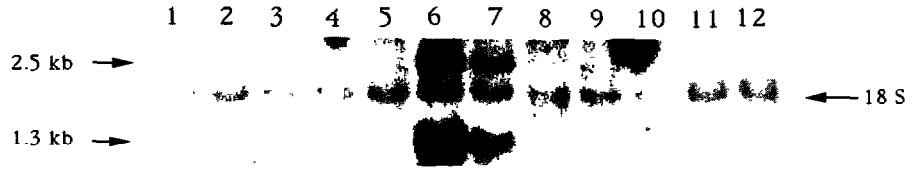

Fig. 3. Detection of IL-6 mRNA by Northern hybridization. Twenty-five μg total RNA were isolated from rat liver at various times after 30% hepatectomy (tracks 1–5) and after 80% hepatectomy (tracks 8–12). Twenty-five mg total RNA were isolated from rat peritoneal macrophages that were stimulated 4 h (track 6) or 24 h (track 7) previously with 10 mg/ml LPS. The 2 rat IL-6 mRNA species of 1.3 kb and 2.4 kb are indicated. Northern gel analysis was probed with ^{32}P-labeled RNA antisense from mouse IL-6 cDNA as described in section 2. The cross-hybridization of the riboprobe with 18S ribosomal RNAs indicate homogeneity in total RNA loading.

function has been shown to be induced in regenerating liver (G. Baffet et al., submitted). Further studies are necessary to pinpoint the interactions between IL-6 and other growth factors, and the possible inhibiting or activating action of IL-6 during hepatic regeneration.

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