Research Article

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Early hepatocyte DNA synthetic response posthepatectomy is modulated by IL-6 *trans*-signaling and PI3K/AKT activation

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Background & Aims: Interleukin-6 (IL-6) is a crucial factor in liver regeneration following partial hepatectomy (PH); however, the role of IL-6 and IL-6 *trans*-signaling in particular, in hepatocyte mitosis remains controversial. IL-6 *trans*-signaling relies upon the release of the soluble IL-6R (sIL-6R), which binds IL-6 to form an agonistic IL-6/sIL-6R complex. Herein we have examined the hypothesis that IL-6 *trans*-signaling plays a crucial and distinct role in liver regeneration following PH.

Methods: The specific IL-6/sIL-6R antagonist, sgp130Fc, was expressed in mice and analyzed for its effect on hepatocyte mitosis following PH. Alternatively, we examined the effect of the IL-6/sIL-6R super-agonist, Hyper-IL-6, or IL-6 expressed either alone or in combination with hepatocyte growth factor (HGF) on hepatocyte mitosis in the absence of PH.

Results: Following PH, the dramatic rise of circulating IL-6 levels is accompanied by a concurrent ~2-fold increase in circulating sIL-6R levels. Ectopic expression of sgp130Fc reduced hepatocyte mitosis by about 40% at early times following PH, while substantially reducing AKT, but not STAT3, activation. But, ectopic Hyper-IL-6 expression in mice without PH was not mitogenic to hepatocytes *in vivo*. Rather, Hyper-IL-6, but not IL-6, markedly increased HGF-induced hepatocyte mitosis. This cooperative effect correlated with greater resistance of HIL-6 than IL-6 to HGF-mediated reduction of AKT activation, rather than changes in STAT3 or MAPK signaling, and was completely blocked by PI3K inhibition.

Conclusions: Following PH, IL-6/sIL-6R cooperates with growth factors, through a PI3K/AKT-dependent mechanism to promote entry of hepatocytes into the cell cycle.

Keywords: Liver regeneration; IL-6 *trans*-signaling; IL-6; sIL-6R; sgp130; HIL-6; PI3K/AKT.

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Abbreviations: HIL-6, Hyper-IL-6; PH, partial hepatectomy.

Introduction

Following two-thirds partial hepatectomy (PH), a coordinated expression of growth factors, including hepatocyte growth factor (HGF), together with priming factors interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), mediate a robust regenerative response, driving hepatocyte cell division [1–3]. IL-6 expression, which dramatically increases shortly following PH [4], appears to be critical to the early onset of liver regeneration and has been shown to mediate both pro-mitotic and pro-survival activities within the regenerative process [5–8], although, the specific role of IL-6 in the mitotic response remains controversial [3,7,8].

IL-6 acts on target cells by binding to the cognate IL-6 receptor (IL-6R), thus triggering gp130 and leading to Jak/STAT, MAPK, and PI3K/AKT activation [1,5]. IL-6R expression is limited to a relatively few cell types in the body, including hepatocytes [9]. However, IL-6R also exists in a soluble form (sIL-6R) that, in a process called IL-6 *trans*-signaling, acts with IL-6 as an agonist on cell types that would not inherently respond to IL-6 alone [9,10]. Healthy individuals also naturally express relatively high levels of a soluble form of gp130 (sgp130) that selectively sequesters IL-6/sIL-6R complexes, but not either protein alone, thus functioning to form a threshold exclusively to IL-6 *trans*-signaling [11]. We have recently shown that a fusion protein of sgp130 with the constant portion of IgG1 (sgp130Fc) could be used to specifically block IL-6 *trans*-signaling without affecting classical IL-6 signaling via the membrane bound IL-6R [12,13].

IL-6 *trans*-signaling has been shown to be crucial to a variety of physiological and pathological processes where the sIL-6R mediates gp130 activation on cells expressing either no or low IL-6R levels [13–16]. However, the relatively high levels of IL-6R expressed on hepatocytes give little support to the notion that the substantial increase in sIL-6R levels observed following PH [17] is of physiological significance to liver regeneration. Nevertheless, reports from our and other laboratories suggest that IL-6 *trans*-signaling and classical signaling in the liver may have disparate physiological outcomes. Ectopic expression of IL-6 and sIL-6R in double transgenic mice, but not IL-6 alone, leads to nodular hepatocellular hyperplasia [18–20]. Furthermore, administration to mice of an IL-6/sIL-6R fusion protein, called Hyper-IL-6 (HIL-6) [21], but not IL-6, significantly accelerates

liver regeneration following PH, and strongly induces liver regeneration following acute hepatic injury [22–24]. These observations suggest that IL-6 *trans*-signaling may have a distinct physiological significance for hepatocyte mitosis. In the present study, we have transiently expressed the specific IL-6/sIL-6R antagonist, sgp130Fc, or super-agonist, HIL-6, in mice in order to test the hypothesis that IL-6 *trans*-signaling plays a crucial role in inducing hepatocyte mitosis during liver regeneration following PH.

The results of this study demonstrate that circulating sIL-6R levels increase rapidly and simultaneously with IL-6 following PH, and that IL-6/sIL-6R complexes make a distinct contribution to early phase hepatocyte replication. Based on this and previous observations, we expected that IL-6/sIL-6R, as opposed to IL-6, to act as a complete mitogen on hepatocytes. Contrary to these expectations, we demonstrate that neither IL-6 nor IL-6/sIL-6R stimulates hepatocyte mitosis *in vivo*. Rather, IL-6/sIL-6R, but not IL-6, cooperates with HGF to enhance hepatocyte mitosis through a PI3K/AKT dependent signaling pathway. Thus, this study establishes a clear role for IL-6 *trans*-signaling in early liver regeneration following PH.

Materials and methods

Animals

Male BALB/c and C57BL/6 mice (19–21 g) (Harlan Laboratories Ltd., Jerusalem) were maintained in an animal facility at a temperature of ~23 °C in a 12-h light–dark cycle, under SPF conditions. Mice received sterile commercial rodent chow and water *ad libitum*. Procedures were performed in accordance with Institutional Animal Care and Use Committee which approved animal treatment protocols. Mice were treated by hydrodynamics-based *in vivo* plasmid DNA transfection essentially as described [15,25]. In experiments involving PH (Supplementary Fig. 1B and F), two-thirds partial hepatectomy was performed 4 days following plasmid DNA transfection under ketamine and xylazine anesthesia in the morning, and consisted of midline laparotomy with separate ligation and removal of the left and anterior median lobes, essentially as described previously [26]. At indicated times after surgery, mice were sacrificed by isoflurane® inhalation, and liver and blood samples were harvested. BrdU (100 mg/kg, i.p.) was injected 3 h before sacrifice. Plasmid DNA doses were 20 μg for pBS-sgp130Fc and pBS-HCRHPI-A. In experiments not involving PH (Supplementary Fig. 1C–E), plasmid DNAs were injected either separately or in combinations at doses of: phAAT-IL-6 (10 μg), phAAT-HIL-6 (2.5 μg), or phAAT-HGF (10 μg, unless indicated otherwise) – made up to 20 μg with control plasmid (pGEM-7). Wortmannin was administered at a dose of 0.7 mg/kg (i.p.) 4 and 24 h post-plasmid DNAs transfection (Supplementary Fig. 1E).

Full methods

Full methods and associated references are available online in supplemental methods at www.jhep-elsevier.com.

Results

IL-6 and sIL-6R levels increase simultaneously following PH

Previous studies have shown that IL-6 levels increase shortly following PH. To examine the relevance of IL-6 *trans*-signaling on liver regeneration, we determined the kinetics of IL-6 and sIL-6R expression following PH by ELISA. Fig. 1 shows that following PH the dramatic increase in serum IL-6 levels is closely accompanied by a roughly twofold increase in circulating sIL-6R protein that reached maximal levels approximately 6 h following PH and was maintained for at least 24 h post-PH. This concurrent rise in circulating levels of both IL-6 and sIL-6R suggested that IL-6 *trans*-signaling may be relevant to the subsequent events of liver regeneration.

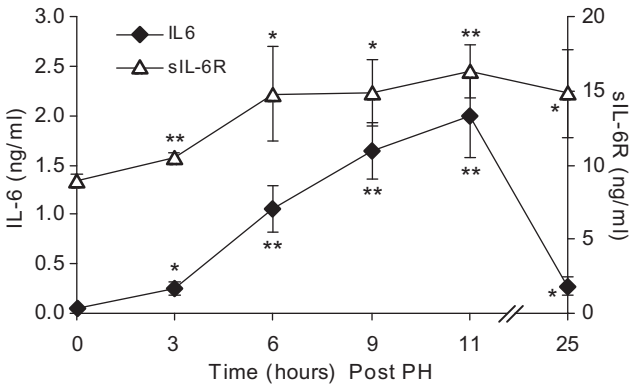

Fig. 1. IL-6 and sIL-6R levels increase simultaneously following partial hepatectomy. ELISA analysis of circulating IL-6 and sIL-6R levels in mouse serum following PH (*p <0.05, **p <0.01 versus baseline values, n = 6). Data are mean ± SEM.

Inhibition of IL-6/sIL-6R reduces hepatocyte DNA synthesis following PH

In order to determine the relevance of the increase in IL-6/sIL-6R levels to the subsequent hepatocyte mitotic response, we next took advantage of the IL-6/sIL-6R antagonist, sgp130Fc, to specifically block IL-6 *trans*-signaling. *In vivo* transfection of the sgp130Fc expression plasmid, pBS-sgp130Fc, resulted in high levels of circulating sgp130Fc protein for at least 2 weeks (Supplementary Fig. 2A), which completely blocked STAT3 activation in hepatocytes when mice were challenged with HIL-6 (Supplementary Fig. 1A and Supplementary Fig. 2B).

We transfected mice with either pBS-sgp130Fc, or control plasmid, and subjected the mice to PH 4 days later (Supplementary Fig. 1B). We elected to subject mice to PH 4 days after transfection because at this time all signs of liver injury resulting from the transfection procedure have typically subsided [25] (and data not shown), and we reasoned that effects of the hydrodynamics transfection procedure on hepatocyte mitosis would be minimal. Indeed, the hepatocyte mitotic index measured by BrdU incorporation following PH did not appear to be affected by *in vivo* transfection *per se* (mitotic index at 24 h = 15.8 ± 4.4 versus 17.9 ± 9.2 BrdU positive hepatocytes per high power field following PH in non-transfected and pBS-HCRHPI-A transfected mice, respectively, n = 10–14; p = ns). In contrast, transfection of mice with pBS-sgp130Fc significantly and consistently reduced the level of mitotically active hepatocytes by about 40% in comparison to control plasmid transfected mice at early times (24 h) following PH (Fig. 2). This inhibition of hepatocyte mitotic response consistently appeared to persist at 48 h post-PH in pBS-sg130Fc transfected mice, but without statistical significance. These results suggest that the concurrent increase in circulating IL-6 and sIL-6R levels following PH indeed leads to IL-6 *trans*-signaling in the liver and, furthermore, indicates that IL-6/sIL-6R functions to enhance the initiation of DNA synthesis in hepatocytes particularly at early times following PH.

IL-6/sIL-6R is not mitogenic to hepatocytes in vivo

This effect of sgp130Fc on the early hepatocyte mitotic response following PH suggested that IL-6 *trans*-signaling, as opposed to classical signaling, may indeed be distinctly mitogenic to hepatocytes *in vivo*. To test this hypothesis, we transfected mice with