IL-22 is involved in liver regeneration after hepatectomy

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The liver is unique in that it has the ability to regenerate after resection or injury despite the fact that hepatocytes normally do not actively divide (12). After partial hepatectomy, mature hepatocytes will replicate until the normal hepatic mass is restored.

Many investigators have studied models of partial hepatectomy to identify cytokines that are involved in hepatocyte proliferation and liver regeneration. For example, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-2, and stem cell factor have all been shown to be important factors for hepatocyte proliferation and/or hepatic regeneration (5, 8, 18, 19, 28). IL-6 is a well-studied cytokine with a significant role in hepatic regeneration after partial hepatectomy (8, 28). Hepatocyte proliferation is initiated through the nuclear translocation of signal transducer and activator of transcription (stat)-3 as a result of increased IL-6 levels after partial hepatectomy (8).

Interleukin-22 (IL-22) is a member of the interleukin-10 (IL-10) family of cytokines (25). It is produced by activated T cells and natural killer (NK) cells (22, 25) and acts via a heterodimeric receptor complex consisting of IL-22 receptor-α (IL-22Rα) and IL-10 receptor-β (IL-10Rβ) (13). Because of the lack of IL-22Rα expression in both resting and activated immune cells, IL-22 does not influence these cells in vitro or in vivo (23). It appears that the IL-22 receptor complex is expressed on number of other nonimmune tissues, including liver, kidney, and digestive and respiratory system tissues (1, 10, 14, 27). At least one role for IL-22 includes the induction of acute phase reactants and participation in the cascade of mediators involved in inflammation. Boniface and colleagues (4) have shown that IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration in human keratinocytes. IL-22 also promotes antimicrobial defense (24).

A recent study supports a potential therapeutic role for IL-22 as a protective factor in hepatocellular injury (17). In both hepatocytes and colon cancer cell lines, IL-22 can activate multiple signaling pathways, including the Janus kinase signal transducer and activator of transcription factor (stat-3) and mitogen-activated protein kinase pathways (14). Brand et al. (5) have demonstrated that low doses of IL-22 significantly increase proliferation in HT-29 cells, a colon cancer cell line. In addition, another recent investigation illustrated that IL-22 can induce hepatocyte proliferation in vitro in a dose-dependent manner (6). This study did not investigate the cellular mechanisms involved in IL-22′s effects in the setting of partial hepatectomy and studied this effect in vitro only. Our present investigation expands on previous information regarding IL-22′s effects in hepatocyte proliferation in that it outlines the kinetics involved in IL-22-initiated hepatocyte proliferation in vivo, documents that there is a correlation between IL-22 upregulation and a stat-3-mediated signal transduction pathway and suggests that IL-22 may play a contributing role in liver regeneration after 70% hepatectomy.

MATERIALS AND METHODS

Materials. Recombinant IL-22 protein, anti-IL-22 antibody, Quantikine mouse IL-6 ELISA kit, and mouse HGF Duoset ELISA Development kit were all obtained from R&D Systems (Minneapolis, MN). Cell proliferation kit was obtained from Amersham Pharmacia Biotech. Anti-stat-1, anti-phospho-stat-1, anti-stat-3, anti-phospho-stat-3, anti-stat-5, and anti-phospho-stat-5 antibodies were obtained from Santa Cruz Biotechnology, (Santa Cruz, CA). Anti-ERK1/2, anti-phospho-ERK1/2, and pro-TGF-α were purchased from Cell Signaling (Beverly, MA). Anti-GAPDH monoclonal antibody was purchased from Chemicon International, (Temecula, CA).

Seventy percent hepatectomy model. Female C57/BL6 mice (6–8 wk of age) were purchased from Jackson Breeding Laboratories (Bar Harbor, ME) and maintained under specific pathogen-free conditions with free access to water and food before each experiment. All experiments were performed in compliance with the standards for animal use and care set by the University of Michigan’s Committee for the Use and Care of Animals.

Animals were anesthetized with isoflurane inhalation and partial (70%) hepatectomy was performed as previously described (4). After midline incision was performed, the median and left lateral lobes of the liver were resected after a 3-0 silk suture ligature was secured around the base of each lobe. Control animals underwent sham laparotomy without resection or manipulation of the liver.
Three animals were used per treatment group per time point and five separate low-power fields were evaluated per animal. The number of BrdU-positive cells per low-field were counted and expressed as means ± SE for each group.

Preparation of whole cell lysates and Western blot analysis. Animals were treated as described above with anti-IL-22 or recombinant IL-22. Mice were euthanized at baseline and at 30, 60, and 180 min after hepatectomy, liver samples were obtained, snap frozen in liquid nitrogen, and stored for preparation of whole cell lysates and subsequent Western blot analysis.

Frozen liver samples were sliced very thinly and thawed in pre-chilled lysis buffer (100 mM Tris, 0.1% SDS, 0.1% Triton X-100, and 15% glycerol), followed by homogenization and sonication. All specimens were maintained at 4°C throughout processing. Next, samples were gently rotated for 30 min and centrifuged at 14,000 g for 15 min at 4°C; supernatants were removed, and the samples were centrifuged again at 14,000 g for 15 min at 4°C. The resulting supernatant contains the total cell lysate. Total protein content was measured with the BCA protein assay kit (Pierce, Rockford, IL).

For Western blot analysis, 60 μg of total cell lysate were electrophoresed on a 10% polyacrylamide gel, transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA), blocked for 1 h at room temperature in 5% dry milk and probed with primary antibodies specific for phosphorylated stat-1 (p-stat-1: 1:100), stat-1 (1:100), p-stat-3 (1:100), stat-3 (1:100), p-stat-5 (1:100), stat-5 (1:100), ERK1/2 (1:500), p-ERK1/2 (1:200), and pro-TGF-α (1:1,000) overnight. Membranes were then washed, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG2a or goat anti-rabbit in a dilution of 1:2,000 for 1 h at room temperature. Antigen-antibody complexes were detected with the enhanced chemiluminescence detection system (ECL, Amersham Pharmacia Biotech, Piscataway, NJ). The same blots were also stripped and reanalyzed by using anti-GAPDH monoclonal antibody as an internal protein loading control.

IL-6, HGF, and IL-22 ELISA. Mice were treated with anti-IL-22 or recombinant IL-22 as previous described and were euthanized at 1, 3, 6, and 24 h after partial hepatectomy. Serum and liver specimens were collected and analyzed for IL-6 and hepatocyte growth factor (HGF) using ELISA as previously described (4). For serum IL-22 measurement, mice underwent sham laparotomy or partial hepatectomy and were euthanized at 1, 3, 6, 12, 24, 48, and 72 h and serum was collected. IL-22 levels were then measured by ELISA as previously described (4).

Statistical analysis. Statistical analysis was performed by the Student t-test. Differences were considered significant if P < 0.05. Data were analyzed by use of Prism 3.0 computer software.
Fig. 3. Bromodeoxyuridine (BrdU) staining in mice undergoing 70% hepatectomy and treatment with anti-IL-22 antibody or control IgG antibody. 

A: mice were treated with IL-22 antibody or control IgG 30 min before partial hepatectomy. Hepatocyte proliferation was measured at 36 h ($P < 0.005$), 48 h ($P < 0.0001$), and 72 h ($P < 0.05$) posthepatectomy. Hepatocyte proliferation was significantly decreased in animals treated with IL-22 antibody compared with animals treated with control IgG (36 h: $P < 0.005$; 48 h: $P < 0.0001$; 72 h: $P < 0.05$). Graphs illustrate means ± SE; 4 animals were used per group and 5 low-power fields (LPF) were observed per mouse. 

B: comparative photographs of BrdU staining in mice treated with control IgG ($a, c, e, g$) and mice treated with IL-22 antibody ($b, d, f, h$) are presented. All pictures were taken at $\times 200$ magnification.
RESULTS

IL-22 and IL-22Rα mRNA expression after partial hepatectomy. Mice underwent 70% hepatectomy or sham laparotomy and quantitative analysis of liver IL-22 and IL-22Rα mRNA expression was performed by real-time RT-PCR. Figure 1 illustrates that hepatic IL-22Rα mRNA expression is significantly increased at 12, 24, and 48 h after partial hepatectomy, compared with sham-operated control animals; levels return to baseline 72 h posthepatectomy. Although hepatic IL-22 mRNA expression did tend to increase after partial hepatectomy, these changes did not reach statistical significance (data not shown).

Serum IL-22 levels after partial hepatectomy. Mice underwent sham laparotomy or partial hepatectomy and were euthanized at 1, 3, 6, 12, 24, 48, or 72 h after 70% hepatectomy; serum was collected, and IL-22 levels measured by ELISA. As illustrated in Fig. 2, increases in serum IL-22 were seen at 6, 12, 24, and 48 h and reached statistical significance at 12 h. This data correlated with the increases seen in hepatic IL-22Rα mRNA levels.

Effects of IL-22 on hepatocyte proliferation after partial hepatectomy. Mice were treated with IL-22 antibody and underwent partial hepatectomy or sham laparotomy, and hepatocyte proliferation was determined by BrdU staining. BrdU staining was significantly decreased in mice treated with IL-22 antibody at 36, 48, and 72 h posthepatectomy, compared with mice treated with saline alone. Treatment with exogenous IL-22 did not induce any significant increases in hepatocyte proliferation after partial hepatectomy (data not shown). Although this may be a technical problem related to a need for more frequent dosing of IL-22, possibly related to a short cytokine half-life, prior studies using other cytokines and chemokines such as TNF or IL-6 resulted in changes in hepatocyte proliferation with a single preoperative dose (18–20). Because the cascade of mediators inducing hepatocyte proliferation is likely initiated early after hepatectomy, a single dose of IL-22 in the perioperative period should be sufficient to initiate the cascade of events leading to hepatocyte proliferation. It is possible that there are already sufficient levels of IL-22 present posthepatectomy to elicit hepatocyte proliferation and additional IL-22 in this setting is not able to further accelerate this process.

Effects of IL-22 on stat-3 phosphorylation after partial hepatectomy. Prior investigations have documented that IL-22 can induce activation of stat-3 in rat hepatoma cells and human primary hepatocytes (6, 13). Our next experiments investigated

Fig. 4. Western blot analysis for signal transducer and activator of transcription-3 (stat-3) activation after partial hepatectomy and treatment with saline or exogenous IL-22. Representative Western blots are illustrated. GAPDH levels are also shown to demonstrate equal loading of the gels. A: significant increase in phosphorylated stat-3 (p-stat-3) levels in mice treated with exogenous IL-22 at baseline and 30 min after hepatectomy, compared with mice treated with saline alone. B: representative densitometry, as a semiquantitative measure of the p-stat-3 levels.

Fig. 5. Serum and hepatic IL-6 protein levels after partial hepatectomy and treated with recombinant IL-22 (rIL-22) or IL-22 antibody. Administration of rIL-22 to mice before partial hepatectomy increased both hepatic and serum levels of IL-6 compared with animals treated with vehicle; these effects were statistically significant at 1 h posthepatectomy (*P < 0.05). Treatment with anti-IL-22 antibody had no significant effects on serum or hepatic IL-6 levels. A: serum IL-6 levels. B: hepatic IL-6 levels. hep, hepatectomy; AG, Antigen; CAB, control antibody.
the effects of exogenous IL-22 on stat-3 activation in vivo after partial hepatectomy using Western Blot analysis. For animals undergoing partial hepatectomy and treatment with saline, increases in p-stat-3 expression are seen at 30, 60, and 180 min (Fig. 4). Furthermore, when sham-operated animals are treated with exogenous IL-22, p-stat-3 level at baseline are significantly increased compared with animals undergoing sham laparotomy alone (Fig. 4). More importantly, in animals undergoing partial hepatectomy and treatment with exogenous IL-22, increases in p-stat-3 levels are seen 30 min after partial hepatectomy, compared with mice undergoing hepatectomy alone (Fig. 4).

The next experiments examined whether IL-22 blockade could inhibit stat-3 activation. Treatment with IL-22 neutralizing antibody had no effects on stat-3 activation after partial hepatectomy; p-stat-3 levels were similar to those seen in animals undergoing hepatectomy and saline injection (controls; data not shown). This suggests that there are likely multiple pathways responsible for stat-3 activation after partial hepatectomy.

**IL-22 does not activate stat-1, stat-5, or ERK1/2 after partial hepatectomy.** We next examined whether IL-22 was involved in stat-1, stat-5, or ERK1/2 activation after partial hepatectomy. Treatment with anti-IL-22 antibody did not significantly change stat-1, stat-5, or ERK1/2 activation after hepatectomy, compared with mice treated with control antibody. Similarly, administration of exogenous IL-22 prior to partial hepatectomy also did not significantly change stat-1, stat-5, or ERK1/2 activation in the liver (data not shown).

**Administration of recombinant IL-22 to mice before partial hepatectomy increases both hepatic and serum levels of IL-6 but does not alter hepatic and serum HGF levels.** As shown in Fig. 5A, administration of rIL-22 significantly increased both serum and hepatic levels of IL-6 1 h after hepatectomy, compared with hepatectomy alone. In contrast, treatment with rIL-22 did not alter hepatic or serum HGF levels (data not shown). Administration of anti-IL-22 antibody prior to hepatectomy did not alter serum or hepatic levels of IL-6 or HGF at any time point (data not shown).

**Administration of anti-IL-22 before partial hepatectomy decreased hepatic TGF-α levels.** Administration of anti-IL-22 decreased hepatic TGF-α levels compared with mice treated with control antibody, as measured by Western blot analysis. As shown in Fig. 6, treatment with anti-IL-22 decreased hepatic TGF-α levels 3 h posthepatectomy, and this persisted out to 24 h posthepatectomy. Administration of rIL-22 before partial hepatectomy did not affect TGF-α levels (Fig. 6).

**DISCUSSION**

IL-22 has been shown to have a variety of effects. It appears to play an important role in inflammation (4, 5, 7, 15) and has also been noted to have proliferative effects in a hepatocyte cell line (6). Although the biological functions of IL-22 and its receptor have been well studied in vitro, there is little information on the biological effects of IL-22 on the liver regeneration after partial hepatectomy in vivo.

The present study shows a significant increase in hepatic IL-22 receptor expression and serum IL-22 after 70% hepatectomy.
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tomy and a significant decrease in liver regeneration after IL-22 blockade. Following 70% hepatectomy, administration of exogenous IL-22 increased stat-3 activation in the liver and IL-22 blockade significantly reduced hepatic activation of this signal transduction factor, concurrent with a significant reduction in BrdU staining, suggesting a decrease in hepatocyte proliferation. These findings suggest that IL-22 is at least partially responsible for signal transduction pathway activation in this model. Previous studies have shown that IL-22 can upregulate IL-6 gene expression (6). The present investigations also demonstrated that recombinant IL-22 significantly increased IL-6 production in both the liver and serum and that IL-22 blockade in the setting of hepatic resection also decreased TGF-α expression, although no decreases were seen in IL-6.

IL-22 is produced by activated T cells and NK cells (25) and acts via the IL-22 receptor complex. The IL-22 receptor complex consists of a tissue-specific receptor component, IL-22Rα and a relatively ubiquitous receptor component, IL-10Rβ (13). Although IL-10Rβ is a promiscuous receptor subunit that is used by multiple other mediators including IL-10, signaling through IL-22Rα appears to be restricted to IL-22 (5). Previous studies have shown that TNF-α and lipopolysaccharide significantly increase IL-22Rα mRNA levels without affecting levels of IL-10Rβ mRNA in HT-29 cells (5), whereas Radaeva and colleagues (17) demonstrated significant IL-22 expression in T-cell mediated hepatitis induced by concanavalin A. In our study we only detected a mild increase in hepatic IL-22 after 70% hepatectomy, but we demonstrated that there is a significant increase in IL-22Rα levels at early time points following partial hepatectomy.

It is well documented that mice deficient in IL-22 regenerate their livers more slowly than normal animals after a variety of injuries and that stat-3 binding is also decreased in these animals (3, 19). It has been shown that TNF-α may stimulate TGF-α expression, which then binds epidermal growth factor receptors in hepatocytes, leading to enhanced proliferation (9). Experiments in primary hepatocytes have shown that at least some of TNF-α’s proliferative effects are related to upregulation of TGF-α (9). These results emphasize the complex and overlapping systems that are involved in hepatic repair and regeneration. Our present study also suggests that IL-22-mediated liver regeneration may be related to TGF-α because IL-22 blockade in this model resulted in decreased hepatic TGF-α levels. Other investigations, as well as our own, have documented that IL-22 upregulates IL-6 gene expression (6, 29). Our present studies showed that IL-22 administration to mice resulted in an increase in both hepatic and serum IL-6 levels. These results suggest that IL-22’s proliferative effects are interrelated with those with IL-6 and TGF-α.

In the liver, stat-3 is primarily activated by IL-6. IL-22 plays an important role in the acute-phase response and also possibly in the promotion of liver regeneration (16, 30). Examination of the downstream signaling events after IL-22 administration in the context of partial hepatectomy demonstrated an increase in stat-3 activation. A significant amount of published evidence supports stat-3-mediated cell survival and proliferation (2, 11, 18–21). Our present investigation shows that IL-22 administration induced stat-3 activation, although IL-22 blockade resulted in no changes in stat-3 activation, suggesting that there are multiple pathways involved in stat-3 activation after partial hepatectomy.

In summary, the goal of this study was to investigate the role of IL-22 in liver regeneration after partial hepatectomy. IL-22Rα was upregulated at the early time points. Mice with treated with neutralizing IL-22 antibody prior to partial hepatectomy showed delayed liver regeneration according to BrdU staining. Administration of exogenous IL-22 prior to hepatectomy increased stat-3 activation, compared with untreated animals. In addition, IL-22 administration increased IL-6 levels and IL-22 blockade decreased TGF-α levels, suggesting that TGF-α and IL-6 may also be involved in this pathway.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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