IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro


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THE LIVER has great regenerative potential (10), and liver regeneration following partial hepatectomy is controlled by a complex interplay of cytokines and growth factors (21). Two known regulators of the priming phase of liver regeneration are the cytokines TNF-α and IL-6, the two key cytokines involved in liver cell regeneration, induce suppressor of cytokine signaling (SOCS)-3 mRNA, which is also induced by partial hepatectomy (12). Recently, we (5) demonstrated STAT activation and induction of SOCS-3 mRNA by IL-22, a novel IL-10-related cytokine, in intestinal epithelial cells. Given its STAT-inducing capacity, we hypothesized a role for this cytokine in liver cell regeneration that is also supported by the protective effects of IL-22 in a murine model of chemically induced hepatitis (46).

IL-22 was originally called IL-10-related T cell-derived inducible factor (IL-TIF) and was described as an IL-9-inducible gene (19). The IL-22 receptor (IL-22R) complex consists of two subunits, IL-22R1 and IL-10R2, which both belong to the class II cytokine receptor family (34). Upon binding to its R1 chain, IL-22 induces a conformational change that enables IL-10R2 to interact with the newly formed ligand-receptor complexes. This, in turn, activates a signal transduction cascade that results in the rapid activation of several transcription factors, including STAT proteins (3, 19, 37).

IL-22Rs are expressed on a variety of tissues, including the kidney, pancreas, and liver (34). Major sources of IL-22 are activated T and natural killer cells (60). As known so far, IL-22 seems to play a role in inflammatory processes, e.g., through upregulation of acute-phase reactants in the liver and hepatoma cells (19).

Although expression of the IL-22R complex has been demonstrated in hepatoma cell lines (37), comprehensive analyses of its expression in hepatic cell lines, including primary human hepatocytes, and of its detailed signal transduction, including its specific functions in hepatocyte regeneration, have not been performed yet. Therefore, elucidating these roles of IL-22 were the aims of this study. In addition, we analyzed the role of SOCS proteins in IL-22-mediated functions and signaling pathways.

MATERIALS AND METHODS

Reagents. The following antibodies were used: phospho-ERK-1/2, phospho-Akt, phospho-STAT5, ERK-1/2, and Akt (from Cell Signaling, Beverly, MA); phospho-STAT1 (BD Transduction Laboratories, Franklin Lakes, NY); phospho-STAT3 (Upstate Biotechnology, Lake Placid, NY), and STAT1, STAT3, and STAT5 (Santa Cruz Biotech-
nology, Santa Cruz, CA). Horseradish peroxidase-conjugated secondary antibodies to mouse or rabbit IgG and chemiluminescent substrate (SuperSignal West Dura Extended Duration Substrate) were from Pierce (Rockford, IL). Recombinant human IL-22 was obtained from R&D Systems (Minneapolis, MN), and recombinant human EGF was from BioMol (Hamburg, Germany). The MEK1 inhibitor PD-98059 and phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin were from Tocris Cookson (Bristol, UK).

**RT-PCR.** RT-PCR and quantitative PCR with primers for human IL-22 were performed as previously described (5). The primers for the PCRs are shown in Table 1.

**Cell culture, stable transfection, and immunoblot analysis.** Human hepatoma cell lines (HepG2, Hep3B, and Huh-7) were cultured in RPMI medium supplemented with 10% FCS, 2 mM glutamine, 1% penicillin-streptomycin, and 0.4% amphotericin B in a 5% CO2 atmosphere. Stable human SOCS-1- and SOCS-3-expressing clones were established as previously described (56). Gel electrophoresis and immunoblot analysis were performed as previously described (8).

**Isolation of primary human hepatocytes.** Tissue samples from human liver resections were obtained from patients undergoing partial hepatectomy. Experimental procedures were performed according to the guidelines of the charitable state-controlled foundation (Human Tissue and Cell Research) with informed patient’s consent (53). The study was approved by the local ethics committee of the Ludwig-Maximilians-University of Munich (53). Hepatocytes were isolated using a modified two-step EGTA-collagenase perfusion procedure as previously described (59). The viability of the isolated hepatocytes was determined by trypan blue exclusion, and cell samples with a viability of >80% were used for cell culture.

**Culture of primary human hepatocytes.** Primary human hepatocytes were plated on a collagen gel layer (BD biocoated collagen I, Becton Dickinson, Heidelberg, Germany) at a density of 1.2 × 10⁴ cells/cm² for 6-well plates and 1.5 × 10⁵ cells/cm² for 12-well plates in appropriate volumes of culture media. The medium consisted of DMEM with 5% FCS, 2 mM l-glutamine, and supplements as follows: 1.7 mM l-arginine, 3.75 ng/ml hydrocortisone, 100 μg/ml streptomycin, and 100 μg/ml penicillin. Cell stimulation experiments were performed as previously described (32). For experiments analyzing IL-22-mediated signal transduction pathways by Western blot experiments, cells were treated according to the following protocol: after cells had been plated for 16 h, medium was replaced by medium without FCS for 24 h. Next, starvation medium was added for 12–16 h and finally replaced by starvation medium for 3 h (32). For FACS and MTS assays, medium was replaced by medium without FCS for 16–24 h after cells had been plated for 16 h. Next, starvation medium with cytokines as indicated was added for 24 h. Cells were incubated at 37°C in a humidified incubator with 5% CO2. The viability of primary hepatocytes during the culture period was monitored by cell morphology using light microscopy and image analysis.

**Cell proliferation analysis by MTS assay.** HepG2 cells were seeded onto 96-well plates at a density of 5,000 or 10,000 cells/well as indicated and were allowed to attach overnight. Cells were stimulated with IL-22 or EGF as indicated or with cytokine-free medium (negative control). The cell proliferation rate was determined by MTS assay on day 2 using a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) according to the manufacturer’s instructions.

**Cell proliferation analysis by [³H]thymidine incorporation assay.** Primary human hepatocytes were labeled by an incubation with 2 μCi of [³H]thymidine (specific activity: 80 mCi/mmol, Amersham, Little Chalfont, UK) for 16 h. Cells were collected and washed on filters (Dunn, Asbach, Germany) using a cell harvester (Skatron, Sterling, VA), and the amount of radiolabeled [³H]thymidine incorporated into DNA was analyzed by determining the ratio of [³H]thymidine uptake in treated wells relative to that in untreated control wells.

**DNA fragmentation and cell cycle analysis.** The rate of apoptotic cell death was quantified by determining DNA fragmentation according to Nicoletti et al. (10). Briefly, cells were incubated for 24 h in hypotonic buffer (1% sodium citrate, 0.1% Triton X-100, and 50 mg/ml propidium iodide) and analyzed by flow cytometry on a FACS Calibur (Becton Dickinson) using CellQuest software. Nuclei to the left of the “G1 peak” containing hypodiploid DNA were considered apoptotic.

**ELISA.** For the quantification of IL-8 release, a BD OptEIA Human IL-8 Elisa Kit II (BD Biosciences, Bedford, MA) was used according to the manufacturer’s instructions.

**In vitro liver cell regeneration assays.** In vitro liver cell regeneration assays were performed as previously described (13, 39). Briefly, HepG2 cells were grown in six-well plates to complete confluence. Using a sterile razor blade, nine standardized wounds were created in each plate. Detached cells were removed by three washes with PBS, and the cell medium was changed from 10% FCS-containing medium to 1% FCS-containing medium. Cells were then stimulated with IL-22 (10 or 100 ng/ml) or PBS. Cells were washed with PBS after 24 h and fixed with ethanol. Numbers of migrated cells were counted under a microscope (Olympus IX50, Hamburg, Germany). For each group (IL-22 stimulated and controls), four dishes were analyzed, whereas for each dish, nine separate fields were counted.

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**Table 1. Primers used for PCR amplification**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 1</th>
<th>Sequence 2</th>
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<tbody>
<tr>
<td>Human IL-22 receptor 1</td>
<td>5′-CTCCACAGGCGCATAGCT-3′</td>
<td>5′-ACATCGACGCTTCAGTGG-3′</td>
</tr>
<tr>
<td>Human IL-10 receptor 2</td>
<td>5′-GGCTGAAATTTGACAGTACG-3′</td>
<td>5′-GAAAGCAGCTGACATAGG-3′</td>
</tr>
<tr>
<td>Human IL-22 binding protein</td>
<td>5′-AGGTCTAATTTACGTCGCCA-3′</td>
<td>5′-CCTGCTGCACTCCATGG-3′</td>
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<tr>
<td>Human IL-22</td>
<td>5′-ATGCCATCTGACTCTCTTG-3′</td>
<td>5′-TCTGAGGCTTCTTCCTAATA-3′</td>
</tr>
<tr>
<td>Human TNF-α</td>
<td>5′-ATGAGCACTGAAACGATG-3′</td>
<td>5′-TCAGACGGGCAATGCTGC-3′</td>
</tr>
<tr>
<td>Human β-actin</td>
<td>5′-GGCAAACGGGAAAGATG-3′</td>
<td>5′-CATCACAGTGCCACTGTA-3′</td>
</tr>
<tr>
<td>Human SOCS-1-3</td>
<td>5′-GCCAGGCGTCGGCTGCGCC-3′</td>
<td>5′-CTGGGCTGCTCGTCACCC-3′</td>
</tr>
<tr>
<td>Human GAPDH</td>
<td>5′-GAGGGCCAGCGCTGCCCC-3′</td>
<td>5′-AGGGTGGCAGTCAGATTT-3′</td>
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SOCS, suppressor of cytokine signaling.

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In vivo model of murine cytomegalovirus infection. C57BL/6 mice were infected intravenously with 1 × 10^6 plaque-forming units of murine cytomegalovirus (MCMV) of the Smith strain (47) in PBS as previously described (4). Control mice received an injection of PBS only. After 45 h, mice were euthanized by CO2 inhalation, and livers were collected. Total RNA of the liver was isolated using TRIZol reagent. This study was approved by the Animal Care and Use Committee of the State of Bavaria (Regierung von Oberbayern) following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Rat in vivo model of hepatic ischemia-reperfusion injury. Eight-week-old male genetically obese (fa/fa) Zucker rats (390 ± 40 g) and their heterozygous littersmates (270 ± 40 g) were obtained from Charles River Wiga and housed under a constant 12:12-h light-dark cycle with free access to water and rat chow (standard diet Altromin 1314). Animals received humane care in compliance with guidelines by the local animal welfare committee and the criteria outlined in the NIH Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23, Revised 1985). Surgery was performed under spontaneous ether inhalation. Arterial blood pressure was continuously monitored via a carotid catheter. Body temperature was kept between 36.5 and 37.5°C by means of a heating pad. A laparotomy was performed, and the common bile duct was cannulated with a polyethylene tube. To avoid splanchic congestion, we used the model of partial liver ischemia (30). Partial liver ischemia was induced by selective clamping of branches of the portal vein and hepatic artery supplying the left and median liver lobes. After 1 h of warm ischemia, the right nonischemic liver lobes (right and caudate) were removed. Immediately thereafter, reflow was initiated by removal of the microclips. After 2 or 6 h of reperfusion, livers were immediately frozen in liquid nitrogen. Total RNA was isolated using TRIZol reagent (GIBCO-BRL/Life Technologies, Gaithersburg, MD). RT-PCR was performed as described above. The primers for rat IL-22 are listed in Table 1.

Sampling of human liver biopsy tissue. Human liver biopsy tissue was taken from patients undergoing diagnostic liver biopsy for medical reasons such as staging of chronic hepatitis C. The study was approved by the Ethics Committee of the Medical Faculty of the University of Munich and adhered to the principles of the Declaration of Helsinki. All participating subjects gave written informed consent before the liver biopsy. A 3-mm-long segment of the biopsy cylinder was immediately stored in TRIZol reagent (GIBCO-BRL/Life Technologies). cDNA was isolated as previously described (8). Quantitative PCR with primers for human IL-22 was performed as previously described (5).

Statistical analysis. Statistical analysis was performed using a two-tailed Student’s t-test. P values of <0.05 were considered as significant.

RESULTS

Primary human hepatocytes and hepatic cell lines express the functional IL-22R complex. To determine if the IL-22R complex consisting of IL-10R2 and IL-22R1 was expressed in liver cells and to utilize a hepatic cell model to study this ligand-receptor system, we analyzed IL-10R2 and IL-22R1 mRNA expression in several human hepatoma-derived cell lines (HepG2, Hep3B, and Huh-7). RT-PCR analysis demonstrated IL-10R2 and IL-22R1 mRNA expression in all cell lines tested (Supplemental Figure S1A).1 Similarly, primary human hepatocytes isolated from three different donor livers expressed IL-10R2 and IL-22R1 mRNA (Supplemental Figure S1B). Next, we analyzed the signal transduction pathways following receptor activation. Previous studies (3, 19, 37) in other cell lines have reported the activation of STAT signaling by IL-22. Accordingly, compared with basal levels of tyrosine phosphorylation of STAT1, STAT3, and STAT5 in unstimulated controls, tyrosine phosphorylation of STAT proteins was significantly stimulated by 100 ng/ml IL-22 in HepG2 cells (Fig. 1, A–C). Moreover, IL-22 (100 ng/ml) induced a transient activation of ERK1/2 (Fig. 2A) and Akt (Fig. 2B). Similarly, IL-22 activated STAT1, STAT3, STAT5, ERK1/2, and Akt kinases in primary human hepatocytes isolated from human donors (Supplemental Figures S2 and S3).1

IL-22 increases liver cell regeneration in vitro. The activation of ERK1/2 and Akt has been linked to increased cell migration (2). Therefore, we analyzed in previously established cell restitution assays (16) if IL-22 promoted hepatic cell migration and liver cell regeneration. To quantify the IL-22-mediated cell migration, we analyzed a total of 36 fields in 4 separate dishes for each group containing >500 migrated cells/group. This experiment demonstrated a significant, dose-dependent twofold increase of the cell migration rate in IL-22-stimulated cells (P < 0.05 for 10 ng/ml IL-22 and P < 0.001 for 100 ng/ml IL-22 vs. unstimulated controls; Fig. 3A), which could be strongly inhibited by a simultaneous treatment with the PI3K inhibitor wortmannin (P < 0.001 for 100 ng/ml IL-22 compared with 100 ng/ml IL-22 + wortmannin treatment).

1 Supplemental information for this article is available online at the American Journal of Physiology-Gastrointestinal and Liver Physiology website.

Fig. 1. IL-22 induces STAT1, STAT3, and STAT5 phosphorylation in hepatic cells. Following stimulation of HepG2 cells with IL-22 (100 ng/ml), STAT1 (A), STAT3 (B), and STAT5 proteins (C) were strongly phosphorylated (p-STAT1, p-STAT3, and p-STAT5). One representative experiment (n = 3) is shown. Similar results were obtained for Huh-7 cells (data not shown).
Treatment with the MEK1 inhibitor PD98059 had no significant influence on the IL-22-mediated cell migration rate (P = 0.07 for 100 ng/ml IL-22 compared with 100 ng/ml IL-22 + PD98059 treatment; Fig. 3A), suggesting a primarily PI3K-dependent mechanism. Treatment with PD98059 or wortmannin alone had no significant effect on cell migration (data not shown).

**IL-22 influences cell proliferation in hepatic cells.** Next, we analyzed if the IL-22-mediated liver cell regeneration was caused by an increased cell proliferation, particularly since ERK1/2 and Akt activation have been shown to mediate antiapoptotic pathways and to increase cell proliferation (5–7, 18, 22). IL-22 at concentrations of 10 and 100 ng/ml significantly increased cell proliferation in HepG2 cells by 28% and 19%, respectively (P < 0.05 compared with unstimulated controls; Fig. 3B). Treatment with the PI3K inhibitor wortmannin significantly impaired IL-22-mediated cell proliferation (P < 0.01 for 100 ng/ml IL-22 compared with 100 ng/ml IL-22 + wortmannin treatment; Fig. 3B), whereas the MEK1 inhibitor PD-98059 had a less pronounced effect that did not reach statistical significance (P = 0.07 for 100 ng/ml IL-22 compared with 100 ng/ml IL-22 + PD98059 treatment; Fig. 3B). This is consistent with our results showing that wortmannin but not PD98059 inhibited IL-22-mediated cell restitution (Fig. 3A). The proliferative effect of IL-22 on hepatocytes was stronger at low cell density compared with higher cell density (Fig. 3C). Almost identical results were obtained using [3H]thymidine incorporation assays (Fig. 3D), which demonstrated a significant increase of cell proliferation at a low cell number (5,000 cells/well) using 10 ng/ml IL-22 (P < 0.05). Although this proliferation-stimulating effect was moderate, it was comparable with that of established hepatocyte growth factors such as EGF (Fig. 3D). Next, we analyzed if IL-22 stimulated the cell proliferation of primary human hepatocytes. In these experiments, primary hepatocytes were isolated from human donors undergoing partial hepatectomy as described in MATERIALS AND METHODS. In previous experiments, we (58) demonstrated an overall low cell proliferation of primary human hepatocytes with low mitotic activity. Similarly, the primary human hepatocytes used in these experiments had low mitotic activity and showed no significant differences in the percentages of cells in the G1, S, and G2 phases after stimulation with IL-22 compared with cells treated with the established hepatocyte growth factor EGF (Fig. 3E). However, despite the low basal cell proliferation rate of primary hepatocytes compared with HepG2 cells, IL-22 at dosages of 10 and 100 ng/ml significantly increased cell proliferation in MTS assays (Fig. 3F). This result was confirmed in [3H]thymidine incorporation assays. IL-22 at 100 ng/ml (and EGF at all concentrations used) significantly increased cell proliferation (P < 0.05; Fig. 3G). Interestingly, in primary hepatic cells, both cell proliferation assays (MTS assay and [3H]thymidine incorporation) demonstrated a dose-dependent increase of cell proliferation with IL-22 that was stronger with 100 than with 10 ng/ml IL-22 (Fig. 3, F and G). In contrast, in HepG2 cells, 10 ng/mL IL-22 consistently had a stronger effect on cell proliferation than 100 ng/ml IL-22 (Fig. 3, B–D).

**IL-22 upregulates gene expression of proinflammatory cytokines and SOCS-3.** IL-6 and TNF-α have been identified as key cytokines involved in liver cell regeneration (14, 63). Therefore, we analyzed if the gene expression of these cytokines was regulated by IL-22. As demonstrated in Fig. 4A, IL-22 upregulated the mRNA expression of the proinflamma-
tory cytokines IL-6, TNF-α, and IL-8. Accordingly, IL-8 protein expression was upregulated 2.8-fold after stimulation with IL-22 (Fig. 4B). Moreover, a previous study (12) has demonstrated a role for SOCS proteins in liver cell regeneration. Therefore, we analyzed if IL-22 also influenced SOCS-1 and SOCS-3 mRNA expression levels in hepatic cells. In these experiments, 100 ng/ml IL-22 rapidly upregulated SOCS-3 mRNA expression in HepG2 cells (Fig. 4A), whereas the expression level of SOCS-1 mRNA did not significantly change (data not shown).

SOCS-1 and SOCS-3 overexpression abrogates IL-22-induced tyrosine phosphorylation of STAT1 and STAT3. Recently, we (9) demonstrated that overexpression of SOCS-1 abrogates IFN-α-induced STAT1 activation. Since IFN-α and IL-22 belong to the group of IL-10-like cytokines and share the IL-10R2 subunit for signaling, we investigated if a similar mechanism applied to IL-22-induced STAT activation. Therefore, we engineered HepG2 cells stably overexpressing SOCS-1 or SOCS-3. Whereas STAT protein phosphorylation was significantly activated by IL-22 in the control clone (pCR3.1/mock), SOCS-1 or SOCS-3 overexpression strongly impaired IL-22-induced tyrosine phosphorylation of STAT1 (Fig. 5A) and STAT3 (Fig. 5B).

SOCS-1 and SOCS-3 overexpression abrogates IL-22-induced liver cell regeneration in vitro. As STAT1 and STAT3 proteins are important mediators of cell motility and migration (41, 50), and having demonstrated that SOCS-1 or SOCS-3 overexpression abrogated IL-22 induced STAT signaling, we next analyzed if IL-22-mediated liver cell regeneration could be inhibited by overexpression of SOCS-1 and SOCS-3. Therefore, we repeated the liver cell restitution assays in HepG2 clones stably overexpressing SOCS-1 or SOCS-3 and compared the results with liver cell regeneration in mock-transfected control clones. The different unstimulated clones did not differ significantly in their cell migration rate (data not shown). In the mock-transfected clones, we observed a 72% increase in cell migration upon stimulation with 100 ng/ml IL-22 (P < 0.001 compared with unstimulated controls; Fig. 5C). This IL-22-mediated effect was strongly abolished by SOCS-1 overexpression (P = 0.35 compared with unstimulated clones; Fig. 5C).
IL-22 mRNA is expressed in human liver disease and its expression is increased in an in vivo model of T cell-mediated hepatic injury but not in ischemia-reperfusion injury. Having demonstrated that IL-22 mediated liver cell regeneration, we next analyzed if IL-22 mRNA expression was regulated during hepatic injury in vivo. Activated lymphocytes are the main source of IL-22 production (62), and IL-22 has demonstrated hepatoprotective effects in models of T cell-mediated hepatitis (46). Therefore, we chose the MCMV hepatitis model, which is an established model of T cell-mediated liver injury (45), to study the regulation of IL-22 mRNA expression. As demonstrated in Fig. 6A, IL-22 mRNA expression was below the detection threshold after 40 cycles in the livers of four uninfected mice, whereas IL-22 mRNA was expressed in all five mice infected with MCMV.

Next, we analyzed IL-22 mRNA expression in a rat in vivo model of ischemia-reperfusion injury that has been traditionally recognized as a model of neutrophil-dependent hepatic injury (36), although recent studies (11, 31) have suggested that CD4<sup>+</sup> lymphocytes may regulate this neutrophil-dependent injury. However, in contrast to the MCMV model of T cell-mediated liver injury, differences between treated and untreated groups were less pronounced in the ischemia-reperfusion experiments. Hepatic IL-22 mRNA expression was detectable in one of the four sham-treated control rats and in three of the four rats with hepatic ischemia-reperfusion injury (Fig. 6B). Next, we repeated the ischemia-reperfusion experiments in obese (fa/fa) Zucker rats, which also serve as a model of steatohepatitis and have increased hepatic injury following ischemia-reperfusion (33). After 2 h, IL-22 mRNA was detectable in all rats treated with hepatic ischemia-reperfusion and three of the four sham-treated control rats (Fig. 6B). After 6 h, IL-22 mRNA expression was detectable in three rats of each group (Fig. 6B).

To analyze if IL-22 mRNA is also expressed in human liver disease in vivo, we collected biopsy tissue from patients with different types of liver disease. The biopsy tissue was taken from patients undergoing diagnostic liver biopsy for medical reasons such as staging of chronic hepatitis C; therefore, and for ethical reasons, no normal controls were included in this analysis. This analysis included liver biopsy tissue from patients with autoimmune hepatitis (n = 2), hepatitis B virus (n = 1), hepatitis C virus (n = 2), primary biliary cirrhosis (n = 2), and primary sclerosing cholangitis (n = 2). IL-22 mRNA expression was detectable in all liver biopsy samples (Table 2). However, IL-22 mRNA expression levels measured by quantitative PCR were significantly higher in the hepatitis samples (autoimmune hepatitis, hepatitis B virus, and hepatitis C virus, mean relative expression: 0.65) than in biopsy samples taken from patients with cholestatic liver disease (primary biliary cirrhosis and primary sclerosing cholangitis, mean relative expression: 0.19, P = 0.001; Table 2).

**DISCUSSION**

The complex process of liver regeneration, including the precise timing and coordination of DNA replication, is controlled by multiple hormonal and cytokine signals. Cytokines and growth factors such as TNF-α, IL-6, transforming growth factor-β, and hepatocyte growth factor have been shown to be involved in the priming and progression of hepatocyte proliferation after liver injury (14, 21, 54, 63). It has been demonstrated that SOCS-3 transcripts and protein are induced during the priming phase of liver regeneration and that this induction is greatly diminished in IL-6 knockout mice (12). Recently, we (5) demonstrated upregulation of SOCS-3 transcripts in intestinal epithelial cells stimulated with IL-22, which prompted us to investigate the role of IL-22 in liver cell regeneration.
Here, we demonstrate that hepatic cell lines express the IL-22R complex consisting of IL-22R1 and IL-10R2, whereas IL-22 itself has proinflammatory functions in hepatic cells. IL-22 increased IL-8 mRNA and protein expression in hepatic cells, confirming the increased chemokine expression after IL-22 stimulation found in another study (17). In addition to the liver (17) and intestine (5), proinflammatory properties of IL-22 have also been reported in the skin (3, 61) and pancreas (24). Moreover, we demonstrated that mRNA expressions of IL-6 and TNF-α, two key cytokines involved in liver cell regeneration, are increased following IL-22 stimulation. In addition, IL-22 activates ERK MAPKs and Akt in hepatic cells, which is consistent with our findings in intestinal epithelial cells (5) and the signaling found in a rat hepatoma cell line (37). In particular, the activation of ERK MAPKs and Akt has been implicated in cell migration (2, 51). Similarly, our experiments demonstrated that IL-22R activation resulted in increased liver cell regeneration due to increased hepatic cell migration and hepatocyte proliferation, which could be blocked using the PI3K inhibitor wortmannin. This is of particular interest because Akt may compensate STAT signaling in liver cell regeneration. For example, recently, compensatory liver regeneration by Akt-mediated hepatocellular hypertrophy has been shown in liver-specific STAT3-deficient mice (25). Moreover, IL-22 activated STAT1 and STAT3, which resulted in increased SOCS-3 mRNA expression in hepatic cells. This is in agreement with the results of a study (35) showing increased SOCS-3 mRNA in a hepatoma cell line following IL-22 stimulation and a very recent study (40) demonstrating STAT1/3 activation after stimulation with IL-22 in the colonic epithelial cell line Colo205. However, we recently demonstrated that SOCS-1 and SOCS-3 overexpression decreased IFN-α and IFN-γ signaling (9, 56), a mechanism that has been implicated in IFN resistance and has also been shown for IL-6 signaling (15). Similarly, in this study, SOCS-1 and SOCS-3 overexpression decreased IL-22-induced STAT signaling, suggesting that IL-22-induced expression of SOCS-3 mRNA serves as a negative feedback mechanism to limit STAT3 activation. In addition, overexpression of SOCS proteins also abolished IL-22-mediated liver cell regeneration. Interestingly, a growth-inhibiting effect of SOCS proteins has been also described in hepatocellular and squamous cell carcinoma (57, 64). Our findings are also supported by a very recent study (42) using a murine model in which deletion of the SOCS-3 gene in hepatocytes promoted the activation of STAT3, resistance to apoptosis, and an acceleration of proliferation, resulting in enhanced hepatitis-induced hepatocarcinogenesis. A role for STAT3 activation has also been recently demonstrated for gastric cancer (27, 29) and several other malignancies (38). Therefore, our findings on cell proliferation and migration shown in the hepatoma cell line HepG2 also have implications for hepatic carcinogenesis. SOCS-2, another member of the SOCS protein family, is a negative regulator of growth hormone signaling (23). Moreover, overexpression of SOCS-1 and SOCS-3, which are rapidly induced and then degraded (1), causes insulin resistance in liver cells (20, 48, 49, 55), potentially contributing to fatty degeneration of the liver as seen in nonalcoholic steatohepatitis. Although overexpression of SOCS proteins has growth-inhibiting functions in these models and decreases IL-22-induced liver cell regeneration, it has been recently demonstrated that SOCS-3 has a hepatoprotective role under certain circumstances. For example, intracellular protein therapy with SOCS-3 inhibited inflammation and apoptosis in different murine models of hepatic inflammation (28). The important role of SOCS proteins in liver regeneration has recently been also demonstrated in SOCS-1 knockout mice, which die before weaning with fatty liver degeneration (52). Very recent findings have suggested that the functions demonstrated here for STAT3 and SOCS-3 are not only limited to liver cell migration and regeneration but are also important in the regeneration of other tissues such as in astrocyte migration and recovery from spinal cord injury (43). Whereas STAT3 knockout limited cell migration, SOCS-3 knockout enhanced astrocyte migration in a murine in vivo model (43), which is consistent with our in vitro findings in hepatic cells.

Recently, it has been demonstrated that IL-22 mRNA and protein expression are significantly elevated in T cell-mediated hepatitis induced by concanavalin A but are less extensively elevated in the carbon tetrachloride-induced liver injury model (46). This is consistent with the increased hepatic IL-22 mRNA expression in our in vivo model of T cell-mediated hepatic injury following MCMV infection. In contrast, we could not demonstrate marked differences in IL-22 mRNA expression in a rat in vivo model of hepatic ischemia-reperfusion injury. Hepatic ischemia-reperfusion results in an acute inflammatory response culminating in the recruitment of activated neutrophils that directly injure hepatocytes. Although recent studies (11, 31) have suggested that CD4+ lymphocytes may regulate this neutrophil-dependent injury, this model is traditionally considered a model of neutrophil-mediated hepatic injury. Activated neutrophils infl
trate the injured liver in parallel with increased expression of adhesion molecules on endothelial cells (36). Therefore, our results from the two in vivo experiments are in agreement with those of another study (60) demonstrating activated T cells and not neutrophils as the main source of IL-22 expression. In addition, overexpression of IL-22 significantly protects against liver injury, necrosis, and apoptosis (44).

Importantly, using human liver biopsies, we demonstrated that IL-22 mRNA is detectable in different disease models of human hepatic inflammation in vivo such as hepatitis B and C, autoimmune hepatitis, and primary biliary cirrhosis, suggesting that the IL-22 ligand-receptor system plays not only a role in artificial models of hepatic injury such as concanavalin A-induced hepatitis but also in human liver diseases. Interestingly, hepatic IL-22 mRNA expression levels were significantly higher in autoimmune and viral hepatitis than in cholestatic liver disease.

In summary, we demonstrated that hepatic cells express the IL-22R complex. Binding of IL-22 to its surface receptor lead to phosphorylation of STAT proteins, Akt, and ERK MAPKs. In addition, IL-22 upregulated the mRNA expression of proinflammatory cytokines and SOCS-3, whereas SOCS-1 and SOCS-3 overexpression abrogated IL-22-induced STAT signaling in hepatic cells and inhibited IL-22-induced liver cell regeneration. IL-22 mRNA expression was detectable in different disease models of human hepatitis. Taken together, these results suggest that IL-22 is a proinflammatory mediator that plays an important role in hepatic cell proliferation and liver cell regeneration.

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Portions of this study have been presented as oral presentations at the annual meeting of the American Gastroenterological Association and Digestive Disease Week (Los Angeles, CA, May 20–25, 2006) and have been published in abstract form in Gastroenterology. Additional portions of this study were presented as an oral presentation at the United European Gastroenterology Week (Berlin, Germany, October 21–25, 2006) and were also published in abstract form in Gut.

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