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

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Brand S, Dambacher J, Beigel F, Zitzmann K, Heeg MH, Weiss TS, Prüfer T, Olszak T, Steib CJ, Storr M, Göke B, Diepolder H, Bilzer M, Thasler WE, Auernhammer CJ. IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. *Am J Physiol Gastrointest Liver Physiol* 292: G1019–G1028, 2007. First published January 4, 2007; doi:10.1152/ajpgi.00239.2006.—The IL-10-like cytokine IL-22 is produced by activated T cells. In this study, we analyzed the role of this cytokine system in hepatic cells. Expression studies were performed by RT-PCR and quantitative PCR. Signal transduction was analyzed by Western blot experiments and ELISA. Cell proliferation was measured by MTS and [^{3}H]thymidine incorporation assays. Hepatocyte regeneration was studied in in vitro restitution assays. Binding of IL-22 to its receptor complex expressed on human hepatic cells and primary human hepatocytes resulted in the activation of MAPKs, Akt, and STAT proteins. IL-22 stimulated cell proliferation and migration, which were both significantly inhibited by the phosphatidylinositol 3-kinase inhibitor wortmannin. IL-22 increased the mRNA expression of suppressor of cytokine signaling (SOCS)-3 and the proinflammatory cytokines IL-6, IL-8, and TNF-α. SOCS-1/3 overexpression abrogated IL-22-induced STAT activation and decreased IL-22-mediated liver cell regeneration. Hepatic IL-22 mRNA expression was detectable in different forms of human hepatitis, and hepatic IL-22 mRNA levels were increased in murine T cell-mediated hepatitis in vivo following cytomegalovirus infection, whereas no significant differences were seen in an in vivo model of ischemia-reperfusion injury. In conclusion, IL-22 promotes liver cell regeneration by increasing hepatic cell proliferation and hepatocyte migration through the activation of Akt and STAT signaling, which is abrogated by SOCS-1/3 overexpression.

interleukin-10-like cytokines; liver regeneration; cell migration; suppressor of cytokine signaling

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 (14, 63), which are increasingly expressed after partial hepatectomy (54, 63). In mice lacking either IL-6 or TNF receptor 1 (p55/TNFR1), liver cell regeneration is impaired after partial hepatectomy (14, 63), which can be restored by a preoperative injection of IL-6 (14). Detailed analysis of IL-6 signaling identified STAT phosphorylation as an essential pathway involved in liver cell regeneration (26). It has been demonstrated that STAT3 is activated after partial hepatectomy as the result of increased IL-6 levels (14, 63), implicating both IL-6 and STAT3 as part of the priming mechanism for hepatocyte proliferation. Moreover, 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-

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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% amphotericine B in a 5% CO_2 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). Human 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.

Table 1. *Primers used for PCR amplification*

	Primer
Human IL-22 receptor 1	
Forward	`5′-CTCCACAGCGGCATAGCCT-3′`
Reverse	`5′-ACATGCAGCTTCCAGCTGG-3′`
Human IL-10 receptor 2	
Forward	`5′-GGCTGAATTTGCAGATGAGCA-3′`
Reverse	`5′-GAAGACCGAGGCCATGAGG-3′`
Human IL-22 binding protein	
Forward	`5′-AGGGTACAATTTCAGTCCCGA-3′`
Reverse	`5′-CGGCGTCATGCTCCATTCTGA-3′`
Human IL-22	
Forward	`5′-GCAGGCTTGACAAGTCCAACT-3′`
Reverse	`5′-GCCTCCTTAGCCAGCATGAA-3′`
Human IL-8	
Forward	`5′-ATGACTTCCAAGCTGGCCGTGGCT-3′`
Reverse	`5′-TCTCAGCCCTCTTCAAAAACTTCTC-3′`
Human TNF-α	
Forward	`5′-ATGAGCACTGAAAGCATG-3′`
Reverse	`5′-TCACAGGGCAATGATCCC-3′`
Human β-actin	
Forward	`5′-GCCAACCGCGAGAAGATG-3′`
Reverse	`5′-CATCACGATGCCAGTGGTA-3′`
Human SOCS-1	
Forward	`5′-CGCCAGCGCCGCTGTCGGCC-3′`
Reverse	`5′-CTGCGGCCTCGTCTCCAGCC-3′`
Human SOCS-3	
Forward	`5′-TTCTGATCCGCGACAGCTC-3′`
Reverse	`5′-TGCAGAGAGAAGCTGCCCC-3′`
Human IL-6	
Forward	`5′-AAAGAGGCACTGGCAGAAAA-3′`
Reverse	`5′-GAGGTGCCCATGCTACATTT-3′`
Human GAPDH	
Forward	`5′-CGGAGTCAACGGATTTGGTCGTAT-3′`
Reverse	`5′-AGCCTTCTCCATGGTGGTGAAGAC-3′`
Murine IL-22	
Forward	`5′-ACCTTTCCTGACCAAACTCA-3′`
Reverse	`5′-AGCTTCTTCTCGCTCAGACG-3′`
Rat IL-22	
Forward	`5′-GTTCTGCTCCCCCAGTCAG-3′`
Reverse	`5′-TCTCTCCACTCTCTCCAAGC-3′`

SOCS, suppressor of cytokine signaling.

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 1.2×10^5 cells/cm^2 for 6-well plates and 1.5×10^5 cells/cm^2 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 mU/ml insulin, 3.75 ng/ml hydrocortisone, 100 μg/ml streptomycin, and 100 U/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, prestarvation 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, prestarvation medium with cytokines as indicated was added for 24 h. Cells were incubated at 37°C in a humidified incubator with 5% CO_2. 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 [^{3}H]thymidine incorporation assay. Primary human hepatocytes were labeled by an incubation with 2 μCi of [^{3}H]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 [^{3}H]thymidine incorporated into DNA was analyzed with a β-counter (LKB/Pharmacia, Uppsala, Sweden). Triplicate cultures were assayed, and results were expressed as mean counts per minute. The stimulation index was calculated as the ratio of counts per minute obtained in the presence of IL-22 or cytokines as indicated to that obtained without cytokines.

Quantification of 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 FACScalibur (Becton Dickinson) using CellQuest software. Nuclei to the left of the "G_1 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.

In vivo model of murine cytomegalovirus infection. C57/BL6 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 CO_2 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 littermates (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 splanchnic 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. 2*A*) and Akt (Fig. 2*B*). 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. 3*A*), 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).

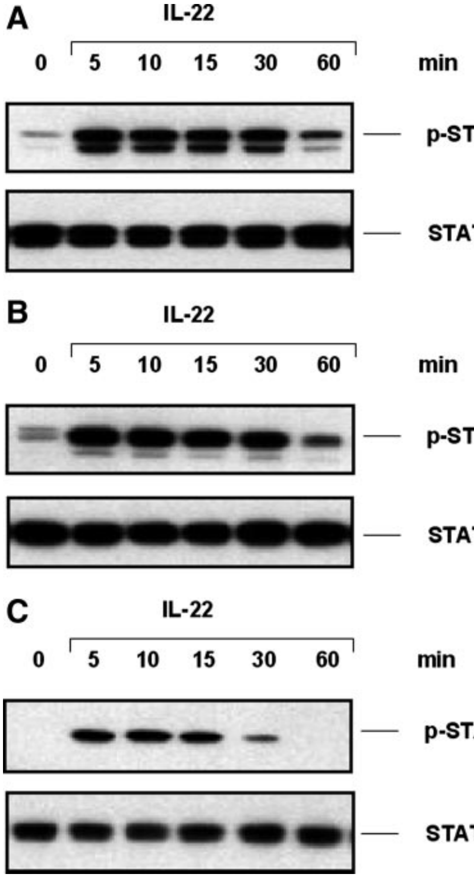

Fig. 1. IL-22 induces STAT1, STAT3, and STAT5 phosphorylation in hepatic cells. Following stimulation of HepG2 cells with IL-22 (100 ng/ml), STAT1 (*A*), STAT3 (*B*), and STAT5 proteins (*C*) were strongly phosphorylated (p-STAT1, p-STAT3, and p-STAT5). One representative experiment ($n = 3$) is shown. Similar results were obtained for Huh-7 cells (data not shown).

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

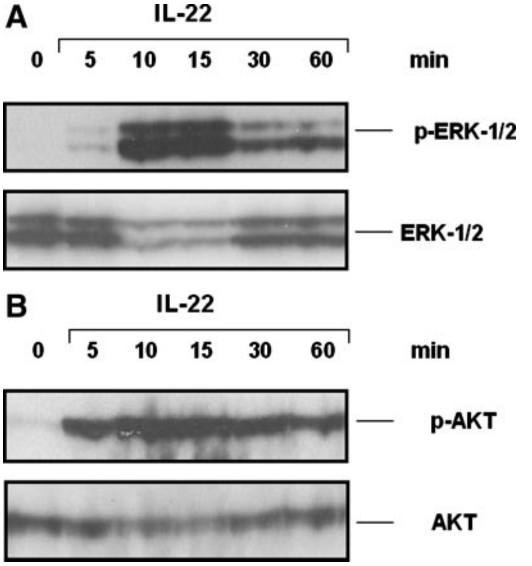


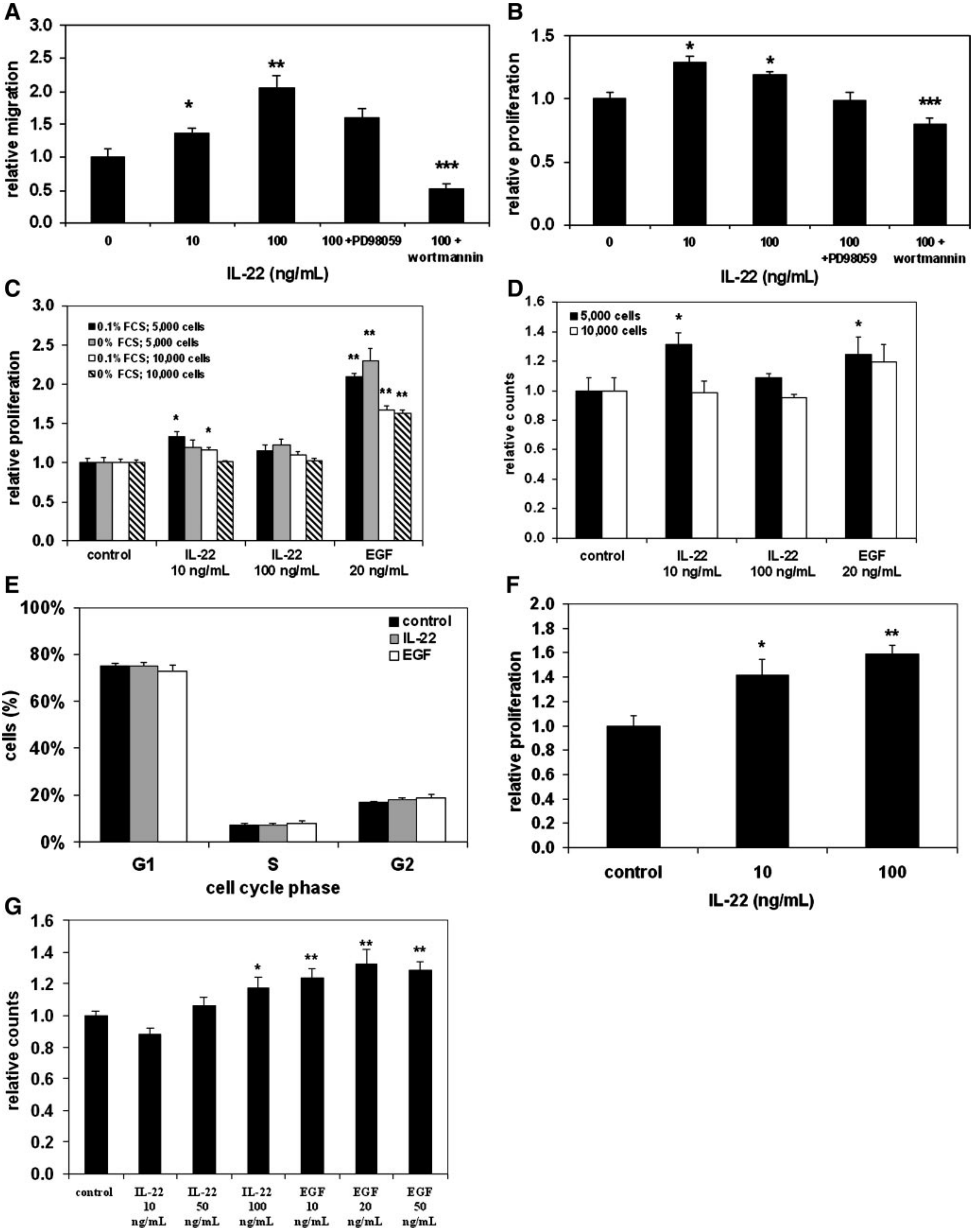
Fig. 2. IL-22 activates ERK MAPKs and Akt in hepatic cell lines. The activation and expression of p-ERK1/2 and p-Akt in HepG2 cells was assessed by immunoblot analysis. *A*: p-ERK1/2 activation after IL-22 stimulation (100 ng/ml). *B*: IL-22 induced Akt phosphorylation. One representative experiment ($n = 3$) is 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 + PD-98059 treatment; Fig. 3*A*), suggesting a primarily PI3K-dependent mechanism. Treatment with PD-98059 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. 3*B*). 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. 3*B*), 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. 3*B*). This is consistent with our results showing that wortmannin but not PD98059 inhibited IL-22-mediated cell restitution (Fig. 3*A*). The proliferative effect of IL-22 on hepatocytes was stronger at low cell density compared with higher cell density (Fig. 3*C*). Almost identical results were obtained using [^{3}H]thymidine incorporation assays (Fig. 3*D*), 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. 3*D*). 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 G_1, S, and G_2 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. 3*F*). This result was confirmed in [^{3}H]thymidine incorporation assays. IL-22 at 100 ng/ml (and EGF at all concentrations used) significantly increased cell proliferation ($P < 0.05$; Fig. 3*G*). Interestingly, in primary hepatic cells, both cell proliferation assays (MTS assay and [^{3}H]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. 4*A*, IL-22 upregulated the mRNA expression of the proinflamma-

Fig. 3. IL-22 induces hepatic cell restitution in vitro and stimulates cell proliferation in hepatic cells. *A*: wounding assays were used to analyze the influence of IL-22 on hepatic cell migration. IL-22 (10 and 100 ng/ml) induced a significant increase of the HepG2 cell migration rate. Preincubation with the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin resulted in decreased migration over the wounding edge, whereas the MEK1 inhibitor PD98059 did not significantly influence IL-22-induced cell migration (*$P < 0.05$ and **$P < 0.001$ compared with unstimulated controls; ***$P < 0.001$ compared with 100 ng/ml IL-22). *B*: cell proliferation significantly increased upon stimulation with 10 and 100 ng/ml IL-22 ($P < 0.05$). Treatment with the PI3K inhibitor wortmannin significantly inhibited the IL-22-mediated effect on cell proliferation (***$P < 0.01$ compared with 100 ng/ml IL-22), whereas PD-98059 had no significant effect. Treatment with PD98059 or wortmannin without IL-22 had no significant effect on the cell proliferation rate (data not shown). *C*: IL-22-mediated cell proliferation is influenced by cell density. Cell proliferation was measured in HepG2 cells using the MTS assay after stimulation with cytokines as indicated. HepG2 cells (5,000 or 10,000 cells) were grown with 0.1% FCS or without FCS as indicated (*$P < 0.02$ and **$P < 0.001$ vs. controls). The experiment was performed in quadruplicate. *D*: [^{3}H]thymidine incorporation assays as described in MATERIALS AND METHODS were performed after stimulation of HepG2 cells with cytokines for 48 h at cell densities as indicated (*$P < 0.05$ vs. controls). Experiments were performed in quadruplicate. *E*: primary hepatocytes have a low mitotic index, and IL-22 and EGF do not significantly influence the cell cycle distribution of primary human hepatocytes. Primary human hepatocytes were stimulated for 24 h with cytokines as indicated. Subsequently, proportions of cells in the G_0/G_1, S, and G_2/M phases were analyzed by flow cytometry. Experiments were performed in triplicate. *F*: IL-22 increases cell proliferation in primary human hepatocytes. Cell proliferation was measured by the MTS assay after stimulation with cytokine-free medium (control), 10 ng/ml IL-22 (*$P = 0.03$), or 100 ng/ml IL-22 (**$P = 0.0004$ vs. control). Six experiments were performed for each cytokine concentration. *G*: analysis of cell proliferation in primary hepatocytes from 3 different donors by [^{3}H]thymidine incorporation assays after stimulation with cytokines as indicated (*$P < 0.05$ and **$P < 0.005$ vs. controls). A total of 18 experiments was performed for each cytokine concentration.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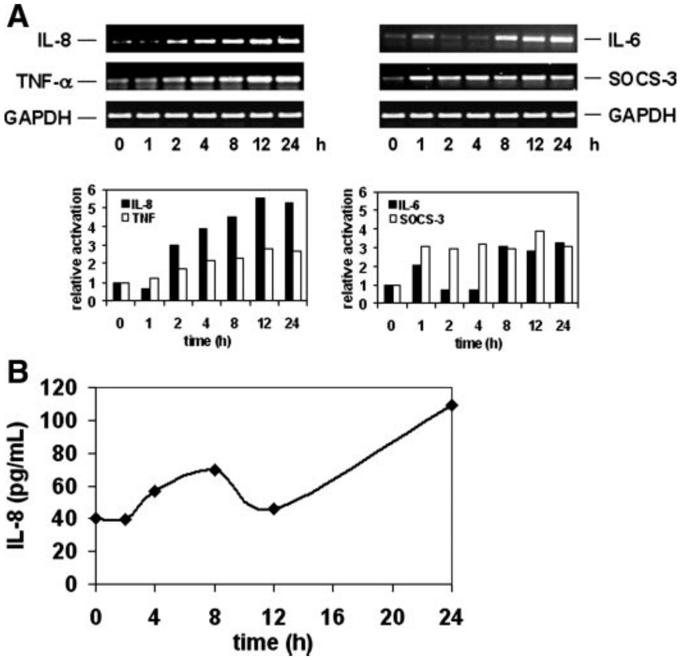

Fig. 4. IL-22 increases the expression of proinflammatory cytokines and suppressor of cytokine signaling (SOCS)-3 in hepatic cells. *A*: regulation of IL-8, TNF-α, IL-6, and SOCS-3 mRNA expression after stimulation of HepG2 cells with IL-22 (100 ng/ml). Whereas IL-8, TNF-α, and IL-6 mRNA levels were induced more slowly, SOCS-3 mRNA levels were rapidly increased already 1 h after stimulation. Densitometric analysis of respective mRNA expression after normalization for GAPDH mRNA expression is demonstrated. *B*: upon stimulation of HepG2 cells with 100 ng/ml IL-22, IL-8 protein levels increased and reached a maximum after 24 h of stimulation. IL-8 protein concentration was determined by ELISA.

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. 4*B*). 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. 4*A*), 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-λ-induced STAT1 activation. Since IFN-λs 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. 5*A*) and STAT3 (Fig. 5*B*).

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. 5*C*). This IL-22-mediated effect was strongly abolished by SOCS-1 overexpression ($P = 0.35$ compared with unstimulated clones; Fig.

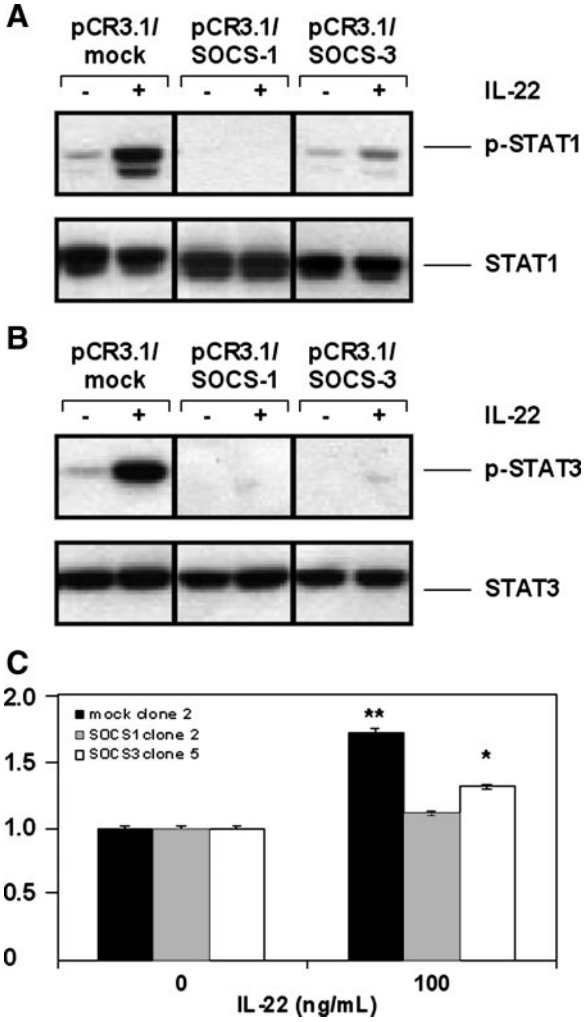

Fig. 5. IL-22-induced STAT activation and cell restitution is abrogated by SOCS-1 or SOCS-3 overexpression. Control HepG2 clones (pCR3.1/mock), SOCS-1-overexpressing clones (pCR3.1/SOCS-1), and SOCS-3-overexpressing clones (pCR3.1/SOCS-3) were incubated with 100 ng/ml IL-22 for 15 min. *A*: STAT1 phosphorylation was inhibited by SOCS-1 or SOCS-3 overexpression with SOCS-1 showing the stronger effect. *B*: SOCS-1 or SOCS-3 overexpression both abolished STAT3 phosphorylation. *C*: "wounding" assays were performed as described in Fig. 4*A* but instead of using untransfected HepG2 cells, control HepG2 clones, SOCS-1-overexpressing clones, and SOCS-3-overexpressing clones were used. IL-22 (100 ng/ml) induced a significant increase of the cell migration rate in control HepG2 clones. This effect was completely abolished by SOCS-1 overexpression, whereas SOCS-3 overexpression had a less pronounced effect on IL-22-mediated cell migration ($^{*}P < 0.05$ and $^{**}P < 0.001$ compared with unstimulated controls).

5*C*). SOCS-3 overexpression significantly decreased the migration rate of IL-22-stimulated cells to 31% above the basal migration rate compared with 72% in mock-transfected clones ($P < 0.01$; Fig. 5*C*).

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. 6*A*, 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$^+$ 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. 6*B*). 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. 6*B*). After 6 h, IL-22 mRNA expression was detectable in three rats of each group (Fig. 6*B*).

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.

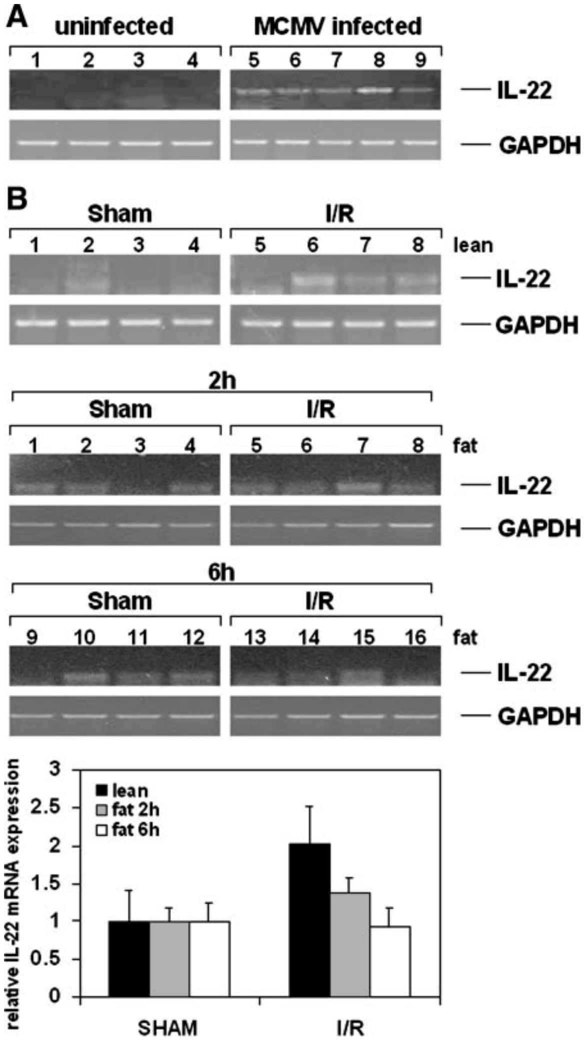

Fig. 6. IL-22 mRNA is increased in an in vivo model of T cell-mediated hepatic injury but not in ischemia-reperfusion (I/R) injury. *A*: hepatic IL-22 mRNA expression was increased in five C57/BL6 mice infected intravenously with 1×10^6 plaque-forming units of murine cytomegalovirus (MCMV) of the Smith strain (47) in PBS compared with four uninfected control mice. Control mice received an injection of PBS only. After 45 h, mice were euthanized, and RT-PCR for hepatic IL-22 mRNA expression performed. *B*: in contrast, differences in hepatic IL-22 mRNA expression were less pronounced in a rat model of hepatic I/R injury with a tendency toward higher IL-22 expression in four lean heterozygous Zucker rats treated with I/R for 2 h compared with four sham-treated controls. No significant differences in IL-22 mRNA expression were found 2 and 6 h after I/R in eight genetically obese (*fa*/*fa*) Zucker rats compared with eight sham-treated controls. Densitometric analysis of rat IL-22 mRNA expression after normalization for GAPDH mRNA expression is demonstrated.

Table 2. *IL-22 mRNA expression in different forms of human liver disease*

Patient	Diagnosis	Relative IL-22 mRNA Expression	Means ± SE		Disease Category
			Same disease	Same category	
1	Autoimmune hepatitis	0.82	0.79±0.04	0.65±0.06	Hepatitis
2	Autoimmune hepatitis	0.75			
3	Hepatitis B	0.49	0.49		
4	Hepatitis C	0.61	0.69±0.08		
5	Hepatitis C	0.76			
6	Primary biliary cirrhosis	0.07	0.19±0.12	0.19±0.06	Cholestatic liver disease
7	Primary biliary cirrhosis	0.31			
8	Primary sclerosing cholangitis	0.28	0.19±0.10		
9	Primary sclerosing cholangitis	0.09			

IL-22 mRNA isolated from liver biopsies from nine patients was analyzed by real-time quantitative PCR. IL-22 mRNA expression levels were normalized to actin expression in the respective cDNA preparation.

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-λ 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 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 infiltrate 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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REFERENCES

1. **Alexander WS.** Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2: 410–416, 2002.
2. **Bonacchi A, Romagnani P, Romanelli RG, Efsen E, Annunziato F, Lasagni L, Francalanci M, Serio M, Laffi G, Pinzani M, Gentilini P, Marra F.** Signal transduction by the chemokine receptor CXCR3: activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt controls cell migration and proliferation in human vascular pericytes. *J Biol Chem* 276: 9945–9954, 2001.
3. **Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F.** IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 174: 3695–3702, 2005.
4. **Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, Diebold J, Diepolder H, Adler B, Auernhammer CJ, Goke B, Dambacher J.** IL-28A and IL-29 mediate antiproliferative and antiviral signals in intestinal epithelial cells and murine CMV infection increases colonic IL-28A expression. *Am J Physiol Gastrointest Liver Physiol* 289: G960–G968, 2005.
5. **Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, Diepolder H, Marquardt A, Jagla W, Popp A, Leclair S, Herrmann K, Seiderer J, Ochsenkuhn T, Goke B, Auernhammer CJ, Dambacher J.** IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. *Am J Physiol Gastrointest Liver Physiol* 290: G827–G838, 2006.
6. **Brand S, Dambacher J, Beigel F, Olszak T, Diebold J, Otte JM, Goke B, Eichhorst ST.** CXCR4 and CXCL12 are inversely expressed in colorectal cancer cells and modulate cancer cell migration, invasion and MMP-9 activation. *Exp Cell Res* 310: 117–130, 2005.
7. **Brand S, Olszak T, Beigel F, Diebold J, Otte JM, Eichhorst ST, Goke B, Dambacher J.** Cell differentiation dependent expressed CCR6 mediates ERK-1/2, SAPK/JNK, and Akt signaling resulting in proliferation and migration of colorectal cancer cells. *J Cell Biochem* 97: 709–723, 2006.
8. **Brand S, Sakaguchi T, Gu X, Colgan SP, Reinecker HC.** Fractalkine-mediated signals regulate cell-survival and immune-modulatory responses in intestinal epithelial cells. *Gastroenterology* 122: 166–177, 2002.
9. **Brand S, Zitzmann K, Dambacher J, Beigel F, Olszak T, Vlotides G, Eichhorst ST, Goke B, Diepolder H, Auernhammer CJ.** SOCS-1 inhibits expression of the antiviral proteins 2′,5′-OAS and MxA induced by the novel interferon-lambdas IL-28A and IL-29. *Biochem Biophys Res Commun* 331: 543–548, 2005.
10. **Brenner DA.** Signal transduction during liver regeneration. *J Gastroenterol Hepatol* 13, *Suppl*: S93–S95, 1998.
11. **Caldwell CC, Okaya T, Martignoni A, Husted T, Schuster R, Lentsch AB.** Divergent functions of CD4+ T lymphocytes in acute liver inflammation and injury after ischemia-reperfusion. *Am J Physiol Gastrointest Liver Physiol* 289: G969–G976, 2005.
12. **Campbell JS, Prichard L, Schaper F, Schmitz J, Stephenson-Famy A, Rosenfeld ME, Argast GM, Heinrich PC, Fausto N.** Expression of suppressors of cytokine signaling during liver regeneration. *J Clin Invest* 107: 1285–1292, 2001.
13. **Ciacci C, Lind SE, Podolsky DK.** Transforming growth factor beta regulation of migration in wounded rat intestinal epithelial monolayers. *Gastroenterology* 105: 93–101, 1993.
14. **Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R.** Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 274: 1379–1383, 1996.
15. **Croker BA, Krebs DL, Zhang JG, Wormald S, Willson TA, Stanley EG, Robb L, Greenhalgh CJ, Forster I, Clausen BE, Nicola NA, Metcalf D, Hilton DJ, Roberts AW, Alexander WS.** SOCS3 negatively regulates IL-6 signaling in vivo. *Nat Immun* 4: 540–545, 2003.
16. **Dignass AU, Podolsky DK.** Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. *Gastroenterology* 105: 1323–1332, 1993.
17. **Donnelly RP, Sheikh F, Kotenko SV, Dickensheets H.** The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. *J Leukoc Biol* 76: 314–321, 2004.
18. **Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME.** Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 275: 661–665, 1997.
19. **Dumoutier L, Louahed J, Renauld JC.** Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J Immunol* 164: 1814–1819, 2000.
20. **Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D, Van Obberghen E.** SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J Biol Chem* 275: 15985–15991, 2000.
21. **Fausto N.** Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 39: 1477–1487, 2004.
22. **Fridell YW, Jin Y, Quilliam LA, Burchert A, McCloskey P, Spizz G, Varnum B, Der C, Liu ET.** Differential activation of the Ras/extracellular-signal-regulated protein kinase pathway is responsible for the biological consequences induced by the Axl receptor tyrosine kinase. *Mol Cell Biol* 16: 135–145, 1996.
23. **Greenhalgh CJ, Rico-Bautista E, Lorentzon M, Thaus AL, Morgan PO, Willson TA, Zervoudakis P, Metcalf D, Street I, Nicola NA, Nash AD, Fabri LJ, Norstedt G, Ohlsson C, Flores-Morales A, Alexander WS, Hilton DJ.** SOCS2 negatively regulates growth hormone action in vitro and in vivo. *J Clin Invest* 115: 397–406, 2005.
24. **Gurney AL.** IL-22, a Th1 cytokine that targets the pancreas and select other peripheral tissues. *Int Immunopharmacol* 4: 669–677, 2004.

25. **Haga S, Ogawa W, Inoue H, Terui K, Ogino T, Igarashi R, Takeda K, Akira S, Enosawa S, Furukawa H, Todo S, Ozaki M.** Compensatory recovery of liver mass by Akt-mediated hepatocellular hypertrophy in liver-specific STAT3-deficient mice. *J Hepatol* 43: 799–807, 2005.
26. **Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L.** Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 334: 297–314, 1998.
27. **Jenkins BJ, Grail D, Nheu T, Najdovska M, Wang B, Waring P, Inglese M, McLoughlin RM, Jones SA, Topley N, Baumann H, Judd LM, Giraud AS, Boussioutas A, Zhu HJ, Ernst M.** Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling. *Nat Med* 11: 845–852, 2005.
28. **Jo D, Liu D, Yao S, Collins RD, Hawiger J.** Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis. *Nat Med* 11: 892–898, 2005.
29. **Judd LM, Bredin K, Kalantzis A, Jenkins BJ, Ernst M, Giraud AS.** STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis. *Gastroenterology* 113: 1073–1085, 2006.
30. **Kawano K, Kim YI, Kaketani K, Kobayashi M.** The beneficial effect of cyclosporine on liver ischemia in rats. *Transplantation* 48: 759–764, 1989.
31. **Khandoga A, Hanschen M, Kessler JS, Krombach F.** CD4+ T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets. *Hepatology* 43: 306–315, 2006.
32. **Klingmüller U, Bauer A, Bohl S, Breitkopf K, Dooley S, Zellmer S, Kern C, Merfort I, Sparna T, Donauer J, Walz G, Geyer M, Kreutz C, Hermes M, Walter D, Egger L, Neubert K, Borner C, Brulport M, Schormann W, Sauer C, Baumann F, Preiss R, Illes P, Nickel PJ, MacNelly S, Godoy P, Wiercinska E, Ciuclan L, Zeilinger K, Heinrich M, Zanger UM, Reuss M, Bader A, Gebhardt R, Maiwald T, Timmer J, von Weizsacker F, Hengstler JG.** Primary mouse hepatocytes for systems biology approaches: a standardized in vitro system for modeling of signal transduction pathways. *Syst Biol* 153: 433–447, 2006.
33. **Koneru B, Reddy MC, dela Torre AN, Patel D, Ippolito T, Ferrante RJ.** Studies of hepatic warm ischemia in the obese Zucker rat. *Transplantation* 59: 942–946, 1995.
34. **Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S.** Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. *J Biol Chem* 276: 2725–2732, 2001.
35. **Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S.** Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. *J Immunol* 166: 7096–7103, 2001.
36. **Kupiec-Weglinski JW, Busuttil RW.** Ischemia and reperfusion injury in liver transplantation. *Transplant Proc* 37: 1653–1656, 2005.
37. **Lejeune D, Dumoutier L, Constantinescu S, Kruijer W, Schuringa JJ, Renauld JC.** Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. *J Biol Chem* 277: 33676–33682, 2002.
38. **Levy DE, Inghirami G.** STAT3: a multifaceted oncogene. *Proc Natl Acad Sci USA* 103: 10151–10152, 2006.
39. **McCormack SA, Viar MJ, Johnson LR.** Migration of IEC-6 cells: a model for mucosal healing. *Am J Physiol Gastrointest Liver Physiol* 263: G426–G435, 1992.
40. **Nagalakshmi ML, Rascle A, Zurawski S, Menon S, de Waal Malefyt R.** Interleukin-22 activates STAT3 and induces IL-10 by colon epithelial cells. *Int Immunopharmacol* 4: 679–691, 2004.
41. **Niwa Y, Kanda H, Shikauchi Y, Saiura A, Matsubara K, Kitagawa T, Yamamoto J, Kubo T, Yoshikawa H.** Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. *Oncogene* 24: 6406–6417, 2005.
42. **Ogata H, Kobayashi T, Chinen T, Takaki H, Sanada T, Minoda Y, Koga K, Takaesu G, Maehara Y, Iida M, Yoshimura A.** Deletion of the SOCS3 gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis. *Gastroenterology* 131: 179–193, 2006.
43. **Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H.** Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med* 12: 829–834, 2006.
44. **Pan H, Hong F, Radaeva S, Gao B.** Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. *Cell Mol Immunol* 1: 43–49, 2004.
45. **Polic B, Hengel H, Krmpotic A, Trgovcich J, Pavic I, Luccaronin P, Jonjic S, Koszinowski UH.** Hierarchical and redundant lymphocyte subset control precludes cytomegalovirus replication during latent infection. *J Exp Med* 188: 1047–1054, 1998.
46. **Radaeva S, Sun R, Pan HN, Hong F, Gao B.** Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 39: 1332–1342, 2004.
47. **Rawlinson WD, Farrell HE, Barrell BG.** Analysis of the complete DNA sequence of murine cytomegalovirus. *J Virol* 70: 8833–8849, 1996.
48. **Rui L, Yuan M, Frantz D, Shoelson S, White MF.** SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* 277: 42394–42398, 2002.
49. **Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA.** Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 278: 13740–13746, 2003.
50. **Silver DL, Naora H, Liu J, Cheng W, Montell DJ.** Activated signal transducer and activator of transcription (STAT) 3: localization in focal adhesions and function in ovarian cancer cell motility. *Cancer Res* 64: 3550–3558, 2004.
51. **Sotsios Y, Whittaker GC, Westwick J, Ward SG.** The CXC chemokine stromal cell-derived factor activates a Gi-coupled phosphoinositide 3-kinase in T lymphocytes. *J Immunol* 163: 5954–5963, 1999.
52. **Starr R, Metcalf D, Elefanty AG, Brysha M, Willson TA, Nicola NA, Hilton DJ, Alexander WS.** Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc Natl Acad Sci USA* 95: 14395–14399, 1998.
53. **Thasler WE, Weiss TS, Schillhorn K, Stoll PT, Irrgang B, Jauch KW.** Charitable state-controlled foundation human tissue and cell research: ethic and legal aspects in the supply of surgically removed human tissue for research in the academic and commercial sector in Germany. *Cell Tissue Bank* 4: 49–56, 2003.
54. **Trautwein C, Rakemann T, Niehof M, Rose-John S, Manns MP.** Acute-phase response factor, increased binding, and target gene transcription during liver regeneration. *Gastroenterology* 110: 1854–1862, 1996.
55. **Ueki K, Kondo T, Kahn CR.** Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24: 5434–5446, 2004.
56. **Vlotides G, Sorensen AS, Kopp F, Zitzmann K, Cengic N, Brand S, Zachoval R, Auernhammer CJ.** SOCS-1 and SOCS-3 inhibit IFN-alpha-induced expression of the antiviral proteins 2,5-OAS and MxA. *Biochem Biophys Res Commun* 320: 1007–1014, 2004.
57. **Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, Dietz A, Wittekind C, Tannapfel A.** SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. *Oncogene* 24: 6699–6708, 2005.
58. **Weiss TS, Jahn B, Cetto M, Jauch KW, Thasler WE.** Collagen sandwich culture affects intracellular polyamine levels of human hepatocytes. *Cell Prolif* 35: 257–267, 2002.
59. **Weiss TS, Pahernik S, Scheruebl I, Jauch KW, Thasler WE.** Cellular damage to human hepatocytes through repeated application of 5-aminolevulinic acid. *J Hepatol* 38: 476–482, 2003.
60. **Wolk K, Kunz S, Asadullah K, Sabat R.** Cutting edge: immune cells as sources and targets of the IL-10 family members? *J Immunol* 168: 5397–5402, 2002.
61. **Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R.** IL-22 increases the innate immunity of tissues. *Immunity* 21: 241–254, 2004.
62. **Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL.** Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2–4 and IL-22R. *J Biol Chem* 275: 31335–31339, 2000.
63. **Yamada Y, Kirillova I, Peschon JJ, Fausto N.** Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 94: 1441–1446, 1997.
64. **Yoshikawa H, Matsubara K, Qian GS, Jackson P, Groopman JD, Manning JE, Harris CC, Herman JG.** SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nat Genet* 28: 29–35, 2001.