G-25 chromatography as well as colorimetric immunoassays. Results: 1. In LR, maximal Itih-4 expression was observed at 30 minutes and 12 hours, predominantly centrizonal in distribution. 2. Itih-4 expression was prominent in early liver development at day 9 and reached a second peak at day 16, being restricted to hepatoblasts, immature hepatocytes and differentiated hepatocytes. 3. A marked increase in Itih-4 labeling was noted in proliferating hepatocytes, but not bile duct cells in liver explant cultures treated with IL-6 (100 units/ml). Itih-4 expression was not altered in explants cultured with TNF alpha. 4. The fluorescence emission spectrum of metal free ITIH-4 showed a maximum at 332 nm with no change on addition of 5 mM Ca. The GST-fusion protein was recovered in the void fraction with no protein binding to the column. Similarly covalent binding of Itih-4 to hyaluronic acid was not detected. Conclusions: In LR, Itih-4 expression corresponds to that of immediate early genes, such as c-myc, c-fos, c-jun, IGFBP-2 and may contribute to the entry of normally quiescent hepatocytes into the early stages of the cell cycle. The markedly high expression of Itih-4 in early liver development and in explants treated with IL-6 suggest a prominent role for Itih-4 in the tissue remodeling that occurs during regenerative and developmental aspects of liver formation.

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The Role of the RON Receptor Tyrosine Kinase in a Murine Model of Fulminant Hepatic Failure

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Virtually nothing is known about the involvement of the RON receptor tyrosine kinase in liver physiology. RON signaling responses have been best characterized in the macrophage, where RON suppresses lipopolysaccharide (LPS)-induced expression of inducible nitric oxide (NO) synthase and NO production. Several studies, in turn, have suggested that NO may participate in murine models of fulminant hepatic failure induced by LPS in galactosamine (GalN)-sensitized animals. Previous studies in our laboratory on mice with a targeted deletion of the tyrosine kinase domain of RON ($TK^{-/-}$ mice) revealed markedly enhanced responses to several murine models of inflammation involving the lung and skin. Given this, we hypothesized that an enhanced inflammatory response in the liver would be observed in $TK^{-/-}$ mice challenged in a LPS-induced, GalN-sensitized murine model of fulminant hepatic failure. Surprisingly, compared to control mice, $TK^{-/-}$ mice challenged with LPS/GalN revealed marked protection to this insult. Control mice had profound elevation of transaminases, hemorrhagic necrosis on histology of the liver and high mortality. In dramatic contrast, experimental $TK^{-/-}$ mice reproducibly had mild transaminase elevation, normal liver histology and rare lethal events, the latter due perhaps to nonhepatic LPS-induced injury. Further analysis by terminal deoxynucleotidase nick end labeling (TUNEL) assay showed a dramatic decrease in the number of TUNEL-positive cells in the liver of $TK^{-/-}$ mice, suggesting protection from the development of apoptosis in response to LPS/GalN. Initial assessment of NO metabolism by immunohistochemistry, with an anti-nitrotyrosine antibody on liver tissue, suggests increased levels of nitrotyrosine deposition in $TK^{-/-}$ mice, both at baseline and in response to LPS/GalN. Based on these studies, and the reported role of NO in the negative regulation of caspase activities involved in apoptotic responses, we hypothesize that protection to LPS/GalN in $TK^{-/-}$ mice is mediated by failure of TK-deficient RON to suppress NO production. Further characterization of $TK^{-/-}$ mice in this LPS/GalN-induced model of fulminant hepatic failure is likely to produce important insights into cellular signaling mechanisms involved with the pathogenesis of this disease process.

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Cholestatic IL-12 Deficient Mice Are Resistant to Endotoxin Induced Liver Injury and Shock

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Patients with obstructive jaundice suffer from postoperative septic complications most likely due to increased susceptibility to endotoxin. IL-12 plays a possible role during endotoxemia. To determine the role of IL-12 in inflammation induced by endotoxin during cholestasis, bile duct ligated (bdl) or sham operated IL-12p35 deficient (IL-12 −/−) and wild type (IL-12 +/+) mice were challenged with endotoxin 2 weeks after surgery. Mice were sacrificed before and 1½, 3, 6, and 24 h after endotoxin challenge (n = 8-10/group/time point). Primary outcome was survival (n = 16/group). Secondary endpoints were cytokine levels (IL-12p40, IFNγ, TNF), degree of liver damage, macrophage activation rate, and level of NO production. All values are mean ± SE, statistics by Wilcoxon and log-rank for survival. Two weeks after surgery, significant cholestasis was achieved (bdl *vs* sham mice: plasma bilirubin [μM] 245 ± 67 *vs* 2 ± 1, $p < .05$). Cholestasis and liver histology did not differ between IL-12 +/+ and IL-12 −/− mice before endotoxin challenge and resulted in hepatic expression of IL-12p40. Induction of endotoxemia lead to rises in cytokine plasma levels, which were higher in bdl than in sham mice. Moreover, in bdl mice endotoxemia caused significantly higher increases in liver macrophage recruitment (immunohistochemistry), hepatocellular injury (ALT), and liver edema (wet/dry ratio). In IL-12 −/− mice, endotoxin-induced increases in cytokines were greatly diminished compared with IL-12 +/+ mice (maximum plasma cytokine levels [ng/ml] for bdl IL-12 −/− *vs* IL-12 +/+ mice were respectively: IL-12p40 22 ± 5 *vs* 37 ± 7, IFNγ 625 ± 68 *vs* 771 ± 69, and TNF 524 ± 83 *vs* 779 ± 94, all $p < .05$). IL-12 deficiency also caused significant reductions in liver macrophage recruitment, NO production, and attenuated the parameters of liver injury, and these changes were more pronounced in bdl than in sham mice. Administration of endotoxin lead to significantly higher mortality in bdl mice compared to sham mice, 65% vs 18%, resp ($p<.001$). Although there was no important difference in mortality between sham IL-12 +/+ and IL-12 −/− mice, endotoxin injection lead to significantly more early mortality in bdl IL-12 +/+ mice than in IL-12 −/− mice ($p<.02$). These data suggest that IL-12 is required for the full induction of liver injury and shock due to endotoxemia during cholestasis. Therefore, more research is needed to gain insight in blocking the effects of IL-12 during surgical trauma since this might be useful as a perioperative strategy in jaundiced patients.

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Inhibition Of 14-3-3 Binding To Keratins Alters Mitotic Progression And Protects From Hepatotoxic Injury

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Background: Keratin polypeptides 8 and 18 (K8/18) are the major intermediate filaments that are expressed in simple-type epithelia. K18 Ser33 phosphorylation regulates keratin binding to the 14-3-3 protein family during mitosis, and interference with such binding in stably transfected cells alters keratin filament organization. The 14-3-3 protein family plays several important functions including binding and modulating cytoplasmic receptors, kinases, apoptosis regulators, and oncogene products. Aim: Study the biologic significance of keratin/14-3-3 binding *in vivo*. Methods: We introduced a mutation in the cloned human (h) K18 gene, which resulted in a K18 Ser33→Ala change. Two transgenic mouse lines were generated ($S33A^1$and $S33A^2$mice) and were compared with previously described transgenic mice that over-express WT hK18 (TG2) or another hK18 phosphorylation mutant (K18 Ser52→Ala, termed S52A mice). Mice were tested for their response to partial hepatectomy, and for susceptibility to microcystin-induced liver injury. Results: The two S33A lines expressed hK18 in the normally expected tissue-specific pattern and at similar levels to control transgenic mice, and hK18 did not bind to 14-3-3 in these mice. In S33A but not TG2 or S52A mice, acinar cell keratin filaments were disorganized, and partial hepatectomy resulted in hepatocyte keratin filament disruption and in partial mitotic arrest, in association with retention of 14-3-3 ζ and σ in the nucleus as determined by immunofluorescence staining. Upon exposure to microcystin, the S33A mutation afforded complete protection from lethality, while 60% of the TG2 mice died from massive liver hemorrhage ($p=0.001$). Conclusion: K18 S33 phosphorylation regulates keratin filament organization in simple epithelia *in vivo*, and predisposes to microcystin-induced hepatotoxic injury. K18/14-3-3 binding modulates hepatocyte mitotic progression partially, possibly by facilitating nuclear redistribution of 14-3-3 proteins during mitosis.

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***In Vivo* And *In Vitro* Effects Of Transforming Growth Factor-β1-Activation-Related Peptide On Hepatocyte Transformation And Hepatic Fibrosis**

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<BACKGROUND> Transforming growth factor-β1 (TGF-β1) is a potent cytokine for hepatic fibrosis. It is reported that inhibition of TGF-β1 prevents progression of fibrosis and enhances hepatocyte regeneration. Thrombospondin-1 (TSP-1) is a major activator of TGF-β1, and a peptide Lys-Arg-Phe-Lys (KRFK) derived from TSP-1 activates latent form of TGF-β1 and a peptide Leu-Ser-Lys-Leu (LSKL) derived from latency-associated protein inhibits the activation of TGF-β1. <AIM> To examine the effects of peptide KRFK and LSKL on hepatocyte transformation *in vitro* and on the progression of hepatic degeneration and fibrosis *in vivo* in dimethylnitrosamine (DMN)-treated rats. <MATERIALS AND METHODS> (I) *In vitro* : Four-week-old male rats were sacrified to isolate small hepatocytes and nonparenchymal cells, and these cells were co-cultured for 4 weeks. These cells were divided into three groups; i) addition of 100 nM LSKL, ii) 100 nM KRFK, and iii) no peptide. After fixation, the cells were immunocytochemically stained for CK8/18 and CK19, and then CK19-positive ratio in those three groups were evaluated. (II) *In vivo* : Four-week-old male rats weighing 70-80g (n=12) were received intraperitoneal injection of 1% DMN at a dose of 10 mg DMN/kg, three consecutive days a week for 4 weeks. The animals were treated simultaneously with one of the following regimen ; i) LSKL 100 μg i.p. daily, ii) KRFK 100 μg i.p. daily and iii) only saline i.p. daily. After 4 weeks of treatment, the degree of damage and fibrosis of the liver in each group was histologically evaluated. <RESULTS> (I) CK8/18 was positively expressed on all hepatocytes. CK19 positive ratio was 29% in LSKL group, 50% in KRFK group and 47% in control group. The percentage of CK19 positive cells in LSKL group was significantly less than in KRFK and control groups. (II) Body weight gain was significantly greater in LSKL group than in KRFK and saline groups. Survival rate after 4 weeks were 3/4 (75%) in LSKL group, 1/4 (25%) in saline group and 0/4 (0%) in KRFK group. In the histological study, hepatocytes degeneration at periportal area and portal fibrosis were more remarkable in KRFK and control groups than in LSKL group. <CONCLUSIONS> The present study has suggestetd that TGF-β1 inactivating peptide LSKL can inhibit the tranformation of hepatocytes into biliary epithelial phenotype *in vitro*, and prevent the progression of hepatic degeneration and fibrosis *in vivo*.

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Early Hepatitis C Viral (HCV) Kinetics- Predicting Sustained Virologic Clearance (SVR)

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Patients(pts) with geno 1 HCV are resistant to IFN as only 12% develop SVR despite 12 months of costly therapy that is fraught with side effects. Studies have shown that HCV RNA decline with IFN is biphasic; within 24 hr(1^{st}phase) there is a 0.5 to 2.0 log decline which reflects the effectiveness(ε) of IFN in blocking viral production(Neumann et al, Science 1998;282,103). Then, viral decline slows reflecting the death of infected liver cells(2^{nd}phase). We have shown that the rate of 2^{nd} phase slope over the 1st month of therapy is an excellent predictor of SVR(Neumann Hep:32,A788, 2000) with a positive predictive value(PPV) of 55% and a negative predictive value(NPV) of 100%. In the present study we sought to determine if 1^{st} phase viral kinetic parameters(ε and viral load at day 1(V1)) correlated with 2^{nd} phase slope and could predict SVR and Non Response(NR) in IFN treated pts within 24 hr. 38 HCV geno 1 infected patients were treated with Infergen for 1 yr. Blood was drawn every 3 hr for RNA measurement (Superquant, NGI) during the 1st day to calculate ε and V1, at wks 1,2,and