Alterations in growth hormone receptor abundance regulate growth hormone signaling in murine obstructive cholestasis


Alterations in growth hormone receptor abundance regulate growth hormone signaling in murine obstructive cholestasis. Am J Physiol Gastrointest Liver Physiol 288: G986–G993, 2005. First published December 16, 2004; doi:10.1152/ajpgi.00287.2004.—Children with cholestatic liver diseases, in particular biliary atresia, may develop an acquired growth hormone (GH) resistance. This is characterized by normal GH secretion, reduced liver GH receptor (GHR) abundance, and reduced circulating insulin-like growth factor I (IGF-I). Consequences include linear growth failure, reduced muscle mass, and increased perioperative morbidity and mortality. However, the molecular basis for altered GH signaling in liver and skeletal muscle in cholestatic liver disease is not known. We hypothesized that reduced IGF-I expression in obstructive cholestasis would be associated with downregulation of the GHR and impaired phosphorylation of signaling transducers and activators of transcription (STAT5). Body composition was determined in C57BL/6J male mice after bile duct ligation (BDL) relative to pair-fed (PF) and ad libitum-fed controls. GHR, STAT5, Sp3, and IGF-I expression and/or DNA binding were assessed using immunoblots, electrophoretic mobility shift assays, and/or real time RT-PCR. Fat-free mass was reduced in PF mice relative to ad libitum-fed controls. BDL led to a further reduction in fat mass and fat-free mass relative to PF controls. TNF-α was increased in liver and skeletal muscle of BDL mice. This was associated with reduced GH-dependent STAT5 activation and IGF-I RNA expression. GHR expression was reduced in BDL mice; in liver, this was associated with reduced Sp3 binding to a GHR gene promoter cis element. Wasting in murine obstructive cholestasis is due to combined effects of reduced caloric intake and biliary obstruction. GH resistance due to downregulation of GHR expression may be attributed primarily to the obstructive cholestasis; therapies that specifically increase GH expression may restore GH signaling in this setting.

jaundice; liver; muscle; insulin-like growth factor I; STAT5

THE CONSEQUENCES OF ALTERED growth hormone (GH) signaling in children with cholestatic liver diseases and cirrhosis may include linear growth failure, osteodystrophy, muscle wasting, altered anabolic metabolism, and increased morbidity and mortality after transplantation (20). This is particularly notable in children with biliary atresia (BA) in whom treatment with GH has failed to improve growth, confirming a functional GH resistance (4, 20). Studies have demonstrated that hepatic GH receptor (GHR) and insulin-like growth factor I (IGF-I) expression and associated circulating IGF-I levels are reduced in children with BA, which could account for this observed GH resistance (6, 22, 36). However, the molecular mechanisms that cause GH resistance in cholestatic liver disease and the relative contribution of undernutrition vs. cholestasis itself have not been defined.

Under normal conditions, the transcription factor signal transducers and activators of transcription (STAT) 5b and its target gene IGF-I are upregulated by GH in liver and extrahepatic tissues, including skeletal muscle, and together provide coordinated control of metabolic functions and linear growth (8, 38). The critical importance of STAT5b for normal postnatal growth has recently been demonstrated in a child with a missense mutation in the STAT5b gene and severe growth failure (26). Conversely, it has been shown that transgenic mice that overexpress the cytokines TNF-α and IL-6 exhibit reduced circulating levels of IGF-I and growth failure (10, 31). We recently determined that these proinflammatory cytokines regulate GH signaling through different molecular mechanisms. Specifically, TNF-α suppressed hepatic GHR gene expression via downregulation of Sp1/Sp3 transcription factor binding to adjacent cis elements in the GHR gene promoter, whereas IL-6 upregulated the cytokine-inducible SH2-containing protein (CIS) and the suppressor of cytokine signaling protein-3 (SOCS-3), thereby inhibiting GH signaling at the postreceptor level (12, 13). Although TNF-α and IL-6 are also upregulated in patients with cholestatic liver diseases and after BDL in rodents, whether these mechanisms also regulate GH signaling in obstructive cholestasis is not known.

Recent studies in skeletal muscle have begun to clarify important similarities and differences with respect to cytokine regulation of liver GH signaling. Myocytes have been shown to express IL-6 and TNF-α in response to inflammatory stimuli, including lipopolysaccharide (LPS) (28). It is this autocrine cytokine signaling that has been hypothesized to contribute to the reduced expression of IGF-I within muscle in models of acute LPS-induced inflammation or sepsis (17, 19, 28). However, in these systems, this has not been associated with impaired GH-dependent activation of STAT5, potentially implicating other molecular targets of GH action (24). In IL-6 transgenic mice and in a rodent sepsis model, reduced muscle IGF-I expression has been associated with upregulation of SOCS-3 (30). Whether these mechanisms could also account for the significant muscle catabolism in obstructive cholestasis was not known.

Bile duct-ligated (BDL) rodents develop biliary cirrhosis and growth failure, which is associated with reduced caloric...
intake and hepatic IGF-I synthesis. BDL therefore represented a useful experimental model of GH resistance due to obstructive cholestasis. In this study, we have examined molecular mechanisms of liver and skeletal muscle GH resistance in experimental obstructive cholestasis and caloric restriction. We hypothesized that downregulation of GHR expression in liver and muscle would lead to impaired GH-dependent STAT5 phosphorylation and IGF-I expression in BDL mice relative to pair-fed (PF) controls. This could then account for an additional reduction in growth and body composition specifically related to the obstructive cholestasis, in addition to the effects of undernutrition. In this study, we have confirmed that liver and muscle GH-dependent STAT5 phosphorylation is impaired in mice with obstructive cholestasis and that this is associated with downregulation of GHR expression.

MATERIALS AND METHODS

Materials. Eight-week-old male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). Human recombinant GH was obtained from Sigma (St. Louis, MO). Actin (catalog no. sc-1615) and SH-protein tyrosine phosphatase (PTP)-1 (catalog no. sc-287) polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). An STAT5-PO4 [anti-phospho-STAT5 A/B (Y694/Y699), catalog no. s4308] antibody was obtained from Sigma. The GHR AL-47 polyclonal antibody, which recognizes the cytoplasmic domain of the GHR, has previously been described (41). Primer/probe sets and reagents for real-time PCR were obtained from Applied Biosystems (Foster City, CA). The NE-PER kit for preparation of nuclear and cytosolic proteins was obtained from Pierce (Rockford, IL). TRizol for preparation of RNA was obtained from Gibco-BRL (Rockville, MD). The Renaissance chemiluminescence kit was obtained from NEN (Wilmington, DE). The DIG electrophoretic mobility shift assay (EMSA) kit was obtained from Boehringer-Mannheim (Indianapolis, IN). ELISA kits for determination of tissue TNF-α abundance were obtained from R & D Systems (Minneapolis, MN). ELISA kits for determination of serum GH levels were obtained from DS Labs (Webster, TX).

Oligonucleotides. EMSA oligonucleotides were synthesized by Invitrogen (Carlsbad, CA). The following synthetic oligonucleotide was used for the L2A EMSA: 5’TTTACCCCGGCCCTTTCCCTC3’. A double-stranded oligonucleotide for EMSA was generated by annealing this synthetic oligonucleotide with the complementary sequence.

BDL and body composition analysis. Eight-week-old male C57BL/6J mice were divided into three groups of eight mice each for BDL, PF, and ad libitum feeding (AL). Animals were maintained in the Yale Animal Research Center and housed in a temperature- and humidity-controlled environment under a constant light cycle with free access to chow and water (with the exception of chow intake in PF mice, which was matched to the reduced chow intake of BDL mice). The protocol was approved by the Yale Animal Care and Use Committee, and all animals received humane care as outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Common BDL was performed under sterile conditions as described previously in a Yale Liver Center Care Facility (29). The common bile duct was exposed, ligated twice close to the hilum of the liver, immediately below the cystic duct, and then cut between the ligatures. AL and PF animals underwent sham surgery, in which the bile duct was exposed but not ligated. The animals were exanguinated, and liver and hindlimb skeletal muscle were harvested 7 days after surgery.

Length, weight, and body composition were determined at baseline and 7 days after surgery. Body composition measurements including fat-free mass (FFM, in g), fat mass (FM, in g), and bone mineral density (in mg/cm²) were obtained utilizing Lunar Piximus dual-energy X-ray absorptiometry scanning in a Yale Musculoskeletal Disorders Core Facility (3). Chow intake was recorded daily. PF mice received the amount of chow consumed by BDL mice on the previous day.

GH administration. GH administration consisted of a single injection (2.5 µg/g body wt ip) administered 30 min before harvesting of liver and hindlimb skeletal muscle. Control mice were injected with an equal volume of sterile PBS. In each group of eight mice, four received GH and four received PBS.

Real-time PCR analysis of GHR and IGF-I gene expression. Total RNA was extracted using TRIzol according to the manufacturer’s recommendations. Concentration and purity were confirmed by spectrophotometry (model DU-640B, Beckmann Instruments, Palo Alto, CA). RNA was stored at −80°C. The following primers and probes were used for the TaqMan RT-PCR assay (synthesized by PE Biosystems): GGATCTTTGTCAAGGCTCTTCAACCTGHR (forward) and TCGAGTGATGCGGATACTCCTC (reverse); TGGCACTGACCAGCACACATTT (TGTGC) for GHRβ probe. GTCACAGCGGCTTAG (forward) and TCGGCTCCGGAGACAAGAC (reverse) for L2. CAGCCCCAAAGCGGACACGA for GHRα probe, GATGTGATGCGGATACTCCTC (forward) and GCTCCGGAGACAACACTCA (reverse) for IGF-I. AGGGCAATCTAGGAGCC (forward) and AAAATCAACACGCGC (reverse) for GAPDH. Each sample was supplement with respective forward and reverse primers and fluorescent probe and made up to 50 µl using TaqMan Master-Mix (Applied Biosystems). Each target probe was amplified in a 96-well plate. All samples were incubated at 50°C for 2 min and at 95°C for 10 min and then cycled at 95°C for 15 s and 60°C for 1 min for 40 cycles. After the standard curves were calculated, the input amounts of cDNA of the unknown samples were calculated for GH, IGF-I, and GAPDH. After the input amount of the probe sample was normalized to the GAPDH expression, the relative amount of the expressed RNA species in each unknown sample was calculated. The PCR products for IGF-I and GAPDH were also analyzed by agarose gel electrophoresis to ensure amplification of a single product of the expected size.

Preparation of nuclear and cytoplasmic proteins, EMSA, and immunoblot analysis. Nuclear and cytoplasmic proteins were prepared utilizing the NE-PER kit according to the manufacturer’s recommendations (Pierce). Oligonucleotide probes were end-labeled, and EMSA was performed utilizing the DIG EMSA kit according to the manufacturer’s recommendations (Boehringer-Mannheim). In supershift assays, 2 µg of polyclonal antibody were added to the gel-shift reaction for 1 h after preincubation of the probe and nuclear proteins for 20 min on ice. Nuclear (20 µg) or cytosolic (40–50 µg) proteins were also loaded onto 7.5% or 12% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA) and subjected to electrophoresis and electrophoresis and electrophoresis on nitrocellulose membranes. Uniformity of protein loading and transfer was assessed by Ponceau staining and by reprobing immunoblots for SH-PTP1 (nuclear proteins) or actin (cytosolic proteins). Nitrocellulose membranes were blocked overnight at 4°C in 20 mM...
Tris·HCl, 150 mM NaCl, 5% nonfat dry milk, and 0.1% Tween 20 (TBST complete). Blots were incubated at room temperature for 1 h with STAT5-Po4, GHR AL-47, actin, or SH-PTP1 (1:1,000) antibodies in TBST complete. Blots were washed and then incubated with anti-rabbit horseradish conjugate antibody (Santa Cruz Biotechnologies; 1:2,000–1:5,000) for 1 h at room temperature. Immune complexes were detected using the NEN Chemiluminescence Plus Western blotting kit and Kodak Biomax light film. Band signal intensity was quantified utilizing NIH Image Analysis software.

ELISA. Liver and muscle tissue TNF-α abundance was determined using protein extracts and an ELISA kit as directed by the manufacturer (R & D Systems). Serum GH levels were determined using mouse serum and an ELISA kit according to the manufacturer’s recommendations (DS Labs).

Statistical analysis. Values are mean±SD of experiments with at least three independent treatments per group. Differences among experimental groups were analyzed by ANOVA or Student’s t-test. Where there were no differences among the groups by ANOVA (P > 0.05), they were subjected to post hoc comparisons. P < 0.05 was considered to be significant.

RESULTS

Relative effects of caloric restriction and obstructive cholestasis on body composition. Children with chronic cholestatic liver diseases typically experience anorexia, weight loss, and muscle wasting; this has been associated with a reduction in hepatic synthesis of IGF-I (15, 16). However, the relative contributions of reduced caloric intake and obstructive cholestasis to alterations in body composition and IGF-I expression in this setting were not known. To test the effect of experimental obstructive cholestasis in relation to caloric restriction, growth and body composition were examined in AL, PF, and BDL mice over a 7-day period. Weight and body composition remained stable over the study period in AL mice (Table 1), which consumed chow at 171(SD31) mg·g⁻¹·day⁻¹. By comparison, a reduction in caloric intake to 84(SD34) mg·g⁻¹·day⁻¹ in PF mice led to a reduction in overall weight, from 23(SD1.7) to 20.7(SD1.5) g, and in FFM, from 20(SD1.7) to 17.1(SD1.7) g. FM was preserved under these conditions. Wasting was more severe in BDL mice [caloric intake 85(SD27) mg·g⁻¹·day⁻¹], with a decrease in overall weight from 23(SD1.7) to 17.4(SD1.2) g. This included reductions in FFM [from 20(SD0.8) to 15.4(SD1.2) g] and FM [from 2.6(SD0.5) to 1.6(SD0.1) g]. Length increased from 8.9(SD0.2) to 9.2(SD0.1) cm in AL mice and did not change in PF or BDL mice. Length was significantly reduced in BDL mice relative to AL-fed mice at the end of the study period. These data demonstrated that caloric restriction alone would reduce overall body weight, primarily via a reduction in FFM, to 90(SD7)% of starting weight, by day 7. Consistent with growth failure during cholestasis, the BDL group showed a more significant weight decrease, to 76(SD5)% of starting weight. This consisted of reductions in FFM and FM, as well as a significant reduction in linear growth velocity relative to AL controls. This suggested that factors (e.g., inflammatory cytokines) that are specifically upregulated in obstructive cholestasis contribute to GH resistance and growth failure in this setting. We therefore then compared GH signaling and IGF-I expression between the PF and BDL groups to identify the molecular basis for the additional growth failure associated with obstructive cholestasis.

Table 1. Growth failure in obstructive cholestasis

<table>
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<tr>
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<th>Weight, g</th>
<th>FFM, g</th>
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<td>Day 0</td>
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<td>Day 0</td>
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<tr>
<td>AL [CI-171(SD31)]</td>
<td>23.8 (SD0.3)</td>
<td>24 (SD0.9)</td>
<td>20.9 (SD0.2)</td>
<td>20.8 (SD1)</td>
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<tr>
<td>PF [CI-84(SD34)]*</td>
<td>23 (SD1.7)</td>
<td>20.7 (SD1.5)*</td>
<td>20.0 (SD1.7)</td>
<td>17.1 (SD1.7)*</td>
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<tr>
<td>BDL [CI-85(SD27)]*</td>
<td>23 (SD1)</td>
<td>17.4 (SD1.2)*</td>
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Values are means (SD); n = 4. Weight, length, chow intake (CI), and body composition of ad libitum-fed (AL), pair-fed (PF), and bile duct-ligated (BDL) mice were determined at baseline (day 0) and 7 days after BDL. FFM, fat-free mass; FM, fat mass. Chow intake over the 7-day time course is expressed as mg·g⁻¹·day⁻¹. *P < 0.05 vs AL; †P < 0.05 vs PF at day 7.
skeletal muscle tissue were harvested after 30 min. Immunoblotts were performed to determine tyrosine-phosphorylated STAT5 (pSTAT5) abundance in nuclear protein isolates from liver and skeletal muscle tissue and then reprobed for SH-PTP1 to control for sample loading. GH administration upregulated liver pSTAT5 nuclear abundance by approximately sixfold in PF mice (Fig. 2A). By comparison, pSTAT5 was not activated by GH in BDL liver. Similarly, GH administration significantly increased pSTAT5 nuclear abundance in skeletal muscle of PF, but not BDL, mice (Fig. 2B). Impaired GH-dependent STAT5 activation could occur at the receptor or postreceptor level. Inasmuch as liver GHR abundance has been shown to be reduced in patients with cholestatic liver diseases, we next determined hepatic GHR RNA expression and protein abundance in PF and BDL mice (22).

Liver GHR expression is reduced in BDL mice. Immunoblot analysis was performed using liver protein extracts from PF and BDL mice and the AL-47 GHR polyclonal antibody. GHR abundance was significantly reduced in BDL mice relative to PF controls (Fig. 3A). The L2 transcript is the predominant murine GHR transcript in liver and extrahepatic tissues, and we previously characterized suppression of L2 expression by TNF-α via downregulation of DNA binding of the Sp3 transcription factor to adjacent cis elements in the L2 promoter (13). To determine whether GHR downregulation after BDL was also at the level of RNA expression, we used real-time PCR to measure total L2 GHR transcripts. Total and L2 GHR RNA transcripts were significantly reduced in BDL mice relative to PF controls (Fig. 3B). The molecular basis for impaired STAT5 phosphorylation in skeletal muscle was not known but could also involve receptor or postreceptor mechanisms. BDL significantly reduced GHR protein abundance relative to PF controls (Fig. 5A). A corresponding reduction in GHR RNA expression occurred under these conditions (Fig. 5B). These data indicated that downregulation of Sp3 binding to the L2 promoter, we examined this potential mechanism in BDL mice (13).

Hepatic Sp3 DNA binding is reduced after BDL. Our previous studies determined that the L2A Sp3 cis element regulates basal and TNF-α-dependent L2 GHR transcript promoter activity in liver (13). In adult mouse liver, Sp3 is much more abundant than Sp1 in nuclear protein isolates and is the predominant positive-acting regulator of L2 gene expression (32). EMSA and immunoblots were thus performed to characterize potential BDL-induced alterations in Sp3 nuclear protein abundance and DNA binding. Sp3 nuclear abundance was modestly reduced after BDL (Fig. 4A), although this difference was not significant. Sp1 protein abundance did not change under these conditions (data not shown). However, as in TNF-α-induced liver inflammation, we found that Sp3 binding to the L2A cis element was significantly reduced after BDL, by ∼50% relative to PF controls (Fig. 4B). Competition with an excess of specific (L2A) and nonspecific (STAT1) unlabeled oligonucleotides and supershift with Sp1, Sp3, and STAT1 antibodies confirmed that the predominant protein comprising the L2A complex in liver nuclear extracts was Sp3 (Fig. 4C). These data confirmed that reduced Sp3 binding to the L2A cis element was associated with downregulation of the GHR gene L2 transcript after BDL.

Skeletal muscle GHR expression is reduced after BDL. The molecular basis for impaired STAT5 phosphorylation in skeletal muscle was not known but could also involve receptor or postreceptor mechanisms. BDL significantly reduced GHR protein abundance relative to PF controls (Fig. 5A). A corresponding reduction in GHR RNA expression occurred under these conditions (Fig. 5B). These data indicated that downregulation of Sp3 binding to the L2 promoter, we examined this potential mechanism in BDL mice (13).

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lation of the GHR also contributed to acquired GH resistance in skeletal muscle in obstructive cholestasis.

DISCUSSION

The majority (~80%) of serum IGF-I is produced in the liver in response to GH activation of the STAT5b transcription factor. Liver-derived IGF-I then circulates throughout the body to regulate GH-dependent anabolic metabolism (9, 40). Local GH-dependent and -independent synthesis of IGF-I in key target tissues, including skeletal muscle, likely also plays a key role in terms of regulating protein synthesis in an autocrine/paracrine manner (18). Resistance to GH upregulation of liver IGF-I is seen in children and adults with obstructive cholestasis and cirrhosis and has been associated with decreased hepatic GHR and IGF-I expression (4, 5, 15, 21, 27, 37). Physiological consequences of this phenomenon can include osteodystrophy, linear growth failure, and muscle catabolism (4, 20). Numerous other metabolic abnormalities in chronic cholestatic liver disease and in the BDL model may also contribute to growth failure and altered hepatic gene expression. These include fat malabsorption with associated fatty acid and fat-soluble vitamin deficiency, as well as deficiency of zinc and accumulation of copper and manganese (1, 7, 14, 16, 39). Future studies that include restoration of GH signaling in BDL mice are required to determine the relative contribution of GH resistance and fat malabsorption to growth failure in this setting.

Our laboratory and several others have begun to investigate the manner by which inflammatory cytokines, including TNF-α and IL-6, which are upregulated in cholestasis, may acutely inhibit GH-dependent STAT5 phosphorylation and IGF-I expression (12, 13, 19, 35). Receptor and postreceptor mechanisms have been characterized in LPS-induced liver inflammation (12, 13). However, the molecular basis for GH resistance in liver and skeletal muscle specifically due to obstructive cholestasis, in addition to effects of undernutrition,

Fig. 2. Growth hormone (GH)-dependent STAT5 activation is impaired in obstructive cholestasis. Immunoblots were performed using nuclear protein from PF and BDL liver (A) and skeletal muscle (B) and polyclonal antibodies for tyrosine-phosphorylated STAT5a/b (pSTAT5) and SH-PTP1. Representative blots for GH-treated and control mice are shown. Signal intensity was determined by densitometry and is expressed as mean(SD) for each group (n = 4). ^P < 0.05 vs. GH treated PF group. OD, optical density; nsp, nonspecific band.

Fig. 3. Liver GH receptor (GHR) abundance and RNA expression are reduced in obstructive cholestasis. A: immunoblots for GHR were performed using liver protein extracts from PF and BDL mice and a polyclonal GHR antibody. Signal intensity was determined by densitometry and is shown as mean(SD); n = 4. B: real-time PCR for GHR and L2 RNA transcripts was performed using total liver RNA from PF and BDL mice. Values are means(SD); n = 4–5. ^P < 0.05 vs. PF group.
was not known. In this study, we have identified a mechanism of tissue GH resistance in obstructive cholestasis due to reduced GHR expression that results in downregulation of local GH-dependent STAT5 activation and IGF-I expression in liver and skeletal muscle.

We previously determined that, in acute, LPS-induced liver inflammation, TNF-α downregulates murine GHR RNA expression via inhibition of Sp3 binding to adjacent gene promoter cis elements, L2A and L2B (13). In this manner, intrahepatic accumulation of proinflammatory cytokines, including TNF-α, may inhibit GH-dependent STAT5 phosphorylation and nuclear translocation, leading to reduced IGF-I expression and GH resistance. However, it was not known whether this mechanism would also occur in the liver in obstructive cholestasis or whether it would extend to affected extrahepatic tissues, including skeletal muscle.

Data demonstrated increased abundance of TNF-α in liver and muscle of BDL mice relative to PF controls. This was accompanied by a significant downregulation of the GHR and associated GH-dependent pSTAT5 nuclear abundance and IGF-I expression in liver and skeletal muscle. Reduced GHR protein abundance was associated with downregulation of GHR gene expression in liver and skeletal muscle and DNA binding of the Sp3 transactivator in liver. This mechanism likely accounts for the GH resistance that was observed in these tissues; however, future studies are required to determine the basis for the reduced Sp3 DNA binding. By comparison, STAT5 activation was relatively preserved in undernutrition (as demonstrated in the PF group), whereas IGF-I RNA expression was only modestly reduced. Physiological effects observed specific to cholestasis included more significant reductions in FFM and FM in BDL mice than in the PF group. Thus we have established that cholestasis leads to alterations in GH signaling in liver and skeletal muscle and associated growth and body composition, which can be specifically related to the liver disease itself, in addition to the well-characterized suppressive effects of reduced caloric intake on these parameters.

In most respects, alterations in GH signaling in vivo were quite similar between liver and skeletal muscle. This indicated that a common factor might mediate GH resistance in both
tissues. Recently, increasing serum TNF-α has been shown to correlate with increasing GH resistance as patients progress from chronic hepatitis to cirrhosis (34). We found that TNF-α was upregulated in liver and skeletal muscle of BDL mice relative to PF controls. Recently, we determined that TNF-α pretreatment of H4IIE rat hepatoma cells reduces the changes in GHR and pSTAT5 abundance that were observed in liver of BDL mice relative to PF controls; this makes it likely that TNF-α contributes to hepatic GH resistance in obstructive cholestasis (11). By comparison, TNF-α pretreatment did not reduce GHR abundance or prevent GH-dependent STAT5 activation in C2C12 myocytes (data not shown). This is in agreement with a recent study by Frost et al. (19), in which TNF-α prevented GH upregulation of IGF-I, but did not alter GH activation of STAT5, in C2C12 mouse muscle cells. It is likely that multiple cytokines and/or bile acids accumulate in liver and skeletal muscle in obstructive cholestasis, and any number of these could be involved in the observed changes in GHR and pSTAT5 abundance. Alternately, C2C12 cells may not contain the signaling pathways that modulate TNF-α regulation of STAT5 activation in vivo. Future studies utilizing TNF-α blockade are required to delineate the specific, nonredundant effects of this cytokine on GH/IGF-I signaling in liver and skeletal muscle in obstructive cholestasis.

The specific basis for the impaired GHR expression and GH-dependent STAT5 activation in liver and muscle is of importance with respect to novel therapies for chronic cholestatic liver diseases, including BA. Studies have shown that children with end-stage liver disease who receive orthotopic liver transplantation (OLT) may then demonstrate an appreciable effect (23). Future studies will determine whether specific cytokine blockade will restore liver and skeletal muscle GH signaling and associated growth and body composition in experimental cholestasis. If so, this may then identify a novel strategy for ameliorating growth failure and wasting in cholestatic liver diseases.

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