Resistance of SHP-null Mice to Bile Acid-induced Liver Damage*  

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The orphan nuclear hormone receptor SHP (gene designation NROB2) is an important component of a negative regulatory cascade by which high levels of bile acids repress bile acid biosynthesis. Short term studies in SHP null animals confirm this function and also reveal the existence of additional pathways for bile acid negative feedback regulation. We have used long term dietary treatments to test the role of SHP in response to chronic elevation of bile acids, cholesterol, or both. In contrast to the increased sensitivity predicted from the loss of negative feedback regulation, the SHP null mice were relatively resistant to the hepatotoxicity associated with a diet containing 0.5% cholic acid and the much more severe effects of a diet containing both 0.5% cholic acid and 2% cholesterol. This was associated with decreased hepatic accumulation of cholesterol and triglycerides in the SHP null mice. There were also alterations in the expression of a number of genes involved in cholesterol and bile acid homeostasis, notably cholesterol 12α-hydroxylase (CYP8B1), which was strongly re-expressed in the SHP null mice, but not the wild type mice fed either bile acid containing diet. This contrasts with the strong repression of CYP8B1 observed with short term bile acid feeding, as well as the effects of long term feeding on other bile acid biosynthetic enzymes such as cholesterol 7α-hydroxylase (CYP7A1). CYP8B1 expression could contribute to the decreased toxicity of the chronic bile acid treatment by increasing the hydrophilicity of the bile acid pool. These results identify an unexpected role for SHP in hepatotoxicity and suggest new approaches to modulating effects of chronically elevated bile acids in cholestasis.

In intracellular and extracellular cholesterol levels are tightly controlled by complex transcriptional control mechanisms based to a large degree on several members of the nuclear receptor family (1–3). The primary route of cholesterol elimination from the body is via bile, based on both direct canalicular excretion of biliary cholesterol as well as conversion of hepatic cholesterol to bile acids (4). Both bile acids and cholesterol are recycled to the liver via the enterohepatic circulation, and the efficiency of this process is a crucial component of bile acid and cholesterol homeostasis. Cholesterol conversion to bile acids occurs via two different pathways: the classic and the alternative pathways, the end products of which are cholic acid and chenodeoxycholic acid. The classic pathway begins with the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1)1 (5, 6), whereas the alternative pathway begins with sterol 27-hydroxylase (CYP27), followed by oxysterol 7α-hydroxylase (CYP7B1) (7, 8). Sterol 12α-hydroxylase (CYP8B1) catalyzes the hydroxylation of the 12α-position of 7α-hydroxy-4-cholesten-3-one in its conversion to cholic acid and is a key determinant of the ratio of cholic acid (hydrophilic) to chenodeoxycholic acid (hydrophobic), and thus the hydrophobicity of the circulating bile acid pool (9). Alterations in the ratio of cholic to deoxycholic acid have been postulated to play roles in cholesterol gallstone formation (10) and the absorption of dietary lipids in the intestine (11).

The flux of bile acids is tightly controlled by nuclear receptors. When hepatic cholesterol levels are high, oxysterols accumulate and activate the LXRb, which stimulate the transcription of CYP7A1 (12, 13). This results in increased bile acid synthesis and the subsequent excretion of cholesterol. When bile acid levels are high, bile acid synthesis is inhibited through a regulatory cascade based on FXR, SHP, and LRH-1 (14, 15). In this pathway the bile acid receptor FXR induces expression of SHP, which negatively regulates the transcriptional activity of other nuclear receptors through a two-step mechanism (16–18). Because SHP is a particularly potent inhibitor of LRH-1 function and LRH-1 is essential for CYP7A1 expression, this induction results in decreased CYP7A1 expression. This model has been confirmed in vivo using both FXR null mice (19) and SHP null mice (20, 21). However, redundant pathways for the control of bile acid homeostasis have also been identified (20, 22–24).

In addition to this regulation of synthesis, nuclear receptors also regulate the flux of bile acid recirculation (25–27). A number of BA transport proteins are involved in this process. These include the Na+/H+-dependent Na+/taurocholate cotransport protein, called NTCP, and the Na+/H+-independent organic anion-transporting polypeptides Oatp1, Oatp2, and Oatp4, all of which import BA from the basolateral surface of hepatocytes. BAs are transported to the canalicular membrane by the intracellular BA-binding protein L-FABP (liver fatty acid-binding protein), 3α-HSD (3α-hydroxysteroid dehydrogenase), and GST (glutathione S-transferase) isoforms. They are excreted from the hepatocyte by the bile salt export pump BSEP and the multidrug-resistance-associated protein Mrp2. In a cholehepatic shunt in bile ductules, a minor portion of BA are reabsorbed via the apical sodium-dependent BA transporter ASBT

1 The abbreviations used are: CYP7A1, cholesterol 7α-hydroxylase; CYP27, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; BA, bile acid; L-FABP, liver fatty acid-binding protein; 3α-HSD, 3α-hydroxysteroid dehydrogenase; GST, glutathione S-transferase; BSEP, bile salt export pump; ASBT, apical sodium-dependent bile acid transporter; I-BAT, sodium-dependent BA transporter ASBT; CH, cholesterol diet; CA, colic acid diet; CON, control diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; mEH, microsomal epoxide hydrolase; AKR, c-Jun NH2-terminal kinase; IL, interleukin; FXR, farnesoid X receptor; PXR, pregnane X; SHP, small heterodimer partner; LRH-1, liver receptor homolog.

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(I-BAT), transported back into the periduodenal capillary plexus by Mrp3, and returned to the hepatocytes. BAs are released from the gallbladder into the intestine where they are reabsorbed by enterocytes, primarily in the ileum. This process depends on ASBT for import, the cysticolic intestinal BA-binding protein I-BABP for intracellular transport, and basolateral Mrp3 for export into the portal circulation. Defects in the expression or transcriptional and post-transcriptional regulation of components of this enterohepatic BA transporter pathway have been recognized as important causes for various cholestatic liver diseases (27).

Elevated concentrations of bile acids within the liver can cause severe liver injury and even liver failure (28). Bile acid toxicity is generally thought to be correlated with relative hydrophobicity, with more hydrophobic bile acids being more cytotoxic, although specific effects of distinct bile acids may depend on additional factors (28). The cytotoxicity of hydrophobic bile acids to hepatocytes has been attributed to membrane-disruptive effects based on their detergent properties (29). Primary bile acids are conjugated to either taurine or glycine before secretion into bile, and taurine-conjugated bile acids are relatively less toxic (30).

SHP plays a crucial role in the acute negative feedback regulation of BA biosynthesis (20, 21). To characterize its role in a more chronic context, we tested the long term effect of diets rich in cholesterol and CA (chow supplemented with 2% cholesterol and/or 0.5% cholic acid) on wild-type and SHP−/− mice. Wild type mice developed severe cholestasis on diets supplemented with CA, whereas CA diet exhibited wasting and decreased adipose weight, although the food intake was similar in wild type and SHP null mice (20, 21). To further characterize the function of SHP in reabsorption of CA into bile, 100 mg of liver was homogenized in chloroform:methanol (2:1), and the resulting homogenate was centrifuged. The extract lipid from liver, 100 mg of liver was homogenized in chloroform:methanol (2:1), and the resulting homogenate was centrifuged. The bile acid (BA) pool size was determined as described previously (32).

RESULTS

Diminished Hepatomegaly in SHP-deficient Mice—Recent results demonstrate that SHP has a crucial role in the acute negative feedback regulation of bile acid biosynthesis in vivo (20, 21). To further characterize the function of SHP in response to chronically elevated BA and cholesterol, both wild type and SHP mutant mice were fed four different diets: control chow (CON) and chow supplemented with 2% cholesterol (CH), 2% cholesterol plus 0.5% cholic acid (CH+CA), or 0.5% cholic acid (CA) for 12 weeks. Wild type and SHP null mice exhibited normal weight gain and growth on the control diet, while the mutant mice showed a modestly diminished growth rate on the cholesterol diet. At the early stages of CH+CA or CA feeding, the mutant mice exhibited a markedly slowed increase in body weight, although the food intake was similar in wild type and SHP null mice (data not shown). At later stages, wild type mice fed the CH+CA diet exhibited wasting and decreased adipose tissue, but SHP null mice showed normal food consumption and adipose tissue weight (data not shown).
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Diminished Hepatotoxicity and Lipid Accumulation in SHP-deficient Mice—In agreement with the gross morphological effects and alterations in liver enzymes, histological examination revealed obvious deleterious effects of long term CH + CA feeding in the wild type animals. In hematoxylin-eosin-stained liver sections, no obvious differences were observed between wild type and SHP null mice on the control diet (Fig. 2, A and B). There was also little difference between the cholesterol-fed wild type and SHP null livers, although the wild type showed an apparent increase in lipid deposition (Fig. 2C) that was confirmed by further studies (see below). On the CH + CA diet, the wild type livers showed inflammatory non-suppurative cholangitis with portal inflammation and ductular proliferation (Fig. 2E). Inflammatory non-suppurative destruction of bile ducts is a feature of various cholestatic diseases in humans. This pathology is caused by defective bile secretion, resulting in cholestasis with accumulation of bile salts and other toxic bile constituents within hepatocytes. This parallels the increase in serum ALT and ALP levels. In striking contrast, the livers of SHP null mice were only mildly affected on the same diet, with intact bile ducts and no hepatocyte damage, inflammation, or disruption of overall architecture, although increased lipid storage was clearly seen (Fig. 2F). There was also evidence of hepatocellular degeneration in livers of wild type mice on the CA diet, which developed centrilobular hyperplasia with degeneration of individual hepatocytes along with some increases in cell size and intercellular sinusoidal spaces (Fig. 2G). These changes were much less evident in SHP null mice (Fig. 2H). Thus, histological analysis confirms the decreased toxicity of CA-containing diets in the SHP null mice.

Oil Red O staining revealed a consistent decrease in neutral lipid content of the SHP null livers on all diets, including the control chow. As expected, staining was increased in both wild type and SHP null livers on the CH and CH + CA diets, and, as also expected from the gross morphology, neutral lipid accumulation was particularly dramatic in the wild type CH + CA-fed livers (Fig. 2, a–h).

Alteration of Lipid Homeostasis in SHP-deficient Mice—The hepatic cholesterol, triglyceride, and free fatty acid levels of wild type mice were all higher than those of SHP null mice maintained on a control diet (Fig. 3, A–C), consistent with the decreased neutral lipid staining (Fig. 2b). These differences were not observed in the livers of young SHP null mice (8–12 weeks) (20), but were obvious in these and other studies with mice at least 20 weeks old. This indicates an age-dependent progressive change in lipid metabolism in the SHP null mice. Cholesterol feeding caused a clear increase in hepatic cholesterol in both wild type (2.5-fold) and SHP null mice (1.5-fold) (Fig. 3A). This was accompanied by an increase in triglycerides (3-fold and 2-fold), but hepatic free fatty acid contents were decreased for both wild type and SHP null mice on the CH diet (Fig. 3, B and C).

In agreement with the enlarged liver mass and distinct color change, hepatic cholesterol was dramatically increased in the CH + CA-fed wild type mice (13.2-fold relative to control diet). Consistent with the Oil Red O staining, SHP null mice showed a lesser total accumulation of hepatic cholesterol than the wild type mice on the CH + CA diet but still exhibited a marked increase (10-fold) (Fig. 3A). No major changes were observed for hepatic triglycerides or free fatty acids in the wild type mice fed the CH + CA diet, but triglycerides were modestly increased in the SHP null mice and free fatty acids were increased by ~3-fold (Fig. 3B).

The CA diet increased hepatic cholesterol levels in a similar extent in both the wild type and SHP null mice, but did not change the hepatic triglycerides levels. Hepatic free fatty acid levels were decreased in both genotypes by this diet.

Interestingly, although there were dramatic and differential alterations in hepatic lipid contents in both wild type and SHP null mice upon different diets, serum lipid levels were relatively unchanged, except for the markedly elevated levels of cholesterol, triglycerides, and free fatty acid levels in wild type mice on CH + CA diet (Fig. 3, D–F). With the previous results, this indicates a significant defect in the capacity of the wild type CH + CA-fed liver, but not the similarly fed SHP null liver, to store and appropriately regulate lipid levels.

Alteration of Bile Acid Homeostasis in SHP-deficient Mice—A number of parameters were assessed to explore potential defects in bile acid homeostasis in the wild type and SHP null animals. The BA pool was approximately twice as large in SHP null mice versus wild type mice on regular chow diet (Fig. 4A), confirming the negative regulatory role of SHP in bile acid biosynthesis. This increase is somewhat greater than

With respect to liver mass, the wild type mice did not differ significantly from SHP null mice on the CON, CH, or CA diets. However, there was a dramatic color change and size enlargement in livers of wild type mice on the CH + CA diet that was not observed in the SHP null mice (Fig. 1, A and B). In addition, the serum levels of the liver enzymes alanine aminotransferase (ALT) (Fig. 1C) and aspartate aminotransferase (AST) (Fig. 1D) were strongly elevated in the wild type, but not the SHP null mice, on either of the CA-containing diets. Thus, the loss of SHP is associated with an unexpected decrease in hepatotoxicity of the CA-containing diets.

**Fig. 1. Cholesterol and cholic acid caused liver damage is diminished in SHP-null mice.** Wild type and SHP null mice were fed control diet (CON), or diet supplemented with 2% cholesterol (CH), or 2% cholesterol plus 0.5% cholic acid (CH + CA), or 0.5% cholic acid (CA) for 12 weeks. A, gross morphology of livers from wild type (+/+) and SHP-deficient (−/−) mice fed chow supplemented with 2% cholesterol plus 0.5% cholic acid for 12 weeks. B, liver mass relative to total body mass of wild type (+/+) and SHP-deficient (−/−) mice fed cholesterol- and CA-supplemented diets. C and D, serum ALT (C) and AST (D) levels from mice fed cholesterol- and CA-supplemented diets.
that observed with younger SHP null mice (20), again suggesting that differences between the wild type and SHP null animals increase with age. No major changes in BA pool size were observed in SHP null mice on any of the diets. In contrast, the BA pool of the wild type mice was increased by 1.4-fold by the CH diet, 3.1-fold by CH/CA, and 2.8-fold for CA. By comparison with the control diet, fecal BA excretion was increased in both wild type and SHP null mice on all three diets. Particularly for the wild type mice, this increase was generally consistent with the increased BA pool size. Urinary and serum BA levels were strongly elevated in the wild type mice on the CH/CA diet (Fig. 4, C and D).

Alteration of Bile Acid Biosynthesis and Transport in SHP-deficient Mice—To explore the mechanism of the resistance of the SHP null mice to CA-induced liver damage, the expression levels of numerous relevant genes were analyzed (Fig. 5). SHP was absent in SHP null mice, as expected, but in contrast to expectations SHP mRNA levels were not altered by the different diets. This is a consequence of the fact that the mice used in this study were fasted overnight before collecting the tissues to minimize effects of metabolic variation on various target genes. We have found that this relatively short term fasting can alter SHP gene expression. Indeed, an obvious induction of SHP expression upon CA diet was observed under non-fasting conditions (data not shown).

As expected from previous results, the expression of the bile acid biosynthetic enzymes CYP7A1, CYP8B1, CYP27, and bile acid receptor FXR was not markedly affected by the loss of SHP in the control fed animals, but CYP7B1 mRNA levels were decreased (20). Expression of CYP7A1 and especially CYP8B1 was increased by the CH diet in wild type mice, consistent with the increased bile acid synthesis. CYP8B1 expression was also elevated in the CH-fed SHP null mice, but CYP7A1 expression was not. The CH diet decreased expression of CYP7B1 by about 2-fold in wild type mice, but this response was also absent in the SHP null mice. Expression of CYP27 and FXR was not affected by genotype or CH feeding. These results show that loss of SHP is associated with moderate alterations in the response of at least some genes to dietary cholesterol, perhaps
as a consequence of the decreased hepatic cholesterol levels in the SHP null livers.

Both the CH+CA and the CA diets strongly reduced CYP7A1, CYP8B1, and CYP7B1 expression in wild type mice, confirming the potent negative feedback regulation of bile acid biosynthesis in these chronic studies. Efficient repression of CYP7A1 and CYP7B1 expression was also observed in SHP null mice on both CA containing diets, although a low level of expression was observed in the knockouts as previously described (20). In contrast to the shorter term studies, however, CYP8B1 expression was restored to almost basal levels in the SHP null mice fed either CA diet. FXR and CYP27 did not show the strong responses observed with the three other bile acid biosynthetic enzymes, although FXR transcripts were decreased in the CH+CA-fed wild type mice and to a lesser extent in the CA-fed animals. This pattern is consistent with a negative effect of SHP on FXR expression, at least in the presence of CA, but may simply be a reflection of the toxicity in these livers. Overall, the patterns of expression of the bile acid biosynthetic enzymes generally confirm the expected responses, with the specific exception of CYP8B1. Thus, the strong difference in CYP8B1 expression in the wild type and SHP null mice on CA feeding provides a potential explanation for the differences in toxicity.

To further explore bile acid transport, the expression of a number of additional hepatic and ileal genes was examined. In addition to a variety of more subtle effects too numerous to describe in detail, expression of a number of genes was strongly affected by the diets. NTCP, the major Na⁺/H⁺-dependent BA transporter of the basolateral surface of hepatocytes was
strongly repressed in wild type mice fed either CA-containing diet. As observed with CYP7A1, this repression was largely, but not completely retained in the SHP null mice, in agreement with the proposed role of SHP in negative regulation of NTCP (33).

Na⁺-independent transport is mediated by the organic anion-transporting polypeptides Oatp1, Oatp2, and Oatp4 (26, 27). Oatp1 expression was decreased in SHP null mice compared with the wild type mice on regular chow or the CH diet. These decreases may be an SHP-independent response to the increased BA pool in the SHP null mice, because Oatp1 expression is known to be repressed by BA (34) and was strongly decreased in mice of both genotypes on both CA-containing diets. This response is consistent with role of Oatp1 in Na⁺-independent uptake of bile salts under normal conditions (26, 27). Oatp2 expression was not affected by the different diets (data not shown), which contrasts with its reported response to cholestasis and other stimuli (35, 36). Although the magnitude of the responses was decreased, the overall pattern of expression of Oatp4 was very similar to that of NTCP, suggesting similar mechanisms for their regulation.

After basolateral uptake, bile salts are rapidly transferred across hepatocytes for canalicular secretion by binding to at least three cytosolic proteins: L-FABP (liver fatty acid-binding protein), 3α-HSD (3α-hydroxysteroid dehydrogenase), and GST (glutathione S-transferase) (27). Expression of those genes was not responsive to the dietary manipulations, except for a potent decrease in L-FABP and a less marked decrease in 3α-HSD in the wild type livers on the CH+CA diet only (data not shown), which may be a reflection of the severe toxicity observed in these animals.

At the apical surface of hepatocytes, conjugated bile acids are actively extruded into the canalicular space by BSEP (bile salt export pump), and di-anionic-conjugated bile acids are also exported by the canalicular ABC transporter Mrp2 (multidrug resistance protein). BSEP expression was modestly lower in CH-fed SHP null mice and in CH+CA-fed wild type mice. The basis for the lack of the expected strong induction in response to the CA-containing diets is not clear. The expression of Mrp2 was similar in mice of both genotypes of mice on regular chow, but was down-regulated on all three CH- or CA-containing diets, which also contrasts with expectations from the response of this gene to activation of FXR or PXR (37). No changes were observed for two additional transporters, MDR2 and MRP3.

The controversial putative BA transporter mEH (microsomal epoxide hydrolase) (38) was examined as well. Surprisingly, the expression of mEH was decreased in SHP null mice on the chow or CH diet. It was also stimulated in the SHP null mice on the CA diet by comparison to the control, with some increase apparent in the wild type CA-fed animals. Intermediate expression was observed in both genotypes on the combined diet. The basis for these previously unreported responses is not clear.

Efficient intestinal reabsorption of bile salts and delivery to the portal blood maintains the enterohepatic recirculation. I-BAT (ileal sodium-dependent bile acid transporter), also referred to as ASBT (apical sodium-dependent bile acid transporter), expressed in apical surface of the distal ileum, plays an important role in the BA uptake. The CH diet greatly stimulated I-BAT expression in the small intestine of wild type mice, but this response was decreased in SHP null mice. The CA diet had an opposite effect; I-BAT expression was strongly decreased in both wild type and SHP null mice. An intermediate level of expression was observed with the combination of CH+CA. These results do not support a direct regulatory role of SHP in I-BAT expression, but clearly demonstrate that I-BAT expression is positively regulated by chronically increased cholesterol levels and negatively regulated by chronically increased CA. The long term treatments used here may account for the discrepancies between these results and previous reports.

The cytosolic bile salt binding protein I-BABP (ileal bile acid-binding protein) gene was one of the first identified FXR targets, and I-BABP expression was induced by the CA diet in both genotypes, as expected. It was also increased by CH alone, and high levels of expression were also observed with CH+CA. Finally, MRP3 is thought to export bile acids from enterocytes to the portal circulation. No change of expression of MRP3 was observed in either wild type or SHP null animals irrespective of diets (data not shown).

**DISCUSSION**

Previous short term studies (1 day to 1 week) of the effects of bile acids and synthetic FXR agonists on wild type and SHP knockout mice established the important role of this unusual orphan nuclear receptor in the negative feedback regulation of bile acid biosynthesis (20, 21). In contrast to expectations based on the proposed central role of SHP in this process, however, both studies also showed that efficient repression of CYP7A1, CYP7B1, and CYP8B1 expression was maintained in SHP null animals on bile acid-containing diets. Two additional bile acid-dependent pathways based on activation of the c-Jun N-terminal kinase (JNK) and the xenobiotic receptor PXR were found to be functional in the SHP null animals and likely contributed to the unexpected maintenance of bile acid negative feedback in the absence of SHP (20).

To further characterize the role of SHP in bile acid and cholesterol homeostasis, we used long term feeding studies to determine the effects of chronic administration of cholesterol alone, cholic acid alone, or both to wild type and SHP null mice. To a large extent these results confirm those of the more acute studies. Thus, expression of both CYP7A1 and CYP7B1 was efficiently repressed in the SHP null mice. As observed previously, however, the SHP null mice show a modest residual level of CYP7A1 expression upon bile acid treatment, which contrasts with the essentially complete loss CYP7A1 expression in the wild type mice. The results described here also provide a relatively comprehensive analysis of the expression of a number of the genes involved in the enterohepatic circulation of bile acids. This integrated study of effects of chronic stresses provides evidence for some previously unreported regulatory effects worthy of further study, such as the strong, opposing responses of IBAT to chronic cholesterol and cholic acid feeding.

One contrast with the previous studies with the SHP null mice is the decreased lipid levels observed in the livers of these older animals (Figs. 2 and 3). These results suggest an intriguing potential function for SHP in triglyceride and fatty acid metabolism that is currently being explored.

These long term studies have also yielded two additional interesting results that are potentially related. The more specific is the re-expression of CYP8B1, but not other bile acid biosynthetic enzymes, in the SHP null animals on the CA diets. In a variety of previous studies (19–21), CYP7A1, CYP7B1, and CYP8B1 all exhibited similarly strong negative responses to bile acids that might be assumed to be due to common regulatory mechanisms. The unexpected re-expression demonstrates that the mechanisms that regulate CYP8B1 expression must differ from those of the other two genes. Although the basis for the recovery of expression could be either indirect or direct in these chronic studies, the simplest explanation is that CYP8B1 is particularly dependent on the FXR/SHP repression pathway. Perhaps chronic elevation of bile acid levels results in desensi-
tization of one or more of the redundant pathways that contribute to the repression observed in the short term studies. Earlier results in FXR knockout mice also showed that bile acid responsiveness of CYP7B1 differs significantly from that of other two genes (19). Thus, it appears that each of these three bile acid biosynthetic genes has a distinct response to bile acids. It is likely that detailed analyses of these individual responses may reveal different contributions of the redundant bile acid signaling pathways.

The second, more general result is the resistance of the SHP null mice to the hepatotoxic effects of the CA diet and, even more dramatically, the severe toxicity of the CH÷CA diet. CH÷CA feeding results in a number of pathological effects in the wild type mice, including strong elevations in the circulating levels of ALT and ASBT, cholesterol, triglycerides, and free fatty acids. There are also significant inflammatory responses that, as expected, are associated with increased expression of appropriate cytokines, such as IL-1α, IL-1β and IL-6 (data not shown). The loss of SHP has effects on expression of a number of genes that can be either direct or indirect and can often be shown). The loss of SHP has effects on expression of a number of genes that can be either direct or indirect and can often be shown. These include genes involved in bile acid synthesis, bile acid transport, and bile acid signaling pathways.

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