Differential Regulation of Hepatic Organic Cation Transporter 1, Organic Anion-Transporting Polypeptide 1a4, Bile-Salt Export Pump, and Multidrug Resistance-Associated Protein 2 Transporter Expression in Lymphocyte-Deficient Mice Associates with Interleukin-6 Production

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ABSTRACT
Cholestasis results from interrupted bile flow and is associated with immune-mediated liver diseases. It is unclear how inflammation contributes to cholestasis. The aim of this study was to determine whether T and B cells contribute to hepatic transporter expression under basal and inflammatory conditions. C57BL/6J wild-type mice or strains lacking T, B, or both T and B cells were exposed to lipopolysaccharide (LPS) or saline, and livers were collected 16 hours later. Branched DNA signal amplification was used to assess mRNA levels of organic anion-transporting polypeptides (Oatp) 1a1, 1a4, and 1b2; organic cation transporter (Oct) 1; canalicular bile-salt export pump (Bsep); multidrug resistance-associated proteins (Mrp) 2 and 3; and sodium-taurocholate cotransporting polypeptide (Ntcp). Real-time polymerase chain reaction analysis was used to correlate changes of transporter expression with interleukin-1b (IL-1b), IL-6, IL-17A, IL-17F, tumor necrosis factor-α (TNF-α), and interferon-γ expression in the liver. LPS treatment inhibited Bsep and Oct1 mRNA expression, and this was abrogated with a loss of T cells, but not B cells. In addition, the absence of T cells increased Mrp2 mRNA expression, whereas B cell deficiency attenuated Oatp1a4 mRNA in LPS-treated mice. Oatp1a1, Oatp1b2, Ntcp, and Mrp3 were largely unaffected by T or B cell deficiency. Lymphocyte deficiency altered basal and inflammatory IL-6, but not TNF-α or IL-1b, mRNA expression. Taken together, these data implicate lymphocytes as regulators of basal and inflammatory hepatic transporter expression and suggest that IL-6 signaling may play a critical role.

Introduction
The liver is a critical immunological and metabolic organ, and is strategically positioned to receive blood that is rich in bacterial and food-derived antigens from the gut via the portal vein. Immune-mediated liver diseases are usually progressive and are associated with significant morbidity and mortality. Cholestasis can result from either a functional defect in bile formation or interrupted bile flow, and is commonly associated with human liver diseases (Hirschfield and Heathcote, 2009; Hirschfield et al., 2010). Genetic and environmental factors, including infections and xenobiotics, can contribute to cholestasis. A major consequence of acute cholestasis is the development of severe liver injury. Hepatocyte and cholangiocyte proliferation that occurs in response to injury in turn leads to periportal fibrosis, biliary fibrosis, and cirrhosis (Hirschfield et al., 2010). The mechanisms by which inflammation contributes to the pathophysiology of cholestasis-mediated liver injury remain unclear.

Bile acids are synthesized in the liver from cholesterol, secreted into the bile, and actively transported to the gut and then back to the liver via enterohepatic circulation. Enterohepatic bile flow is essential for the emulsification of dietary fat, fat-soluble vitamin absorption, and elimination of toxic compounds, and is regulated through a coordinated hepatobiliary sinusoidal (basolateral) and canalicular (apical) transport system (Merritt and Donaldson, 2009). Organic cation transporters contribute to bile salt excretion and biliary flow regulation. Bile salts are synthesized in the liver and then secreted into the bile, where they can be returned to the liver through the enterohepatic circulation. This process is essential for the absorption of fat and fat-soluble vitamins.

A B B R E V I A T I O N S: Bsep, bile-salt export pump; BW, body weight; Ct, threshold cycle; ΔCt, target Ct values; IFN-γ, interferon-γ; IL, interleukin; KO, knockout; LPS, lipopolysaccharide; Mrp, multidrug resistance-associated protein; μMT, IgM transmembrane tail exon; NK, natural killer; NKT, natural killer T; Ntcp, Na+-taurocholate cotransporting polypeptide; Oatp, organic anion-transporting polypeptide; Oct, organic cation transporter; PCR, polymerase chain reaction; Rag, recombination-activating gene; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; TLR, Toll-like receptor; tm1, targeted mutation 1; TNF-α, tumor necrosis factor-α; Treg, regulatory; WT, wild-type.
receptor 4 (TLR4) and 10% B cells (Dong et al., 2007). TLRs are widely expressed in a large number of innate and adaptive immune cells for protection against harmful pathogens and autoreactive self-antigens from foodstuffs and commensal bacteria. Lymphocyte activation in Kupffer cells. This model predicts that proinflammatory cytokines, in turn, signal through their respective membrane receptors to suppress transporter expression and function (Geier et al., 2006; Lickteig et al., 2007; Mulder et al., 2009). Although the underlying mechanisms remain unclear, LPS-induced alteration of transporter function appears to be immune-mediated and the direct cause of cholestasis. Thus, LPS-induced cholestasis provides a valuable experimental tool to study mechanisms of hepatic transporter regulation directly, rather than secondarily in response to the accumulation of bile constituents resulting from biliary obstruction.

The liver receives approximately 70% of its blood supply from the intestine through the portal circulation, and is thus exposed to potential gut-derived immune modulators including bile acids and bacteria. Accordingly, the liver is selectively enriched in a large number of innate and adaptive immune cells for protection against harmful pathogens and autoreactive self-antigens from foodstuffs and commensal bacteria. Lymphocyte subpopulations in normal human livers comprise approximately 35% T cells, 30% natural killer (NK) T cells (NKT), 20% NK cells, and 10% B cells (Dong et al., 2007). TLRs are widely expressed by immune cells, including conventional CD4\(^+\) T helper cells, cytotoxic T lymphocytes, B cells, natural forkhead box P3\(^+\) regulatory T cells, and NKT cells. This enables responsiveness to microbes through pattern- and cytokine-associated molecular patterns or cell injury via danger-associated molecular patterns for cytokine production during LPS-induced cholestasis. Substantial evidence has recently implicated that TLR4 signaling contributes to the activation of T cells, B cells, and NKT cells (Pone et al., 2010; Kim et al., 2012; Reynolds et al., 2012). Importantly, whereas some cytokines, e.g., TNF-\(\alpha\) and IL-1\(\beta\), are thought to be proinflammatory and detrimental, others, including IL-6, are also anti-inflammatory and suppress liver injury (Sun et al., 2004). The objective of this study was to test the hypothesis that lymphocytes contribute to the expression of hepatic transporter expression under basal and acute inflammatory conditions.

Materials and Methods

Animal Models. Male adult C57BL/6J wild-type (WT) and homozygous T cell–deficient B6.129S2-T-cell receptor \(\alpha\) chain; targeted mutation 1 (tm1), Peter Mombaerts (Terra\(^{tm1}Limm\)) (TLR-\(\alpha\) KO), B cell–deficient B6.129S2-IgM; tm1, University of Cologne (IgM\(^{tm1}Limm\)) (IgM transmembrane tail exon (\(\mu\)MT) deficient), and T and B cell–deficient B6.129S7 recombinant activating gene 1 (tm1, Peter Mombaerts (Rag1\(^{tm1}Limm\)) (Rag1 KO) mice (8–10 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.A SC.C7 TCR transgenic Rag2 KO mice (Seder et al., 1992) were obtained from the National Institute of Allergy and Infectious Diseases contract facility (Taconic Farms, Germantown, NY). All mice were acclimated to the housing conditions for at least 14 days prior to use. Groups for LPS-induced cholestasis studies were designated WT (n = 6), TLR-\(\alpha\) KO (n = 6), \(\mu\)MT (n = 6), and Rag1 KO (n = 6). Groups for sex-preference expression studies were designated C57BL/6J (n = 3) and B10.A TCR Rag2 KO (n = 3). All mice were maintained in 12-hour light/dark cycles and allowed water and standard chow ad libitum. All animals were used according to the guidelines of the University of Missouri Animal Care and Use Committee and in accordance with the US National Institutes of Health and the American Association for Laboratory Animal Care International.

LPS Model of Cholestasis. Four groups (WT, TLR-\(\alpha\) KO, \(\mu\)MT, and Rag1 KO) of adult (8–10 weeks old) age-matched male mice (22–31 g) underwent treatment. LPS (4 mg/kg in a volume of 5 \(\mu\)l) or sterile saline vehicle was administered to mice by i.p. injection. Livers were excised 16 hours after LPS administration, snap frozen in liquid nitrogen, and stored at −80°C until RNA isolation. Mice were maintained under specific pathogen-free conditions in University of Missouri animal facilities that are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All protocols were approved by the University of Missouri Animal Care and Use Committee. Mice were monitored for signs of distress during the study (abdominal distention, respiratory difficulty).

Chemicals. LPS (from Escherichia coli serotype O55:B5) and all other chemicals, unless otherwise indicated, were purchased from Sigma-Aldrich (St. Louis, MO). One milligram of LPS was added to 0.5 ml of sterile saline to create stock solutions and frozen (−20°C) prior to use.

RNA Preparations. Total RNA was isolated from mouse liver using RNAzol B reagent (Tel-Test, Inc., Friendswood, TX) per the manufacturer’s recommendations. RNA concentrations were determined by UV spectrophotometry, and the integrity of the RNA was confirmed by ethidium bromide staining after agarose gel electrophoresis.

Branched DNA Assay. Specific oligonucleotide probes for Oatp1a1, Oatp1a4, Oatp1b2, Oct1, Ntcp, Bsep, Mrp2, and Mrp3 genes (Augustine et al., 2005) were diluted in lysis buffer supplied by the manufacturer. The substrate solution, lysis buffer, capture hybridization buffer, amplifier, and label probe buffer used in the analysis were all obtained from the Quantigene Discovery Kit (Panomics, Fremont, CA). The assay was performed in 96-well format with 10 \(\mu\)g of total RNA added to the capture hybridization buffer and 50 \(\mu\)l of the diluted probe set. The total RNA was then allowed to hybridize to the probe set overnight at 53°C. Hybridization steps were performed per the manufacturer’s protocol on the following day. Luminescence of the samples was measured with a Quantiplex 320 branched DNA luminometer interfaced with Quantiplex Data Management Software, version 5.02 (Bayer, Walpole, MA).

Real-Time Reverse-Transcription Polymerase Chain Reaction. Real-time quantification of IL-1\(\beta\), interferon–\(\gamma\) (INF-\(\gamma\)), TNF-\(\alpha\), IL-6, IL-17A, and IL-17F relative to \(\beta\)-actin mRNA was performed using SYBR Green polymerase chain reaction (PCR) master mix (Applied Biosystems, Foster City, CA) and an ABI PRISM 7900HT sequence detector (Applied Biosystems). Total RNA (5 \(\mu\)g) was reverse transcribed to cDNA using SuperScript III and random hexamer primers (Invitrogen, Carlsbad, CA) in a 20-\(\mu\)l reaction. PCR was performed using the following primers: IL-1\(\beta\) primers 5′-GAAAAGCTTCCACCTCCTGATG-3′ and 5′-GCCGCTTTCATATTACACAGG-3′, INF-\(\gamma\) primers 5′-CTGCCACGGCACAGTCTTATG-3′ and 5′-TGCTACTCCCTTTGCGCTTGC-3′.
TNF-α primers 5'-GACCCTCACTCAGATCATCTTCT and 5'-CCACCTGGTTGTGTTGCTACGA, IL-6 primers 5'-TGTTCTATACCTCTCAAGTCGGAAG and 5'-GGCAACCTCTTCTCATTCCCAC, IL-17A primers 5'-GCTCCAGAAGGCCCTCAGACT and 5'-CCAGGTTTTCCTGCCATTGGA, IL-17P primers 5'-CTGGAGGATAACACTGTTGAGAT and 5'-TGCTGGAATGGCGACGGAGTTGTC, and β-actin primers 5'-ATGGTGGAAGTGGTCAAGAA and 5'-CCATGTCGTCCAGTTGGATCA. PCR reactions incorporated 5 pmol forward and reverse primer and used the following cycling conditions: 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 60 seconds followed by dissociation curve analysis. For the negative controls, the reverse-transcriptase step was omitted and PCR was performed directly from the RNA. The normalized threshold cycle (Ct) values were subtracted from the target Ct values of each sample (ΔCt). Relative levels of target mRNA were calculated as 2−ΔΔCt.

**Statistical Analysis.** Statistical analysis was performed using InStat 3 software (GraphPad Software, La Jolla, CA). Data are shown as the mean ± S.E. The Mann-Whitney, Wilcoxon matched-pairs test, or unpaired two-tailed t test was applied to compare two groups; the one-way analysis of variance with Bonferroni’s multiple comparisons test was used to compare three or more means. Best fit of correlation was measured by the root mean square. All P values <0.05 were considered statistically significant. In the figures and tables, P values are displayed according to the following scheme: **P < 0.01; *P < 0.05.

**Results**

A genetic loss of function approach was used to determine the regulatory role of lymphocytes in hepatic transporter steady-state mRNA expression prior to and after LPS treatment. Uptake and efflux transporters investigated in this study were chosen on the basis that they encompass sinusoidal and canalicular membrane localization and respond to inflammation. Given that transporter and cytokine expression are sex-dependent and adult female responses are likely influenced by the estrous cycle (Klaassen and Aleksunes, 2010; Kwekel et al., 2010), male mice were used. Hepatic transporter expression has also been shown to be age-dependent (Fu et al., 2012); therefore, despite C57BL/6J Rag1 KO mice being smaller in size relative to WT, TCR-α KO, and μMT mice (P < 0.01), age-matched mice were studied (Fig. 1). Unfasted body weights were recorded during the acclimatization period (days −14, −10, and −5), on the day of treatment (day 1), and at necropsy (16 hours post-treatment). The administration of LPS resulted in a loss of body weight in WT, TCR-α KO, and μMT mice, but not Rag1 KO mice, amounting to 12.97% ± 1.86% (P < 0.05), 16.22% ± 0.99% (P < 0.01), 13.36% ± 1.05% (P < 0.01), and 8.44% ± 1.18% (P < 0.05) of saline controls, respectively (Fig. 1). Overall, these results suggest that mice lacking T and B cells likely respond differently to acute LPS exposure.

Consistent with previous studies (Klaassen and Aleksunes, 2010), basal steady-state mRNA expression of sinusoidal Oatp1a1, Oatp1a4, Oatp1b2, Ntcp, Oct1, and Mrp3 and canalicular Mrp2 and Bsep transporters was readily detected (Table 1). Oatp1a4 expression was reduced in TCR-α KO mice relative to WT mice (P < 0.05). Although not significant, basal Oct1 expression was reduced in Rag1 KO mice. In comparison, basal Mrp3, Mrp2, and Bsep expression was lower in TCR-α KO, μMT, and Rag1 KO mice compared with WT mice. Meanwhile, basal Oatp1a1, Oatp1b2, and Ntcp expression was unaffected by lymphocyte deficiency. Of the transporter genes studied, Oatp1a4 mRNA was uniquely downregulated in the absence of T cells, but not B cells or the combined deficiency of T and B cells (Table 1), suggesting that B cells, in a T cell–independent manner, may augment Oatp1a4 expression. In summary, these data demonstrate that T and B cells contribute to the maintenance of steady-state mRNA expression levels of selective transporters in the liver of adult male C57BL/6J mice in the absence of inflammation, infection, or autoimmunity.

We next investigated the effect of T cell and B cell deficiency on transporter mRNA expression during inflammation. Figure 2 demonstrates augmented Mrp2 mRNA and a loss of LPS-induced suppression of Bsep mRNA in TCR-α KO mice following acute LPS exposure. We further investigated the potential for lymphocytes to impact hepatic sinusoidal transporter expression following LPS treatment. Figure 3 shows that LPS treatment did not alter Oatp1b2, Ntcp, or Mrp3 mRNA in any of the genotypes. In contrast, Oatp1a1 mRNA was reduced in response to LPS treatment in all four genotypes. Meanwhile, LPS-induced inhibition of Oct1 and Oatp1a4 was observed only in WT and B cell–deficient mice, respectively (Fig. 3). Overall, these data demonstrate that lymphocytes contribute to differential regulation of hepatic transporter expression in a transporter- and inflammation-dependent manner.

TNF-α, IL-1β, and IL-6 likely contribute to the regulation of hepatic drug transporters during injury. Given that these cytokines are produced by multiple cell types, including lymphocytes, we next asked whether T and B cells influence the expression of these soluble mediators in the liver. For these
Table 1: Basal transporter gene expression in liver isolated from male WT mice and mice lacking T cells (TCRα KO), B cells (μMT), or both T and B cells (Rag1 KO) Results represent the mean relative light units/10 μg total RNA ± S.E. (n = 3).

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Human Gene</th>
<th>Accession #</th>
<th>Nuclear Receptor</th>
<th>Ligand</th>
<th>WT</th>
<th>TCRα KO</th>
<th>μMT</th>
<th>Rag1 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatp1a1 (Oatp1)</td>
<td>Slco1a1</td>
<td>NM_013797</td>
<td>FXR, RXR, SHP, HNF4α, GR</td>
<td>BA (↑)</td>
<td>69.56 ± 7.78</td>
<td>74.09 ± 13.39</td>
<td>63.43 ± 8.85</td>
<td>64.26 ± 4.16</td>
</tr>
<tr>
<td>Oatp1a4 (Oatp2)</td>
<td>Slco1a4</td>
<td>NM_030687</td>
<td>FXR, SHP, HNF4α</td>
<td>SHP (↑)</td>
<td>5.81 ± 0.88</td>
<td>0.90 ± 0.41**</td>
<td>6.03 ± 0.61</td>
<td>5.03 ± 0.92</td>
</tr>
<tr>
<td>Oatp1b2 (Oatp4)</td>
<td>Slco1b2</td>
<td>NM_020495</td>
<td>FXR</td>
<td>BA (↑)</td>
<td>82.27 ± 17.96</td>
<td>88.53 ± 19.38</td>
<td>63.80 ± 3.33</td>
<td>85.07 ± 7.10</td>
</tr>
<tr>
<td>Ntcp (Ntcp)</td>
<td>Slc10a1</td>
<td>NM_011387</td>
<td>FXR, RXR, SHP, HNF4α, GR</td>
<td>Retinooids (↑)</td>
<td>64.04 ± 11.60</td>
<td>57.95 ± 2.83</td>
<td>76.06 ± 8.80</td>
<td>80.73 ± 18.80</td>
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<tr>
<td>Oct1</td>
<td>Slc22a1</td>
<td>NM_009202</td>
<td>FXR, PPARα, HNF4α</td>
<td>OC (↑)</td>
<td>192.93 ± 29.88</td>
<td>124.42 ± 20.75</td>
<td>151.48 ± 30.08</td>
<td>104.84 ± 3.80</td>
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<tr>
<td>Mrp3</td>
<td>Abcc3</td>
<td>NM_029600</td>
<td>FXR, CAR, XFR, VDR</td>
<td>BS, Bili, PB, phytoestrogens (↑)</td>
<td>15.12 ± 3.58</td>
<td>11.24 ± 4.80</td>
<td>11.89 ± 3.13</td>
<td>5.56 ± 1.00</td>
</tr>
<tr>
<td>Mrp2</td>
<td>Abcc2</td>
<td>NM_013806</td>
<td>FXR, CAR, XFR</td>
<td>BA, Bili, OSH, Xenobiotics (↑)</td>
<td>29.87 ± 8.59</td>
<td>20.40 ± 1.91</td>
<td>13.13 ± 3.27</td>
<td>14.55 ± 3.65</td>
</tr>
<tr>
<td>Bsep</td>
<td>Bsep, SGP, PFIC-2</td>
<td>NM_021022</td>
<td>FXR, RXR</td>
<td>BA (↑)</td>
<td>50.48 ± 8.52</td>
<td>21.81 ± 4.35</td>
<td>31.27 ± 0.38</td>
<td>38.67 ± 15.93</td>
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</table>

Abch, ATP-binding cassette, subfamily C; Abcc, ATP-binding cassette, subfamily C; BA, bile acid; Bili, bilirubin; BS, bile salts; CAR, constitutive androstane receptor; cMoat, canalicular multiorganic anion transporter; FXR, farnesoid X receptor; GSH, glutathione; GR, glucocorticoid receptor; HNF4α, hepatocyte nuclear factor α; OC, organic cation; PB, phenobarbital; PFIC-2, progressive familial intrahepatic cholestasis; PPARα, peroxisome proliferator-activated receptor α; PXR, pregnane X receptor; RARα, retinoid acid receptor α; RXR, retinoid X receptor; SHP, short heterodimer partner; Slc, solute-carrier; SPGP, sister of P-glycoprotein; VDR, vitamin D receptor; ↑, increases transporter expression; ↓, decreases transporter expression.

*Reviewed in Beuers et al., 2001; Chandra and Brouwer, 2004; Shu et al., 2008; Halilbasic et al., 2013.

**P < 0.05 relative to WT.
further investigated the contribution of NKT cells to the regulation of sex-biased expression of hepatic bile acid transporters (Buist and Klaassen, 2004; Maher et al., 2005; Cheng et al., 2007; Klaassen and Aleksunes, 2010). For these experiments, male and female WT C57BL/6J and B10.A TCR transgenic Rag2 KO mice were used to determine the influence of combined genetic deficiency of CD8$^+$, B, NKT, and natural CD4$^+$ T regulatory (Treg) cells on hepatic transporter expression. We first compared WT C57BL/6J and CD8$^+$, B, NKT, and natural Treg cell–deficient (henceforth referred to as NKT-deficient) males and females (Table 2). Although hepatic transporter mRNA expression was unaltered in NKT-deficient males, increased Mrp3 mRNA was observed in NKT-deficient female mice relative to WT controls (Table 2). In summary, the previous analyses provide comparable between males and females. Mrp3 mRNA expression was female-predominant in WT as well as NKT-deficient mice (Table 2). In summary, the previous analyses provide evidence that lymphocytes may further function in a sex-bias manner to modulate hepatic transporters.

**Discussion**

The possibility that lymphocytes may control hepatic transporter expression has not been addressed until now. Using a genetic loss-of-function approach, we provide evidence suggesting that T and B cells contribute to the regulation of hepatic Mrp2, Bsep, Oct1, and Oatp1a4 transporter expression. We further associate elevated IL-6 production during conditions of T cell deficiency with abrogated suppression of Bsep and Oct1 expression during acute inflammation. In addition, we provide evidence that lymphocyte-mediated regulation of hepatic transporter expression is sex-biased. Given that transporters can be significantly affected by drug-drug interactions or genetic polymorphisms, and the immune system has been linked to sex-biased human liver disorders, the contribution of lymphocytes to changes in drug transporter activity warrants further attention. These findings contribute novel insights to the cellular basis of cytokine production in the liver and to the mechanisms of inflammation-induced liver diseases.

Previous studies demonstrating that altered hepatic transporter expression in response to LPS have implicated that Kupffer cells directly respond to LPS through TLR4-dependent signaling to upregulate TNF-α, IL-6, and IL-1β transcription (Green et al., 1996; Kubitz et al., 1999; Kim et al., 2000; Cherrington et al., 2004; Li et al., 2004; Augustine et al., 2005; Li and Klaassen, 2005). In theory, these presumed proinflammatory cytokines, in turn, signal through their respective receptors to repress transcription of genes encoding hepatic transporters, causing bile acid accumulation and cholestatic liver disease. We now demonstrate that T and B cells play substantial, yet differential, roles in IL-6, but not IL-1β, and TNF-α production during basal and acute inflammatory conditions. IL-6 has been implicated in both proinflammatory and anti-inflammatory responses (Scheller et al., 2011), and most studies have investigated proinflammatory contributions by IL-6. However, our data illustrating down- and upregulated IL-6 in WT and T cell–deficient mice, respectively, are consistent with recent reports suggesting that B cell–induced production of IL-6 prevents downregulation of hepatic transporters to reduce liver injury associated with acute inflammation (Taub, 2003; Klein et al., 2005). The
molecular mechanisms by which IL-6 provides protection in the liver are not well understood, and this is further con- 
ounded by a poor understanding of how T cells propagate 
hepatic inflammation. Nonetheless, it is well established that 
lack of gp130 expression in mice. **

Regarding the role of lymphocytes in regulating transporter 
expression, our findings do, in fact, support our hypothesis 
that lymphocytes contribute to the transcriptional control of 
hepatic bile acid transporters. Given that most lymphocytes, 
including T, B, NK, and NKT cells, are thought to provide 
critical defense against invading pathogens and modulate liver 
and it remains to be determined whether TLR4 signaling 
pathways differ between lymphocytes and macrophages, par-

Thus, it is unclear whether 
B cell–induced IL-6 production directly targets T cells or 
these results suggest that hepatocytes may be the 
critical recipient of the IL-6 signaling during acute TLR4-

Although not well appreciated, recent evidence has estab-
lished that T, B, and NKT cells also express TLR4 (Gururajan 
et al., 2017). Thus, lymphocytes also retain the capacity to rapidly respond to 
endotoxin and other microbes, including commensal bacteria 
(Tough et al., 1997; Godfrey and Rossjohn, 2011; Reynolds et al., 
2012). Little is known about TLR4 signaling in lymphocytes, 
and it remains to be determined whether TLR4 signaling 
pathways differ between lymphocytes and macrophages, par-

Our results are in agreement with previous studies demonstrat-
ing downregulation of sinusoidal and canicular transporters 
in response to LPS, as previously reviewed (Klaassen and 
Alekunes, 2010).

Without doubt, hepatic transporter expression is critical for 
effective elimination of conjugated bilirubin and bile acids, as 
well as xenobiotics and their metabolites, to maintain me-
tabolic homeostasis and simultaneous removal of toxic waste. 
However, it is also appreciated that transient repression of 
hepatic efflux transporter function during acute inflammation,
such as sepsis or gallstones, offers protection to the liver by enhancing the retention of antioxidants such as glutathione (Roeb et al., 2003; Kong et al., 2012). Thus, it is the sustained disruption of these efflux pumps, in combination with uptake transporters, which exacerbates pathology in liver diseases. It is appreciated that administration of LPS in experimental mouse models reproduces the common clinical features of sepsis, including downregulation of transporter mRNA. Therefore, is altered hepatic transporter expression in response to lymphocyte deficiency pathogenic or protective? This is a particularly important question given the liver provides a unique environment that favors immunological tolerance. Of particular importance, CD4^+ forkhead box P3^+ Tregs are mediators of immune tolerance, express Toll-like receptors including TLR4, exert enhanced suppressor function in response to LPS treatment, and have previously been shown to contribute to extrahepatic cholestasis (Caramalho et al., 2003; Katz et al., 2011). Additional studies to identify which lymphocytes are involved and gain a better understanding of their mechanism(s) of action during acute and chronic exposure are required to appropriately address this question. Here, we have clearly determined that hepatic transporters are sensitive to

**TABLE 2**

<table>
<thead>
<tr>
<th>Transporter</th>
<th>WT Males</th>
<th>TCR-a Tg Rag2 KO Males</th>
<th>WT Females</th>
<th>TCR-a Tg Rag2 KO Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatp1a1</td>
<td>89.07 ± 26.73</td>
<td>100.32 ± 17.10</td>
<td>37.87 ± 22.75</td>
<td>4.41 ± 1.93</td>
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<td>Oatp1a4</td>
<td>6.80 ± 1.30</td>
<td>5.09 ± 1.11</td>
<td>44.53 ± 5.91</td>
<td>27.09 ± 3.15</td>
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<tr>
<td>Oatp1b2</td>
<td>91.75 ± 8.18</td>
<td>93.07 ± 3.75</td>
<td>61.29 ± 14.74</td>
<td>50.60 ± 20.58</td>
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<tr>
<td>Ntcp</td>
<td>64.49 ± 12.90</td>
<td>97.89 ± 10.96</td>
<td>146.58 ± 21.35</td>
<td>163.08 ± 4.55</td>
</tr>
<tr>
<td>Oct1</td>
<td>128.78 ± 26.81</td>
<td>154.74 ± 14.71</td>
<td>182.76 ± 56.49</td>
<td>254.43 ± 49.54</td>
</tr>
<tr>
<td>Mrp3</td>
<td>6.31 ± 0.53</td>
<td>7.22 ± 1.98</td>
<td>9.30 ± 0.97</td>
<td>17.46 ± 3.07**</td>
</tr>
<tr>
<td>Mrp2</td>
<td>28.85 ± 3.17</td>
<td>25.71 ± 4.91</td>
<td>24.38 ± 13.87</td>
<td>41.69 ± 5.16</td>
</tr>
<tr>
<td>Bsep</td>
<td>74.97 ± 12.99</td>
<td>60.70 ± 1.32</td>
<td>128.67 ± 56.67</td>
<td>102.47 ± 9.06</td>
</tr>
</tbody>
</table>

**P < 0.01, comparing sex-specific WT and TCR-a Tg Rag2 KO mice.**

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**Fig. 5.** Schematic depicting differential regulation of hepatic transporter expression by T and B cells. Bsep and Mrp2 transporters are expressed on hepatocyte canalicular membranes and export monovalent tauro- and glyco-conjugated (conj.) hepatic bile acids (BAs) and divalent sulfated (sulpho-) or glucuronidated (glucurono-) BAs amidated with a taurine or a glycine, organic anions (OAs), reduced glutathione (GSH), and bilirubin (bili) out of the hepatocyte into the bile canaliculi. Mrp3, similar to Mrp2, is an ATP-binding cassette transporter, but unlike Mrp2, is localized on hepatocyte basolateral surfaces and functions to efflux bile and organic acids during periods of bile acid overload, e.g., gallstones or dysfunctional Mrp2 and/or Bsep. Ntcp, Oatp1a1, Oatp1a4, and Oatp1b2 are positioned at the basolateral membrane and facilitate enterohepatic cycling of BAs and OAs. Oct1 is also expressed at the basolateral membrane and functions in a similar manner, except it transports conj. BAs and organic cations (OCs) from the portal vein.
modulation by lymphocyte activity. Importantly, although we and others propose that LPS-induced cytokines directly target hepatocytes and ductal epithelial cells to alter hepatic transporter mRNA, it has also been established that transporters, in particular ATP-binding cassette transporters, are expressed on lymphocytes. Therefore, given that lymphocytes also express TLR4, we cannot ignore the possibility that alteration of lymphocyte transporter expression may contribute to inflammation in the liver, as has been previously suggested (van de Ven et al., 2009; Giraud et al., 2010; Verbrugge et al., 2012).

As hepatic bile acid transporter expression and immune response in other liver diseases are also both sex-dependent, a second aspect of this study involved elucidating whether lymphocytes contribute to the sex-biased basal expression of hepatic transporters. Exacerbated basal Mrp3 expression in female mice only suggests that lymphocytes may contribute to sex-biased hepatic transporter expression and implicates a particularly important role for CD4+ T cells. Consistent with this, conventional CD4+ T cells rapidly produce IL-2 upon activation. IL-2 receptor signaling is predominantly mediated through STAT5a and STAT5b signaling pathways (Lin et al., 2012; Liao et al., 2013), and hepatocytes respond to IL-2 signaling (Sunnan et al., 2004). A role for STAT5 signaling in sex-specific hepatic transporter expression has been identified for Ntcp (Clodfelter et al., 2006, 2007; Zhang et al., 2009). Signaling in sex-specific hepatic transporter expression has also been identified for Ntcp (Clodfelter et al., 2006, 2007; Zhang et al., 2009). In conclusion, the present study implicates that T and B cells regulate hepatic transporters (Fig. 5). In addition, we further implicate a protective role for IL-6 and identify lymphocytes as modulators of sex-biased hepatic transporter expression. Given that targeted therapies that affect lymphocyte function could adversely affect bile flow, it is critical that further work be done to identify the lymphocytes that regulate transporter function.

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