Toxicogenomic Analysis Reveals Profibrogenic Effects of Trichloroethylene in Autoimmune-Mediated Cholangitis in Mice

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ABSTRACT

Epidemiological studies suggest that exposure to environmental chemicals increases the risk of developing autoimmune liver disease. However, the identity of specific chemical perpetrators and the mechanisms whereby environmental chemicals modify liver disease is unclear. Previous studies link exposure to trichloroethylene (TCE) with the development of autoimmune liver disease and exacerbation of autoimmunity in lupus-prone MRL mice. In this study, we utilized NOD.c3c4 mice, which spontaneously develop autoimmune cholangitis bearing resemblance to some features of primary biliary cirrhosis. Nine-week-old female NOD.c3c4 mice were given TCE (0.5 mg/ml) or its vehicle (1% Cremophor-EL) in drinking water for 4 weeks. TCE had little effect on clinical chemistry, biliary cyst formation, or hepatic CD3+ T-cell accumulation. Hepatic microarray profiling revealed a dramatic suppression of early growth response 1 (EGR1) mRNA in livers of TCE-treated mice, which was verified by qPCR and immunohistochemical staining. Consistent with a reported link between reduced EGR1 expression and liver fibrosis, TCE increased hepatic type I collagen (COL1A1) mRNA and protein levels in livers of NOD.c3c4 mice. In contrast, TCE did not increase COL1A1 expression in NOD.ShiLtJ mice, which do not develop autoimmune cholangitis. These results suggest that in the context of concurrent autoimmune liver disease with a genetic basis, modification of hepatic gene expression by TCE may increase profibrogenic signaling in the liver. Moreover, these studies suggest that NOD.c3c4 mice may be a novel model to study gene-environment interactions critical for the development of autoimmune liver disease.

Key words: trichloroethylene; autoimmunity; cholangitis; liver; mouse

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by autoimmune-mediated destruction of intrahepatic bile ducts, which causes cholestasis (i.e., disrupted bile flow), hepatic injury, and inflammation. If left unchecked, persistent hepatic autoimmunity ultimately progresses to liver fibrosis, cirrhosis, and liver failure. Patients with PBC, predominantly older females, present with elevated levels of serum IgM, antimitochondrial antibodies to pyruvate dehydrogenase complex, and hepatic accumulation of autoreactive T cells (Shimoda et al., 2006). Ursodeoxycholic acid is the only Food and Drug Administration (FDA) approved drug for the treatment of PBC. However, this drug does not fully prevent the progression of disease in all patients, often resulting in a need for liver transplantation (Nishio et al., 2000). The lack of other pharmacologic thera-
pies for this disease highlights a need for better understanding of how genetic and environmental risk factors both contribute to the initiation and progression of PBC (Flores and Mayo, 2014).

Strong epidemiological evidence supports the connection between environmental exposures and PBC. For example, the prevalence of PBC is increased in populations exposed to environmental toxins, particularly those found in Superfund sites, public health hazard areas designated for immediate remedial action (Ala et al., 2006). Notably, trichloroethylene (TCE) is a persistent environmental contaminant found in ground water and in abundance in Superfund sites associated with PBC clusters (Fay and Mumtaz, 1996), and has been classified as a human carcinogen, regardless of the route of exposure (Chiu et al., 2013; Guha et al., 2012). TCE metabolites generated by cytochrome P450 enzymes in the liver (i.e., trichloroacetaldehyde) stimulate T-cell activation in vitro (Gilbert et al., 2004). In addition, strong experimental evidence links TCE to the development of autoimmune liver disease in rodent models (Chiu et al., 2013; Cooper et al., 2009). Specifically, TCE exposure exacerbated autoimmunity in lupus-prone mice (i.e., MRL mice), with liver histopathology in TCE-exposed MRL mice indicative of periportal lymphocytic inflammation (Khan et al., 1995). Collectively, these studies suggest that TCE could contribute to the pathogenesis of hepatic autoimmune disease.

Strong genome-wide association studies have begun to delineate the link between autoimmune liver disease risk loci in the context of interacting genetic and environmental factors (Liu et al., 2012; Mells et al., 2013); however, the impact of exposure to environmental contaminants, such as TCE, on the progression of autoimmune liver disease with a complex genetic basis is not known. To address this, we utilized NOD.3c4 mice, which were derived via chromosomal rearrangement of specific loci from C57 mice into the non-obese diabetic mouse background (Koara et al., 2004). NOD.3c4 mice spontaneously develop B- and T-cell-dependent autoimmune cholangitis, are reported to produce auto-antibodies common to PBC, and develop several liver histological features similar, but not identical, to autoimmune liver disease in humans (Leung et al., 2012). Their genetic counterparts, NOD.ShiLtJ mice, do not develop autoimmune liver disease and were used as a genetic comparator for this study.

We determined the effect of TCE exposure on the progression of liver disease in NOD.3c4 mice. Utilizing a toxicogenomic approach, we tested the hypothesis that TCE-elicited hepatic gene expression modifies the pathogenesis of spontaneous autoimmune cholangitis.

**MATERIALS AND METHODS**

**Mice.** Nine-week-old female NOD.3c4 and NOD.ShiLtJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed at an ambient temperature of 22°C with alternating 14/10-h light/dark cycles and provided water and rodent chow ad libitum (Teklad 8604; Harlan, Indianapolis, IN) in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility at the University of Kansas Medical Center. All animal procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

**Treatment.** Mice were exposed to 0.5 mg/ml TCE (Sigma-Aldrich, St. Louis, MO) or vehicle (1% Cremophor-EL, Sigma-Aldrich) in drinking water. This concentration approximated the maximum daily occupational TCE exposure (Gilbert et al., 2009), and has been shown previously to induce hepatic autoimmunity in lupus-prone MRL mice (Cai et al., 2008; Gilbert et al., 2009; Griffin et al., 2000b). Water was exchanged twice weekly, as described (Griffin et al., 2000a), to minimize degradation of TCE. After 4 weeks of TCE exposure, mice were anesthetized using isoflurane, and blood was collected from the caudal vena cava into an empty syringe for the collection of serum. The liver was removed, washed in saline, and sections were flash-frozen in liquid nitrogen for gene expression profiling. Sections for histopathology and immunohistochemistry were fixed in 10% neutral-buffered formalin and then embedded in paraffin or affixed to a cork using optimal cutting temperature compound (Fisher Scientific, Pittsburgh, PA), and immersed for approximately 3 min in liquid nitrogen-chilled 2-methylbutane (Fisher Scientific). The remaining liver was snap frozen in liquid nitrogen.

**Serum clinical chemistry.** Blood samples were spun at 10000 x g for 2 min for collection of serum. Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were determined according to the manufacturers’ protocols (Infinity ALT/GPT, Thermo Scientific, Waltham, MA; ALP, Pointe Scientific, Canton, MI). Total bile acid concentration in serum was determined using a colorimetric assay (Bio-Quant, San Diego, CA). For each assay, data were collected using an Infinite M200 plate reader (Tecan, Durham, NC).

**Histopathology and immunohistochemistry.** Formalin-fixed liver sections were cut at 5 μm and stained with hematoxylin and eosin (H&E). At least two to three sections of the liver from the left lateral lobe were evaluated by light microscopy to quantitatively determine the extent of biliary hyperplasia and biliary cyst area. Formalin-fixed liver sections were stained with picrosirius red by the Michigan State University Investigative Histopathology Laboratory. Immunofluorescent staining for type I collagen and cytokeratin-19 (CK-19) was performed as described (Sullivan et al., 2010). For CD3+ T-cell staining, sections were fixed with acetone (–20°C) for 10 min, allowed to dry, and rehydrated with PBS for 5 min. The sections were then blocked with 10% goat serum in PBS for 1 h at room temperature and incubated with rat anti-mouse CD3 antibody (100202, BioLegend, San Diego, CA) diluted 1:100 in block buffer for an additional 2 h. The sections were then washed with PBS and incubated with donkey anti-rat Alexa Fluor 594-conjugated antibody (Life Technologies, Carlsbad, CA), diluted 1:500 for 2 h at room temperature. Early growth response 1 (EGR1) immunohistochemical staining on formalin-fixed sections was performed by the Michigan State University Investigative Histopathology Laboratory according to published procedures (Allen et al., 2010; Pritchard et al., 2011). EGR1 immunofluorescent staining on frozen sections was performed as described previously and utilized for quantification of EGR1-positive nuclei (Allen et al., 2010; Pritchard et al., 2011). Prolong Gold (DAPI-containing) Antifade reagent (Life Technologies) was applied to the tissues prior to cover slipping. Fluorescent staining in liver sections was visualized using an Olympus DP70 microscope (Olympus, Lake Success, NY) and merged (as appropriate) using Olympus DP Manager software. Type I collagen and CK-19 staining was quantified using Scion Image (Scion Corporation, Frederick, MD) as described previously (Sullivan et al., 2010), utilizing approximately 10 low-power images (100X) for each tissue. The percentage of pixels containing positive signal (i.e., collagen staining) was expressed as a fold change relative to vehicle NOD.ShiLtJ mice.
FIG. 1. Exposure to TCE does not affect biliary cyst formation or serum clinical chemistry in NOD.c3c4 mice. Nine-week-old female NOD.ShiLtJ or NOD.c3c4 mice were exposed to 0.5 mg/ml TCE or vehicle (1% Cremophor-EL) in drinking water for 4 weeks. (A) Representative hematoxylin and eosin and (B) cytokeratin-19 (CK-19, 200X) stained liver sections from vehicle-treated NOD.ShiLtJ and NOD.c3c4 mice. (C) Quantification of CK-19 positive pixels and (D) biliary cyst area, both expressed as percent of hepatic area. Levels of (E) alanine aminotransferase (ALT), (F) alkaline phosphatase (ALP), and (G) bile acids were measured in the serum. NA: not available. Data are expressed as mean ± SEM. *p ≤ 0.05 versus treatment-matched mice of the opposite genotype; #p ≤ 0.05 versus vehicle-treated mice within the same genotype; n = 4–9 mice per group.

RNA isolation, cDNA synthesis, and quantitative real-time PCR. Total RNA was isolated from approximately 20 mg frozen liver from each mouse (i.e., RNA was not pooled) using TRI Reagent (Molecular Research Center Inc., Cincinnati, OH) according to the manufacturer’s protocol. One microgram of high-quality RNA (A_{260}/A_{280} > 1.9) was used for the synthesis of cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) and a C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Hepatic gene expression was determined using iTaq Universal SYBR Green Supermix (Bio-Rad) or TaqMan gene expression assay (IDT, Coralville, IA) and analyzed using Bio-Rad CFX Connect Real-Time System.
Exposure to TCE does not alter accumulation of hepatic CD3+ T-cells in NOD.c3c4 mice. Nine-week-old female NOD.ShiLtJ or NOD.c3c4 mice were exposed to 0.5-mg/ml TCE or vehicle (1% Cremophor-EL) in drinking water for 4 weeks. (A) Hepatic sections from vehicle- and TCE-exposed NOD.c3c4 mice were stained for CD3+ T-cells (200X) and (B) quantified. Images were converted to grayscale and inverted such that CD3+ staining is dark. Data are expressed as mean ± SEM. *p ≤ 0.05 versus treatment-matched mice of the opposite genotype; n = 4–9 mice per group.

The expression level of each gene was normalized to the geometric mean of hypoxanthine-guanine phosphoribosyltransferase (HPRT) and 18S ribosomal RNA (18S) levels, and the relative expression level was determined using the comparative \( \Delta\Delta Ct \) method. Mouse type I collagen (COL1A1) primer sequences were 5'-GAGCGGAGAGTACTGGATCG-3' (forward) and 5'-GCTTCTTTTCTTTGGGTC-3' (reverse). Mouse tissue inhibitor of metalloproteinase 1 (TIMP1) primer sequences were 5'-GAGACACACCGAGAGATACC-3' (forward) and 5'-AGCTTCTTTTCTTTGGGTC-3' (reverse). Mouse integrin \( \beta 6 \) (ITG\( \beta 6 \)) primer sequences were 5'-CTCACCGGTTAGTACTGGATCG-3' (forward) and 5'-AAATGAGCTCTCAGGGCAGC-3' (reverse). Mouse transforming growth factor \( \beta 1 \) (TGF\( \beta 1 \)) primer sequences were 5'-CTCCCGGTGCTTCTAGTGC-3' (forward) and 5'-GCCTTAGTTTGGACAGGATCTG-3' (reverse). Mouse EGR1 primer sequences were 5'-AGCCGCCGATGATGAGAAG-3' (forward) and 5'-GATGGCATTTTCGGAGGGGA-3' (reverse). Mouse HPRT primer sequences were 5'-AAGCCTAAGATGAGCGCAAG-3' (forward) and 5'-TTCTAGGAGATGAGGCAAC-3' (reverse). Mouse 18S primer sequences were 5'-GTAACCGGTTAGAACCAC-3' (forward) and 5'-CCATCCAAATCGGTAGTAGCG-3' (reverse).

Whole-genome microarrays and data analysis. Total RNA extracted from hepatic sections of the vehicle- and TCE-exposed NOD.c3c4 mice was analyzed using whole-genome Affymetrix QuantiGene 2.0 array chips at the University of Kansas Medical Center Genome Microarray Facility. Detailed protocols for sample preparation can be found at [http://www.kumc.edu/genomics/microarray-facility/protocols.html](http://www.kumc.edu/genomics/microarray-facility/protocols.html). Data were analyzed at the University of Kansas Medical Center Bioinformatics Core. The full microarray data set is provided as Supplementary table 1.

**RESULTS**

Exposure to TCE does not Affect Biliary Cyst Formation or T-Cell Accumulation in Livers of NOD.c3c4 Mice

Lymphocyte-driven biliary cyst formation is a hallmark histological indicator of hepatic autoimmunity in NOD.c3c4 mice (Koarada et al., 2004). NOD.c3c4 mice, but not NOD.ShiLtJ mice, spontaneously developed biliary cyst and portal inflammation, as marked by H&E-stained liver sections (Fig. 1A). CK-19 staining, a marker of hepatic bile ducts, was markedly greater in livers of NOD.c3c4 mice compared with NOD.ShiLtJ mice (Fig. 1B). Notably, biliary cysts were also CK-19 positive in livers of NOD.c3c4 mice. TCE exposure did not alter CK-19 staining or biliary cyst area in either mouse strain (Figs. 1C and D). Similarly, serum levels of ALT or ALP were not different between exposure groups and between genotypes (Figs. 1E and F); however, serum bile acids were significantly higher in NOD.c3c4 mice exposed to TCE relative to vehicle-treated NOD.c3c4 group (Fig. 1G).

One of the characteristic features of the autoimmune biliary disease in NOD.c3c4 mice is hepatic accumulation of CD3+ T-cells. Importantly, treatment with the anti-CD3 antibody partially protects NOD.c3c4 mice from the autoimmune liver disease (Irie et al., 2006). Mouse CD3+ T-cell numbers were unaffected by TCE in NOD.ShiLtJ mice (Fig. 2B). An increased accumulation of CD3+ T-cells was seen in livers of NOD.c3c4 mice, primarily near biliary epithelial cells in areas of cyst formation (Figs. 2A and B). TCE exposure did not affect CD3+ T-cell accumulation in the livers of NOD.c3c4 mice (Figs. 2A and B).
FIG. 3. Exposure to TCE suppresses EGR1 expression in livers of NOD.c3c4 mice. Nine-week-old female NOD.ShiLtJ or NOD.c3c4 mice were exposed to 0.5 mg/ml TCE or vehicle (1% Cremophor-EL) in drinking water for 4 weeks. (A) Hepatic levels of EGR1 gene expression were determined by qPCR. (B) EGR1 protein (arrows) in vehicle and TCE-exposed mice was detected using immunohistochemical staining (200X), (C) quantified using immunofluorescent staining, and expressed as an average number of EGR1-positive cells per high power field. Data are expressed as mean + SEM. *p ≤ 0.05 versus treatment-matched mice of the opposite genotype; #p ≤ 0.05 versus vehicle-treated mice within the same genotype; n = 4–9 mice per group.

Impact of TCE on Hepatic Gene Expression in NOD.c3c4 Mice
Liver samples from NOD.c3c4 vehicle and TCE-exposed mice were analyzed by whole-genome Affymetrix gene chips. Remarkably, application of a broadly accepted, stringent filtering criterion (i.e., fold change > 1.5 and p-value < 0.05) identified TCE-elicited differential expression of only eight genes, including induction of heat shock protein 8 (Hsp8, 1.54-fold) and suppression of cytochrome P450, family 26, subfamily 1, polypeptide 1 (Cyp26a1, −1.53-fold), coiled-coil domain containing 25 (Ccdc25, −1.70-fold), ankyrin repeat domain 1 (Ankrd1, −1.80-fold), NADPH oxidase 4 (Nox4, −2.08-fold), 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (Hmgcr, −2.23-fold), nuclear receptor subfamily 0, group B, member 2/small heterodimer partner (Nr0b2/Shp, −2.92-fold), and Egr1 (−2.97-fold).

The full microarray data set is available as Supplementary table 1.

Exposure to TCE Suppresses EGR1 Expression in the Livers of NOD.c3c4 Mice
Hepatic microarray profiling revealed that Egr1 was the most suppressed gene among the TCE-exposed NOD.c3c4 mice relative to vehicle-treated NOD.c3c4 mice. Verification of the Egr1 transcript expression using quantitative PCR (qPCR) revealed markedly higher levels of Egr1 mRNA in livers of NOD.c3c4 mice compared with NOD.ShiLtJ mice and further confirmed the TCE-mediated suppression of Egr1 in NOD.c3c4 mice (Fig. 3A). EGR1 protein staining in liver sections of NOD.c3c4 mice revealed positive nuclear staining in both hepatocytes and peribiliary cells. The staining was less evident in TCE-exposed NOD.c3c4 livers,
FIG. 4. Exposure to TCE induces profibrogenic gene expression in livers of NOD.c3c4 mice. Nine-week-old female NOD.ShiLtJ or NOD.c3c4 mice were exposed to 0.5 mg/ml TCE or vehicle (1% Cremophor-EL) in drinking water for 4 weeks. Hepatic levels of (A) TGFβ1, (B) TGFβ2, (C) TIMP1, and (D) ITGβ6 gene expression were measured by qPCR. Data are expressed as mean ± SEM. *p ≤ 0.05 versus treatment-matched mice of the opposite genotype; #p ≤ 0.05 versus vehicle-treated mice within the same genotype; n = 4–9 mice per group.

particularly in hepatocytes (Figs. 3B and C), in agreement with suppression of hepatic Egr1 gene expression.

Exposure to TCE Induces Profibrogenic Signaling and Type I Collagen Deposition in Livers of NOD.c3c4 Mice

Several gene expression changes elicited by TCE exposure in NOD.c3c4 mice were suggestive of a potential connection to profibrogenic pathways, including suppression of Egr1 and Shp (Supplementary table 1). EGR1 deficiency significantly increased hepatic collagen deposition in models of chronic toxicant-induced liver fibrosis (Pritchard and Nagy, 2010; Sullivan et al., 2012). SHP-deficiency increased toxicant-induced liver fibrosis in mice (Fiorucci et al., 2004; Smalling et al., 2013; Zhang et al., 2011; Zhang et al., 2014). Collectively, our observation that TCE exposure caused a robust suppression of these genes prompted us to determine whether TCE could amplify liver fibrosis in NOD.c3c4 mice, even in the absence of an obvious increase in hepatocellular injury.

Analysis of hepatic profibrogenic gene expression by qPCR revealed modest (~1.7-fold) but significant increase in expression of Tgfβ1 in TCE-exposed NOD.c3c4 mice (Fig. 4A). Interestingly, expression of other profibrogenic genes, including Tgfβ2, Timp1, and Itgβ6, was significantly induced in NOD.c3c4 mice, but not further increased by TCE exposure (Figs. 4B–D). However, induction of Col1a1 mRNA, deposition of type I collagen protein, and picrosirius red staining were each further increased in TCE-exposed NOD.c3c4 mice (Figs. 5A–C). Collagen deposition was primarily localized to areas of ductular proliferation and biliary cysts. Of importance, TCE exposure did not increase hepatic type I collagen deposition in livers of NOD.ShiLtJ mice (Fig. 5C).

DISCUSSION

Classifying the toxic effects of TCE and establishing risks associated with TCE exposure in humans remains a pressing concern in the United States and abroad (Chiu et al., 2013). Among potential adverse effects of TCE exposure is exacerbation of autoimmunity, for which a number of studies have identified a strong connection (Cooper et al., 2009; Griffin et al., 2000a; Khan et al., 1995). Epidemiological studies have indicated an increased prevalence of PBC around toxic waste sites where TCE is present (Ala et al., 2006). Experimental evidence links TCE exposure to autoimmune hepatitis in the lupus-prone MRL+/+ mouse model (Gilbert et al., 2009). Liver histopathology in affected mice indicated a marked perportal lymphocytic infiltrate, consistent with autoimmune cholangitis (Gilbert et al., 2009; Irie et al., 2006), and reflecting the potential coupling of TCE exposure to PBC in humans. Collectively, both human and animal studies suggest that TCE exposure could trigger or worsen autoimmune liver disease in humans, particularly in conditions where the portal tract is a target of immunity.

Based on the potential connection between PBC and TCE exposure, we hypothesized that TCE could exacerbate autoimmune liver disease in a mouse model of spontaneous autoimmune cholangitis. Comparative analysis of clinical and histopathological endpoints by multiple reports revealed that NOD.c3c4 mice spontaneously develop autoimmune liver disease resembling select features of PBC in patients (Chuang et al., 2008; Irie et al., 2006; Leung et al., 2012; Oertelt et al., 2007). NOD.c3c4 mice develop chronic destruction of small bile ducts, hepatic T-cell infiltration, elevated levels of serum IgM, and frequently manifest antimitochondrial antibodies against in-
FIG. 5. Exposure to TCE induces type I collagen deposition in livers of NOD.c3c4 mice. Nine-week-old female NOD.ShiLtJ or NOD.c3c4 mice were exposed to 0.5 mg/ml TCE or vehicle (1% Cremophor-EL) in drinking water for 4 weeks. (A) Hepatic levels of COL1A1 gene expression were determined by qPCR. (B) Total collagen levels in vehicle and TCE-exposed mice were detected using picrosirius red staining (top 100X; bottom 400X). (C) Type I collagen protein was detected by immunofluorescent staining (red; 400X), quantified, and normalized to the CK-19 area (green). Data are expressed as mean ± SEM. *p ≤ 0.05 versus treatment-matched mice of the opposite genotype; #p ≤ 0.05 versus vehicle-treated mice within the same genotype; n = 4–9 mice per group.
It was surprising that TCE did not have a larger impact on overall pathology and lymphocytic infiltration in NOD.c3c4 mice. Although these changes are often tightly coupled to autoantibody levels, additional studies will be required to assess both qualitative and quantitative shifts in autoantibodies by TCE exposure in this mouse model. Notably, where TCE-elicited autoimmunity has been reported previously, it has been investigated largely as a trigger in susceptible mice. It is plausible that the genetic basis for autoimmunity in NOD.c3c4 mice is so strong as to mitigate the requirement for a chemical trigger for liver disease. Remarkably, despite the lack of obvious potential of cellular injury, TCE exposure elicited a number of gene expression changes and increased collagen deposition in NOD.c3c4 mice, including COL1A1 and TGFβ1, both of which have been tightly linked to liver fibrosis in both humans and animal models (Bataller and Brenner, 2005; Jin et al., 2011; Milani et al., 1991; Sullivan et al., 2010). Early fibrosis was reported to occur in only a small number of NOD.c3c4 mice (Irie et al., 2006), rendering the observation that TCE exacerbates this feature of disease quite exciting. Of importance, TCE exposure did not cause fibrosis in livers of NOD.ShiLtJ mice, in which TCE has been shown to not increase autoimmunity (Ravel et al., 2005). Taken together, the results in NOD.c3c4 mice suggest that TCE has the potential to act as a disease modifier in the presence of concurrent hepatic autoimmunity.

Global gene expression analysis of TCE-treated NOD.c3c4 livers identified a few select TCE-induced transcriptional changes suggestive of a role for TCE in liver fibrosis. Most importantly, we identified a significant decrease in the mRNA and protein levels of the multifunctional transcription factor EGR1, which has been demonstrated to inhibit the pathogenesis of hepatic fibrosis in mice (Pritchard and Nagy, 2010; Sullivan et al., 2012). Specifically, in a model of xenobiotic-induced cholestasis, EGR1 deficiency exacerbated hepatic fibrosis as evidenced by increased type I collagen deposition in association with elevated expression of profibrogenic gene, ITGβ6 (Sullivan et al., 2012). Similarly, exposure to carbon tetrachloride significantly exacerbated fibrosis in EGR1-deficient mice relative to wild-type animals (Pritchard and Nagy, 2010). The mechanism whereby EGR1 suppresses hepatic fibrosis is not known, and to some extent complicated by the tissue-specific nature in which it regulates profibrogenic responses, as EGR1 is known to promote pulmonary fibrosis and fibroblast activation. Notably, the vast majority of EGR1-positive nuclei in livers of NOD.c3c4 mice appeared to be in hepatocytes, consistent with bile acid induction of hepatocyte EGR1 expression (Allen et al., 2010). In agreement with its inhibition of EGR1 mRNA induction, TCE exposure dramatically reduced hepatocellular EGR1 staining, although the mechanism for this inhibition is not known. Overall, these findings suggest that EGR1 may act as a negative regulator of TCE-mediated hepatic fibrosis in a model of autoimmune liver disease. Additional studies determining the effect of genetically imposed EGR1 deficiency on liver disease in NOD.c3c4 mice would be required to definitively identify the role of EGR1 suppression in liver fibrosis.

A previous study found that TCE exposure did not exacerbate autoimmunity in NOD.ShiLtJ mice (Ravel et al., 2005). In agreement, exposure to TCE had no effect on profibrogenic gene expression or hepatic collagen deposition in NOD.ShiLtJ mice, suggesting that exposure to this environmental contaminant selectively modifies the progression of liver disease only in the context of autoimmunity. Accordingly, this indicates the possibility that autoimmune liver disease may have a unique impact on TCE metabolism in NOD.c3c4 mice, particularly in light of recent toxicogenomic and metabolomic evidence suggesting that interindividual differences exist in TCE metabolism and molecular signaling in the liver (Bradford et al., 2011). Moreover, CYP2E1-derived metabolites are implicated in the initiation of hepatic autoimmunity by TCE (Griffin et al., 2000). It is therefore possible that unique genetic makeup of NOD.c3c4 mice rendering them susceptible to autoimmune liver disease can directly affect TCE signaling and TCE metabolism. Identification and assessment of novel changes in TCE metabolism directly affected by genetic background with simultaneous autoimmunity is required to provide additional clues to understanding of the TCE-mediated exacerbation of autoimmune liver disease.

In summary, our studies revealed an important mechanistic insight into TCE-mediated modification of autoimmune cholangitis leading to hepatic fibrosis in genetically predisposed mice. Collectively, the results suggest that exposures to environmental contaminants have the potential to modify disease through gene-environment interactions, leading to exacerbation of the pathogenesis of autoimmune liver disease.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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