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Lymphocytes contribute to biliary injury and fibrosis in experimental xenobiotic-induced cholestasis

Nikita Joshi[a,b,c], Anna K. Kopec[b,c], Holly Cline-Fedewa[b], James P. Luyendyk[a,b,c,*]

[a] Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI 48824, USA
[c] Institute for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

The etiology of chronic bile duct injury and fibrosis in patients with autoimmune cholestatic liver diseases is complex, and likely involves immune cells such as lymphocytes. However, most models of biliary fibrosis are not autoimmune in nature. Biliary fibrosis can be induced experimentally by prolonged exposure of mice to the bile duct toxicant alpha-naphthylisothiocyanate (ANIT). We determined whether lymphocytes contributed to ANIT-mediated biliary hyperplasia and fibrosis in mice. Hepatic accumulation of T-lymphocytes and increased serum levels of anti-nuclear-autoantibodies were evident in wild-type mice exposed to ANIT (0.05% ANIT in chow). This occurred alongside bile duct hyperplasia and biliary fibrosis. To assess the role of lymphocytes in ANIT-induced biliary fibrosis, we utilized RAG1$^{-/-}$ mice, which lack T- and B-lymphocytes. ANIT-induced bile duct injury, indicated by increased serum alkaline phosphatase activity, was reduced in ANIT-exposed RAG1$^{-/-}$ mice compared to ANIT-exposed wild-type mice. Despite this reduction in biliary injury, ANIT-induced bile duct hyperplasia was similar in wild-type and RAG1$^{-/-}$ mice. However, hepatic induction of profibrogenic genes including COL1A1, ITGβ6 and TGFβ2 was markedly attenuated in ANIT-exposed RAG1$^{-/-}$ mice compared to ANIT-exposed wild-type mice. Peribiliary collagen deposition was also reduced in ANIT-exposed RAG1$^{-/-}$ mice. The results indicate that lymphocytes exacerbate bile duct injury and fibrosis in ANIT-exposed mice without impacting bile duct hyperplasia.

1. Introduction

Among the key functions of the liver is production and delivery of bile to the gall bladder (McCuskey and Sipes, 2010). The synthesis of bile is complex and involves not only the synthesis of bile acids by hepatocytes, but also regulation of bile composition and flow by bile duct epithelial cells (BDECs; cholangiocytes) (Chen et al., 2008; Morell et al., 2013; O'Hara et al., 2013). BDECs line intrahepatic bile ducts, forming a conduit separating bile, which is both toxic and proinflammatory, from the liver parenchyma (Kanz, 2010; Perez and Briz, 2009; Sipka and Bruckner, 2014). BDEC injury is one trigger of cholestatic liver disease, wherein bile flow out of the liver is disturbed, leading to elevated plasma levels of bile acids (Chen et al., 2008; Li and Crawford, 2004; Morell et al., 2013; O'Hara et al., 2013). Although BDEC injury is a well-appreciated etiology of several cholestatic liver diseases in humans, the mechanisms responsible for biliary injury are not completely understood, and vary from increased pressure (i.e., obstructive cholestasis) to immune-mediated events in conditions such as primary biliary and primary sclerosing cholangitis (PBC and PSC) (Hirschfield et al., 2013; Lazaridis and LaRusso 2015; Li and Crawford, 2004; Lindor et al., 2009, 2015; Trauner et al., 1998).

Experimental models serve as an important platform to trace the mechanisms of chronic liver injury and fibrosis triggered by injury to intrahepatic BDECs (Kopec et al., 2016). Although obstructive cholestasis (i.e., bile duct ligation) and genetic models (Mdr2$^{-/-}$ mice) are utilized, BDECs can also be chronically injured by administration of certain xenobiotics. For example, owing to its

Abbreviations: ANIT, alpha-naphthylisothiocyanate; CK-19, cytokeratin-19; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TGFβ, transforming growth factor beta; COL1A1, type I collagen; ITGβ6, integrin beta 6; $\alpha_V\beta_6$, alphaVbeta6 integrin; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; BDEC, bile duct epithelial cell.

* Corresponding author at: Department of Pathobiology & Diagnostic Investigation, Michigan State University, 1129 Farm Lane, 253 Food Safety and Toxicology Building, East Lansing, MI 48824, USA.
E-mail address: luyendyk@cvm.msu.edu (J.P. Luyendyk).

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unique metabolism and transport, the xenobiotic alpha-naphthylisothiocyanate (ANIT) is selectively toxic to BDECs (Becker and Plaa 1965; Jean et al., 1995; Plaa and Priestly 1976). In contrast to acute ANIT exposure in mice (Hill et al., 1999), chronic exposure of mice to ANIT elicits bile duct injury, hyperplasia and fibrosis alongside induction of mixed lymphocytic hepatic inflammation

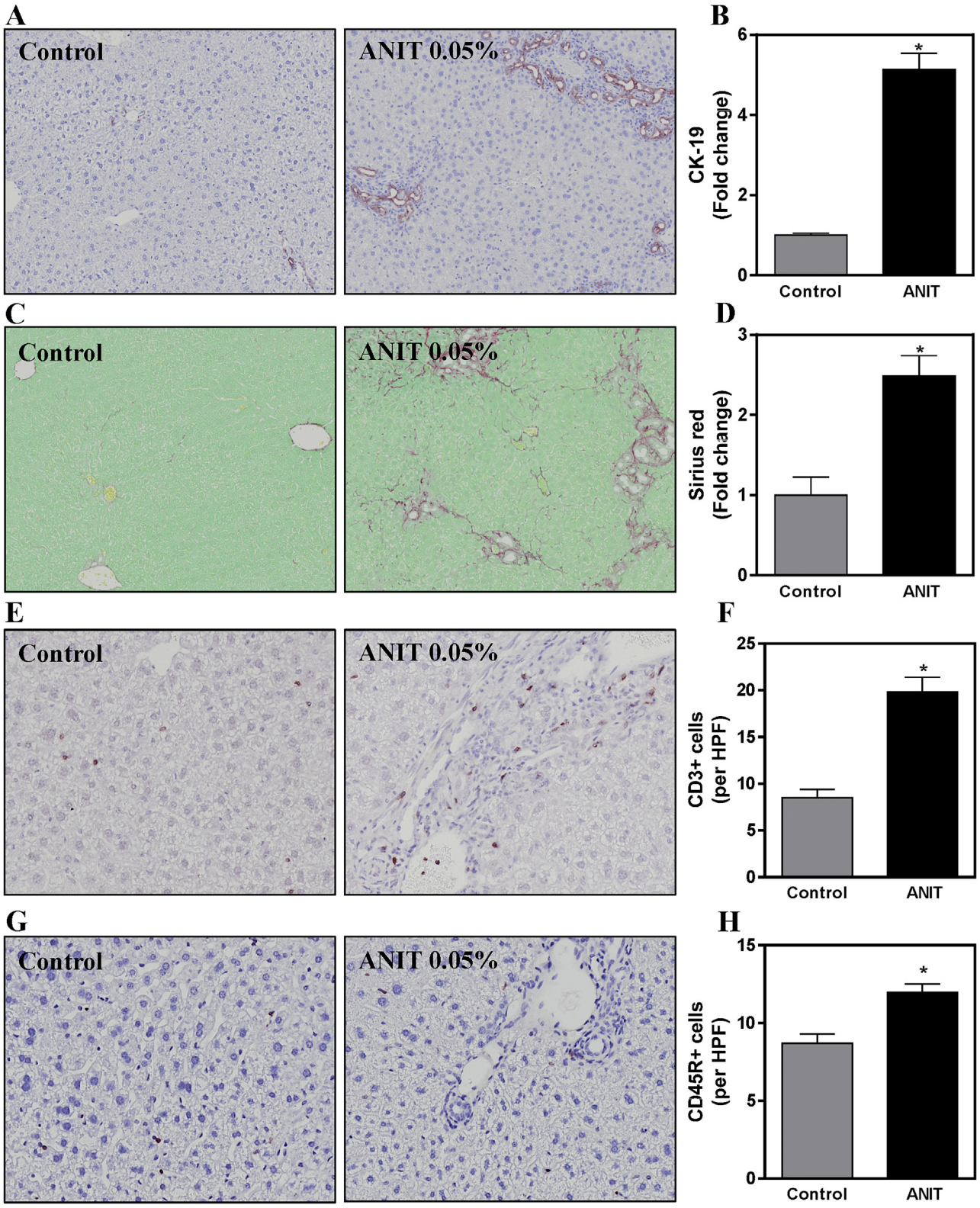

Fig 1. Hepatic lymphocyte accumulation, biliary hyperplasia and liver fibrosis in wild-type mice after chronic ANIT exposure: Wild-type mice were fed standard control rodent chow or diet containing 0.05% ANIT for 4 weeks. Representative photomicrographs (100×) show liver sections stained for (A) Cytokeratin-19 (CK-19, brown), (C) Sirius red (red), (E) CD3 T-lymphocytes (brown) and (G) CD45R B-lymphocytes (brown). Staining for (B) CK-19, (D) Sirius red, (F) CD3 T-lymphocytes and (H) CD45R B-lymphocytes per high power field (HPF, 200×) were quantified as described in Materials and Methods. Data are expressed as mean + SEM; n = 5–10 mice per group. *$p < 0.05$ vs. control diet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)