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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Dysregulated Intrahepatic CD4$^+$ T-Cell Activation Drives Liver Inflammation in Ileitis-Prone SAMP1/YitFc Mice

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SUMMARY

Ileitis-prone SAMP1/YitFc mice display concomitant immune-mediated liver inflammation, which is driven by hyperactivated intrahepatic T_H1 CD4$^+$ T effector cells. Locally impaired hepatic immunosuppression rather than recruitment of gut-derived cells is likely responsible for the heightened activation status of these cells.

BACKGROUND & AIMS: Liver inflammation is a common extraintestinal manifestation of inflammatory bowel disease (IBD), but whether liver involvement is a consequence of a primary intestinal defect or results from alternative pathogenic processes remains unclear. Therefore, we sought to determine the potential pathogenic mechanism(s) of concomitant liver inflammation in an established murine model of IBD.

METHODS: Liver inflammation and immune cell subsets were characterized in ileitis-prone SAMP1/YitFc (SAMP) and AKR/J (AKR) control mice, lymphocyte-depleted SAMP (SAMPx*Rag-1*$^{-/-}$), and immunodeficient SCID recipient mice receiving SAMP or AKR donor CD4$^+$ T cells. Proliferation and suppressive capacity of CD4$^+$ T-effector (Teff) and T-regulatory (Treg) cells from gut-associated lymphoid tissue (GALT) and livers of SAMP and AKR mice were measured.

RESULTS: Surprisingly, prominent inflammation was detected in 4-week-old SAMP livers before histologic evidence of ileitis, whereas both disease phenotypes were absent in age-matched AKR mice. SAMP liver disease was characterized by abundant infiltration of lymphocytes, required for hepatic inflammation to occur, a T_H1-skewed environment, and phenotypically activated CD4$^+$ T cells. SAMP intrahepatic CD4$^+$ T cells also had the ability to induce liver and ileal inflammation when adoptively transferred into SCID recipients, whereas GALT-derived CD4$^+$ T cells produced milder ileitis but not liver inflammation. Interestingly, SAMP intrahepatic CD4$^+$ Teff cells showed increased proliferation compared with both SAMP GALT- and AKR liver-derived CD4$^+$ Teff cells, and SAMP intrahepatic Tregs were decreased among CD4$^+$ T cells and impaired in in vitro suppressive function compared with AKR.

CONCLUSIONS: Activated intrahepatic CD4$^+$ T cells induce liver inflammation and contribute to experimental ileitis via locally impaired hepatic immunosuppressive function. *(Cell Mol Gastroenterol Hepatol 2015;1:406–419; http://dx.doi.org/10.1016/j.jcmgh.2015.05.007)*

Keywords: Hepatic CD4$^+$ T Cells; IBD-Associated Liver Inflammation; Liver Sinusoidal Endothelial Cells; Regulatory T Cells; SAMP1/YitFc Mice.

Liver disease constitutes one of the most frequently occurring extraintestinal manifestations of IBD, with primary sclerosing cholangitis (PSC) representing the most common hepatobiliary-associated ailment. Besides PSC, a wide range of liver pathologies have been reported in IBD patients, including PSC/autoimmune hepatitis (AIH) "overlap syndrome," IgG4-associated cholangitis, primary biliary cirrhosis, fatty liver, granulomatous hepatitis, cholelithiasis, and portal vein thrombosis.[1] Despite its prevalence, the precise etiology of liver inflammation during IBD is currently unknown.

To date, the most widely accepted hypothesis of IBD-associated liver disease, particularly PSC, is that it occurs as a consequence of aberrant adhesion molecule expression that promotes the recruitment of memory T cells, originally activated in the gut, to the liver where they drive hepatic

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Abbreviations used in this paper: AIH, autoimmune hepatitis; AKR, AKR/J; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APC, antigen-presenting cell; BM, bone marrow; BMC, bone marrow chimera; CCL25, CC chemokine ligand 25; DC, dendritic cell; FoxP3, forkhead box protein 3; FACS, fluorescence-activated cell sorting; GALT, gut-associated lymphoid tissue; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; MAdCAM-1, mucosal addressin cell adhesion molecule-1; MHC, major histocompatibility complex; MLN, mesenteric lymph node; NPLC, nonparenchymal liver cells; PSC, primary sclerosing cholangitis; SAMP, SAMP1/YitFc; SCID, severe combined immunodeficiency; Teff, effector T cell; Treg, regulatory T cell.

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inflammation.[2] Specifically, expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and CC chemokine ligand 25 (CCL25), normally restricted to the intestinal endothelium and small bowel epithelium, respectively, and which comprise the conventional gut "postal code,"[3] have been implicated as key mediators in this process. In PSC, for example, MAdCAM-1 and CCL25 are aberrantly expressed by the portal and sinusoidal endothelium and suggest the importance of these homing molecules for the migration of T cells to the liver.[4,5] Together, these observations support the existence of an enterohepatic lymphocyte circulation that facilitates trafficking of T lymphocytes from the gut to the liver that can then promote liver inflammation.

Alternatively, emerging evidence suggests that T-lymphocyte priming can also occur locally in the liver during both homeostatic and pathologic conditions.[6–8] Liver-resident antigen-presenting cells (APC), such as liver sinusoidal endothelial cells (LSEC), dendritic cells (DC), and Kupffer cells (KC), are capable of interacting with naïve T cells and have the ability to induce regulatory T cell (Treg) differentiation and function,[9,10] thereby promoting hepatic immune tolerance.

Of the many available models of IBD, only few allow investigation of the earliest events associated with the onset and natural course of disease. SAMP1/YitFc (SAMP) mice represent a well-characterized model of spontaneous, chronic intestinal inflammation, whose primary disease location (ie, the terminal ileum), histologic features, and response to standard therapies closely resemble Crohn's disease, one of the idiopathic forms of IBD.[11,12] The SAMP strain was derived from several generations of brother-sister mating of parental AKR mice with ileitis but not colitis developing spontaneously without chemical, genetic, or immunologic manipulation.[11–13] Our initial findings of liver disease noted lymphocytic infiltration of hepatic portal tracts in 20- to 30-week-old SAMP mice,[13] previously reported by others;[14] however, this observation was never further characterized by either group. Accordingly, SAMP mice represent an ideal model to evaluate the onset and progression of liver inflammation in the presence of chronic ileitis and to investigate the relationship and potential mechanism(s) linking gut and liver pathologies.

Herein, we report both portal and lobular liver inflammation that occurs *before* the onset of gut inflammation in SAMP mice, an established mouse model of Crohn's disease-like ileitis. Further characterization of intrahepatic immune cells confirmed specific expansion of type 1 helper T (T_H1)-polarized $CD4^+$ T cells that drive severe liver as well as ileal inflammation when adoptively transferred into naïve SCID recipients. Importantly, intrahepatic $CD4^+$ T cells do not display a gut-tropic phenotype, suggesting that local over-activation rather than recruitment of gut-activated $CD4^+$ T cells may be responsible for the liver disease in SAMP. Indeed, impaired in vitro suppressive function of hepatic Tregs was observed in SAMP mice. Taken together, our results challenge the current paradigm that IBD-associated liver disease represents a secondary event to gut inflammation and raise the possibility that liver inflammation during IBD may develop as a consequence of impaired immune tolerance within the host liver, which may also influence the course of chronic intestinal inflammation in individuals predisposed to IBD.

Materials and Methods

Mice

Original AKR/J (AKR), B6.129S7-Rag1^{tm1Mom}/J (*Rag-1*$^{-/-}$), and C3Smn.CB17-Prkdcscid/J (SCID) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). SAMP founders were originally provided by S. Matsumoto (Yakult Central Institute for Microbiological Research, Tokyo, Japan).[11,13] The SAMP*Rag-1*$^{-/-}$ strain was generated by backcrossing *Rag-1*$^{-/-}$ mice onto the SAMP background for 10 generations. All experimental mice were subsequently bred (>20 generations) and maintained under specific pathogen-free conditions, fed standard laboratory chow (Harlan Teklad, Indianapolis, IN), and kept on 12-hour light/dark cycles. All procedures followed Association for Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by Case Western Reserve University's institutional animal care and use committee.

Liver Enzyme Levels

Sera from experimental mice were assayed 1:5 for alkaline phosphatase (ALP) (BioVision, Mountain View, CA), and undiluted for alanine aminotransferase (ALT) (Genzyme Diagnostics P.E.I., Charlottetown, PE, Canada) according to manufacturer's instructions.

Histology and Immunohistochemistry

Liver and ileal tissues were processed for histology[11,14–20] and evaluated by trained pathologists (T. Roskams, J. Mize) using a novel liver (Table 1) and an established ileal[14,16–22] scoring system. Immunolocalization of T cells was performed using αCD3 (ab5690/1:50) primary antibodies (Abcam, Cambridge, MA), and visualized using the Dako EnVision System (Dako, Carpinteria, CA). Images were obtained on an Axiophot microscope and assembled by Axiovision Release 4.5 (Carl Zeiss, Thornwood, NY).

Nonparenchymal Liver Cell Isolation

Nonparenchymal liver cells (NPLCs) were isolated by perfusing mice with Hank's balanced salt solution through the hepatic portal vein, after which livers were removed and homogenized. Homogenates were incubated with agitation at 37°C for 30 minutes in a solution of collagenase type IV and DNase I in Hank's balanced salt solution (Sigma-Aldrich, St. Louis, MO). Resulting cell suspensions were centrifuged at 300 rpm at 4°C, passed through μm nylon filters, and centrifuged again at 1200 rpm at 4°C for 10 minutes. ACK Lysing Buffer (Invitrogen, Carlsbad, CA) was added to cell pellets for 5 minutes at room temperature and similarly centrifuged. Cells were washed twice and used for immediate experimentation, or either further purified by centrifugation at 2500 rpm for 30 minutes through a 40%/70% Percoll gradient (Sigma-Aldrich) or enriched for $CD4^+$ T cells (described herein), after which the cells were washed twice and then used for experiments.

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