ORIGINAL ARTICLE

Persistence of cirrhosis is maintained by intrahepatic regulatory T cells that inhibit fibrosis resolution by regulating the balance of tissue inhibitors of metalloproteinases and matrix metalloproteinases

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Fibrosis is the result of the abnormal accumulation of the extracellular matrix and ineffective clearance of fibroplasia. $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) are immunosuppressive lymphocytes that are highly expressed in the fibrotic tissues and peripheral blood of patients with cirrhosis or hepatocellular carcinoma. The role of Tregs in the progression of liver fibrosis is not well understood. Our experiments reveal that abundant of Tregs was scattered around sites of fibroplasia. Conversely, the depletion of Tregs promoted the resolution of liver fibrosis. As a consequence of Tregs depletion, the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) was altered; MMP9 and TIMP1 were reduced, whereas MMP2 and MMP14 were enhanced. The MMP9/TIMP1, MMP13/TIMP1, and MMP14/TIMP2 ratios were significantly increased in association with fibrosis resolution. Kupffer cells (KCs) are the main source of MMP. We observed that when KCs were cocultured with Tregs, the Tregs were able to inhibit MMP expression of KCs even at a low ratio; and anti–transforming growth factor-β (TGF-β) significantly reversed the inhibition of Tregs on MMP. Meanwhile, we also found that after Tregs depletion, TGF-β levels decreased in the mice liver, unlike in fibrosis. Furthermore, double depletion of both KCs and Tregs did not cause fiber resolution in mice. Thus, our results demonstrate that the persistence of liver cirrhosis is maintained by increased Tregs in the sites of fibroplasia and the subsequent regulation of the MMP/TIMP balance and that the suppression of KC-mediated MMP expression contributed to the regulatory process. (Translational Research 2015;■:1–13)

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Abbreviations: α-SMA = α-smooth muscle actin; CCl_4 = carbon tetrachloride; CTL = cytotoxic lymphocyte; CTLA-4 = cytotoxic T-lymphocyte–associated protein 4; ECM = extracellular matrix; GITR = glucocorticoid-induced TNF receptor; HCC = hepatocellular carcinoma; HSCs = hepatic stellate cells; KCs = Kupffer cells; LPS = lipopolysaccharide; MMP = matrix metalloproteinase; MNCs = mononuclear cells; PSC = primary sclerosing cholangitis; TGF-β = transforming growth factor-β; TIMP = tissue inhibitors of metalloproteinase; Tregs = regulatory T cells

AT A GLANCE COMMENTARY

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Background

Regulatory T cells (Tregs) are immunosuppressive lymphocytes that are highly expressed in the fibrotic tissues and peripheral blood of patients with cirrhosis. The role of Tregs in the progression of liver fibrosis is not well understood.

Translational Significance

We found the persistence of liver cirrhosis is maintained by increased Tregs in the sites of fibroplasia and the subsequent regulation of the matrix metalloproteinase/TIMP balance and that the suppression of Kupffer cell–mediated matrix metalloproteinase expression contributed to the regulatory process. Moreover, this study indicated manipulation of Tregs using a specific antibody might also be explored as an option for the treatment of cirrhosis.

INTRODUCTION

Regulatory T cells (Tregs) are a group of immunosuppressive T lymphocytes that are critical for peripheral immune homeostasis.[1] The suppressive functions of Tregs are largely the result of forkhead box P3 (Foxp3), a master transcription factor that interacts with multiple cotranscriptional regulators to determine immune suppression.[2] In mammals, defects of the Foxp3 pathway may cause a variety of autoimmune disorders.[3] Significant increases in Foxp3 are positively correlated with the intensity of inflammation.[4] The effects of Tregs include the immunosuppression of target cells such as $CD4^+$ and $CD8^+$ T cells and dendritic cells.[5] Indeed, Tregs regulate immune responses against silica particles via the suppression of inflammatory cells in the early stage,[6] and hepatic Tregs together with Kupffer cells (KCs) create a local suppressive microenvironment that prevents the establishment of the cytotoxic lymphocyte response.[7] A new article reported that Tregs modulate the interaction between natural killer cells and hepatic stellate cells (HSCs) by acting on either cell type.[8] Tregs maintain peripheral tolerance and control inflammation, and they also limit beneficial responses by suppressing sterilizing immunity and limiting antitumor immunity.[5] Large numbers of Tregs are often found in chronically infected tissues as well as in sites of fibrosis and tumors and may play certain function. For instance, increased Tregs are found in the peripheral blood of patients with hepatocellular carcinoma or chronic hepatitis virus infection.[9-11] Moreover, elevation of Tregs is related to the loss of immune surveillance in the tumor microenvironment and tissue fibrosis.[12] However, the specific, potentially immunosuppressive role of Tregs in liver fibrosis remains unknown.

In the present study, we sought to explore this issue further. We found that Tregs inhibited fiber degradation to maintain the persistence of liver fibrosis, by affecting the balance of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) through neutralization or depletion of Tregs. Then we used in vitro cell coculture experiments to investigate the ability of Tregs to suppress the secretion of MMPs by KCs. Furthermore, we depleted KCs and Tregs of mice with liver fibrosis, and the fibrotic tissue did not alter. Thus, the evidence presented here supports our hypothesis that the accumulation of Tregs is involved in the maintenance of liver fibrosis by regulating the MMP/TIMP balance, and its suppression of MMP expression by KCs contributes to the regulatory process.

METHODS

Animals. Six- to eight-week-old male C57BL/6 wild-type mice were obtained from the Laboratory Animal Center, Academy of Military Medical Science, Beijing, China. Animals were maintained under controlled conditions (22°C–24°C, 12-hour light/dark cycle) and were fed standard laboratory chow and water. Animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the Capital Medical University in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Animal protocols. Liver fibrosis was induced by repeated i.p. administration of carbon tetrachloride