Hepatobiliary & Pancreatic Diseases International 000 (2018) 1–7

Contents lists available at ScienceDirect

Hepatobiliary & Pancreatic Diseases International

journal homepage: www.elsevier.com/locate/hbpd

Original Article/Liver

$CD4^+Foxp3^+CD25^{+/-}$ Tregs characterize liver tissue specimens of patients suffering from drug-induced autoimmune hepatitis: A clinical-pathological study

Li-Mei Qu[a], Shu-Hua Wang[b], Kun Yang[a], David R. Brigstock[c,d], Li Sun[a], Run-Ping Gao[a,*]

[a] Department of Hepatic-Biliary-Pancreatic Medicine, First Hospital of Jilin University, Changchun 130021, China
[b] Department of Surgical Gastroenterology, First Hospital of Jilin University, Changchun 130021, China
[c] The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, USA
[d] Division of Pediatric Surgery, Department of Surgery, The Ohio State University, Columbus, OH 43205, USA

ARTICLE INFO

Article history:
Received 29 April 2017
Accepted 28 December 2017
Available online 19 February 2018

Keywords:
Drug-induced liver injury
Autoimmune hepatitis
Drug-induced autoimmune hepatitis
Liver biopsy
Regulatory T cells

ABSTRACT

Background: The diagnosis of drug-induced autoimmune hepatitis (DIAIH) and its differentiation from idiopathic autoimmune hepatitis (AIH) is challenging. This study aimed to differentiate DIAIH from AIH by comparing the biochemical changes, histological features, and frequencies of $CD4^+Foxp3^+CD25^{+/-}$ regulatory T cells (Tregs) in liver tissues or peripheral blood lymphocytes.

Methods: A total of 15 DIAIH patients and 24 AIH patients who underwent liver biopsies at initial presentation were enrolled in this study. The liver histological changes were assessed by HE staining. The phenotypic recognition and distribution of $CD4^+Foxp3^+CD25^{+/-}$ Tregs in liver tissues were evaluated by single/double immunostains in serial sections. The $CD4^+Foxp3^+CD25^{+/-}$ Tregs in peripheral blood were analyzed by flow cytometry.

Results: The median values of ALT and AST were 404.50 U/L and 454.10 U/L in DIAIH patients and 309.50 U/L and 315.00 U/L in AIH patients, respectively. More importantly, for the first time we found that patients with DIAIH had higher levels of serum ALT and AST, more severe degree of lobular inflammation, higher frequencies of zone 3 necrosis and higher number of lobular $CD4^+Foxp3^+CD25^-$Tregs compared with AIH ($P < 0.05$). Furthermore, there were positive correlations in DIAIH between the degree of lobular inflammation and either the AST/ALT level or the number of lobular $CD4^+Foxp3^+CD25^-$ Tregs ($P < 0.05$). However, the frequency of peripheral blood $CD4^+Foxp3^+CD25^{+/-}$ Tregs were not significantly different between DIAIH and AIH.

Conclusions: The differences of ALT, AST and the number of lobular $CD4^+Foxp3^+CD25^-$ Tregs between patients with DIAIH and those with AIH are clinically helpful in differentiating these two diseases in their early stage.

Introduction

Idiopathic autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized by elevated liver enzymes, hypergammaglobulinemia, autoantibodies, intrahepatic lymphoplasmacytic infiltration and high relapse rate after withdrawal of immunosuppressant [1,2]. Drug-induced autoimmune hepatitis (DIAIH) is similar to classical AIH. However, remission is maintained in DIAIH after withdrawal of immunosuppressant [3]. Currently, the differentiation DIAIH from AIH in early onset patients is still challenging [2–4].

Over the last decade, the pathogenesis of AIH received much attention, but it remains to be clarified. The transcription factor Forkhead box P3 (Foxp3) has been recognized as a lineage specification factor of regulatory T cells (Tregs), which not only has a highly restricted expression within the $CD4^+$ subset of T cells but also regulates their differentiation and function [5]. Recently a subset of $CD4^+Foxp3^+$ T cells expressing CD25 (named as $CD4^+Foxp3^+CD25^+$ Tregs), which accounts for 5–10% of the entire $CD4^+$ T cell population, was discovered to play a key role in maintaining immune equilibrium via its potent suppressor activity [6,7]. More recently, several studies [8–11] showed that a subset of $CD4^+Foxp3^+CD25^-$ Tregs was augmented in the

* Corresponding author.
E-mail address: gao_runping@126.com (R.-P. Gao).

https://doi.org/10.1016/j.hbpd.2018.02.004
1499-3872/

Please cite this article as: L.-M. Qu et al., $CD4^+Foxp3^+CD25^{+/-}$ Tregs characterize liver tissue specimens of patients suffering from drug-induced autoimmune hepatitis: A clinical-pathological study, Hepatobiliary & Pancreatic Diseases International (2018), https://doi.org/10.1016/j.hbpd.2018.02.004

peripheral blood in T cell-mediated autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis and type 1 diabetes mellitus. However, the frequencies of $CD4^+Foxp3^+CD25^{+/-}$ Tregs in DIAIH and AIH remain to be clarified.

In this study, we aimed to differentiate DIAIH from AIH by comparing the biochemical changes, histological features, and frequencies of $CD4^+Foxp3^+CD25^{+/-}$ Tregs in liver tissues or peripheral blood lymphocytes.

Methods

Patients

We enrolled 15 well-characterized DIAIH patients and 24 AIH patients diagnosed in our Liver Unit between May 2008 and May 2014 and followed them for one year. None of the patients underwent immunosuppressive therapy prior to liver biopsy. Diagnosis was made at initial presentation by exclusion of competing etiologies and assessment of relevant clinical and histological data at the time of diagnosis. The diagnosis of AIH was made according to international AIH group score system in which the cut off score is 16 points [12]. All 24 AIH patients were classical AIH with typical histological features including marked portal inflammation, interface hepatitis, and varying degrees of lobular hepatitis [13]. Nonclassical phenotypes of AIH at presentation were excluded, which included acute severe AIH (acute presentation with high level bilirubin and signs of acute liver failure with an INR >1.5 at any time but without histological evidence of cirrhosis) and histological atypical AIH (AIH-PBC or AIH-PSC overlap syndrome) [14–17]. The diagnosis of DIAIH was made by the time correlation of the drug intake on the onset of hepatitis and by an aggregate score for each patient of >16 points according to international AIH group score system and by the exclusion of other causes of hepatitis (i.e., HBV, HCV, HEV, EBV). The presences of the following two criteria were also necessary for diagnosis of DIAIH: (i) liver injury resolves on withdrawal of medication that triggered the disease, with or without immunosuppressive therapy to induce remission; (ii) no relapse within one 1 year after withdrawal of all immunosuppressants [18].

Histological evaluation

The 39 liver biopsy slides from all of the patients were stained by HE and evaluated using a standardized histological scoring system by three experienced hepatopathologists who were blinded to the clinical information. The following data were collected: the number of lymphocytes, plasmacytes, neutrophils or eosinophils in three lobules and portal tracts, the average number of inflammatory cells per lobule or portal tract. The presences of bile duct proliferation, rosette formation, zone 3 necrosis, and focal necrosis were evaluated and scored as “yes/no”. Cellular edema, ballooning degeneration and acidophilic degeneration were divided into mild, moderate or severe. Interface hepatitis or lobular inflammation was divided into mild, moderate or severe. Hepatic inflammation was assessed by the use of hepatic activity index (HAI) score [19] while fibrosis was staged with Scheuer system (ranging from 0 = no fibrosis to 4 = cirrhosis) [20].

Immunopathological evaluation

The frequency and distribution of Tregs in the liver biopsy sections were evaluated by double immunostaining with mouse anti-human Foxp3 monoclonal (Abcam, Cambridge, USA) and rabbit anti-human polyclonal CD25 (MXB, Fuzhou, China) antibodies, and by immunostaining with mouse anti-human CD4 monoclonal antibody (MXB, Fuzhou) in adjacent serial sections. The experimental procedures for recognition of antigen-specific Foxp3 and CD25 as well as CD4 were carried out using a DouSP™ KIT (MXB, Fuzhou) according to the manufacturer’s instructions. Development of the chromogenic color reaction was accomplished using 5-Bromo-4-Chloro-3-Indolyl Phosphate/nitroblue tetrazolium (BCIP/NBT) or 3-amino-9-ethylcarbazole (AEC) (MXB, Fuzhou). The average number of $CD4^+Foxp3^+CD25^{+/-}$ Tregs for each lobule or portal tract was calculated from representative photomicrographs ($\times 400$) of single/double immunostaining for the cells in three lobules and three portal tracts from serial sections following single/double immunostaining. The relationship between the number of $CD4^+Foxp3^+CD25^-$ Tregs and the degree of lobular inflammation was analyzed.

Flow cytometry analysis

Blood samples were collected from 9 of 15 DIAIH patients, 8 of 24 AIH patients, and from 8 healthy controls one day before or on the same day of liver biopsy and before immunosuppressive therapy. Total 25 mL of each blood sample was topped up with MACS buffer (PBS, 0.5% BSA, 2.5 mmol/L EDTA) to a total volume of 35 mL, layered onto 15 mL of human lymphocyte separation medium (Dakewe, Shenzhen, China) and centrifuged at 400 g for 30 min. The mononuclear cells were collected and washed twice with MACS buffer. To quantify Tregs in blood samples, flow cytometry (BD, FACSCalibur, San Jose, USA) was performed on mononuclear cells stained with mouse anti-human CD4 conjugated to FITC, anti-CD25 conjugated to APC, or anti-Foxp3 conjugated to PE (BD, FACSCalibur, San Jose). The number of $CD4^+Foxp3^+CD25^{+/-}$ Tregs in a total number of 1×10^4 $CD4^+$ T cells was then analyzed with Flowjo 7.6.1 software.

Statistical analysis

Data are presented as median (interquartile range, IQR) or number (%). The Wilcoxon–Mann–Whiteney test was used to compare continuous variables and the Fisher’s exact test was used to compare categorical data. Wilcoxon rank sum test was used to compare nonparametric variables. All reported P values are two-tailed. A $P<0.05$ was considered statistically significant. For correlation analysis, the Pearson’s correlation coefficient was calculated. Statistical analyses were done using SPSS version 18 (IBM, Armonk, USA).

Results

General characteristics of the patients

Clinical characteristics of patients with DIAIH and AIH at initial presentation are summarized in Table 1. All DIAIH patients were female, with a median age of 53.12 years (46.43–57.18). Total 95.8% (23/24) of AIH patients were female, with a median age of 54.26 years (46.53–65.17). The median values of ALT and AST were 404.50 U/L (299.00–734.75) and 454.10 U/L (287.00–767.00) in DIAIH patients and 309.50 U/L (158.32–574.75) and 315.00 U/L (177.95–396.33) in AIH patients, respectively. The ALT and AST values were significantly higher in DIAIH than those in AIH patients ($P<0.05$). However, there was no significant difference in other biochemical data between the two groups. All DIAIH and AIH patients were positive for ANA. The median values of ANA titres were 1:320 (1:320–1:1000) in DIAIH and 1:1000 (1:320–1:1000) in AIH, respectively, without significant difference between two groups. There were positive for SMA in one of the AIH patients and two of the DIAIH patients. But all patients tested negative for anti-LKM-1 as well as SLA/LP antibodies.

Please cite this article as: L.-M. Qu et al., $CD4^+Foxp3^+CD25^{+/-}$ Tregs characterize liver tissue specimens of patients suffering from drug-induced autoimmune hepatitis: A clinical-pathological study, Hepatobiliary & Pancreatic Diseases International (2018), https://doi.org/10.1016/j.hbpd.2018.02.004