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Evaluation of intrahepatic regulatory T cells to understand their roles in the progression of liver damage in patient with hepatitis

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Abstract. Infection with hepatitis viruses, especially HBV and HCV is a global health problem. Inadequacy and inefficiency of immune responses contribute to the chronicity of the diseases and play an important role in the progress of liver injury. This paper aimed to evaluate the frequency of immune cells in the liver of patients infected with HBV and HCV and analysed the correlation between pathological findings and clinical course of the diseases. The currently on going study recruited participants who were 18 years old or older and presented to a tertiary hospital in Surakarta, Indonesia since August 2017. Clinical and laboratory data were extracted from the patients' medical records. The biopsy procedure was performed on patients' liver as referred by the doctors who treat them. Samples were sent to the Pathology Anatomy Laboratory for assessment of the disease progression and the evaluation of immune cells in the area of portal triad. An immunohistochemistry staining was conducted to enumerate the frequency of immune cells expressing CD4⁺, CD8⁺, CD25⁺ and Foxp3⁺ which were associated with the presence of T lymphocytes within the subgroups of T helper, T cytotoxic, and T regulatory cells, respectively. From six liver biopsy samples, we detected one unknown hepatitis case, one case of acute viral hepatitis B, three cases of chronic viral hepatitis B without fibrosis, and one case of chronic viral hepatitis C METAVIR score 1. The frequency of cells expressing CD4⁺ and CD8⁺ were predominant (>50%), followed by Foxp3⁺ expression (26-50%); whereas cells expressing CD25⁺ were being rarely detected (0-5%). These findings suggest that when the liver injury is minimal, the T helper and cytotoxic lymphocytes are proliferated and activated, which may promote the differentiation of regulatory T cells expressing CD25⁺ and Foxp3⁺ to minimize immune-mediated liver damage.

Keywords: intrahepatic regulatory, t-cells, liver damage, hepatitis



1. Introduction

Hepatitis disease has been known as one of the health problems in the community. There are several types of hepatitis, namely hepatitis A, B, C, D, and E. Hepatitis A and E often present as outbreaks, transmitted through the fecal-oral route, and often being acute self-limiting diseases. In contrast, hepatitis B, C, and D are transmitted parenterally, often become chronic infection which eventually ends up as liver cirrhosis and malignancy. As a matter of fact, chronic infection occurs in more than 70% of HCV-infected adults and around 90% of HBV-infected neonates acquired the virus through vertical transmission [1]. According to the World Health Organization, there were 240 million people living with chronic hepatitis B and 150 million people have chronic hepatitis C, causing more than a million deaths per year [2, 3]. The mechanisms which cause high prevalence of chronic hepatitis B and C are not yet to be elucidated, but it has been known that individuals with HBV and HCV chronic infection have a higher risk for developing liver cirrhosis and hepatocellular carcinoma.

Previous studies showed that T cell response is crucial to control HBV and HCV infection [4-7]. An adequate response of T cells is needed to facilitate spontaneous resolution during acute HBV or HCV infection and to prevent chronic persistent infection. Indeed, the decrease of CD4⁺ and CD8⁺ T cells disrupt viral clearance. On the other hand, T cells also contribute to the development of liver injury during HBV and HCV infection. Liver infiltration of T cells accompanied by the increase of serum alanine aminotransferase (ALT) during acute HBV and HCV infection indicates the important role of T cells in liver injury [84]. Unfortunately, the exact mechanism of T cells-mediated liver injury is not well understood since it is uncommon and perhaps unethical to examine the liver tissue of HBV- or HCV- infected patients during the early stage of infection unless there is a strong justification to perform the liver biopsy procedure.

Regulatory T cells (Tregs) consist of specific T cell population that can suppress the activation, proliferation, differentiation and other effector functions of various immune cells, including T cells, B cells, natural killer (NK) cells, and dendritic cells. In particular, Tregs ensure immunological tolerance by suppressing auto-reactive T cells and also control excessive immune activation following infection by pathogens. In HBV and HCV infection, Tregs play an important role in modulating the antiviral response of T cells and immune-mediated host injury during acute and chronic infection [9]. This paper aimed to evaluate the frequency of immune cells in the liver of patients infected with HBV and HCV and analyze the correlation between pathological findings and clinical course of the diseases.

2. Method

This study is conducted at Dr. Moewardi hospital, a tertiary hospital in Surakarta, Indonesia. As the main hospital in Central Java province, the hospital serves more than 34 million populations and is equipped with the most updated facilities and supported by various specialists and consultants (sub-specialists). In addition to its role in health service, the hospital is also a center of teaching and research and has a strong collaboration with the adjacent medical faculty.

This study is part of a larger research project studying the molecular epidemiology of blood-borne viruses [11, 12]. The protocol has been approved by the Human Research Ethics Committee at Dr Moewardi hospital (No. 548/ VIII/ HREC/ 2017). Our research group has collected blood samples from HIV patients since 2009, and starting from August 2017 we collected liver biopsy samples from patients presenting to Dr Moewardi hospital with hepatitis. Data retrieved from medical records include clinical information as well as laboratory and radiology findings. The liver samples were sent to the Pathology Anatomy Laboratory for the assessment of disease progression and the evaluation of immune cells in the area of portal triad using a standard procedure for immunohistochemistry staining. The assessment of disease severity was done and reported by a pathologist at Dr Moewardi hospital and the enumeration of immune cells in portal triad was performed by another trained pathologist at Faculty of Medicine, Universitas Sebelas Maret by using quantification and scoring system established by a previous study [13]. For the evaluation of immune cells in the patients liver, we determined the

proportion of CD4⁺, CD8⁺, CD25⁺ and FoxP3 cells which represent the subsets of T cells, including T helper, T cytotoxic, and regulatory T cells. The clinical course of disease was determined from the report made by the doctors who treat the patients (clinical diagnosis) as well as from the Pathology Anatomy specialist who interpreted the results of liver biopsy.

3. Results

Six patients, aged between 29 and 63 years old, were involved in this currently ongoing study. The results of this study are summarized in Table 1 and the representative figures are presented in Figure 1-4 (400x magnification), in which the expression of CD4⁺ and CD8⁺, CD25⁺ and FoxP3⁺ is indicated by brown color in immunohistochemistry staining. We found that CD4⁺ and CD8⁺ T cells are the predominant immune cells, followed by Foxp3⁺ and CD25⁺ cells.

Table 1. Individual characteristics, clinical and laboratory data of study participants.

Patient ID	Demographic data (initial, sex, age)	Clinical diagnosis	Pathology anatomy diagnosis	Frequency of immune cells
001	N, F, 29	Hepatitis B	Chronic hepatitis B, mild, without fibrosis	CD25: 0-5% CD8: >50% CD4: >50% FoxP3: 26-50%
002	HS, F, 49	NA	Chronic hepatitis B, mild, without fibrosis	Cannot be assessed because the specimen did not contain the portal triad
003	S, F, 41	NA	NA	CD25: 0-5% CD8: >50% CD4: >50% FoxP3: 0-5%
004	SS, F, 56	NA	Chronic hepatitis C, METAVIR score (1)	CD25: 0-5% CD8: 26-50% CD4: >50% FoxP3: 0-5%
005	D, M, 58	Hepatitis A	The appearance of ground glass hepatocytes commonly seen in chronic hepatitis B, without fibrosis	CD25: 0-5% CD8: >50% CD4: >50% FoxP3: 0-5%
006	M, F, 63	Hepatitis B	Acute viral hepatitis B	CD25: 0-5% CD8: >50% CD4: >50% FoxP3: 0-5%

F: female, M; male, NA: not available

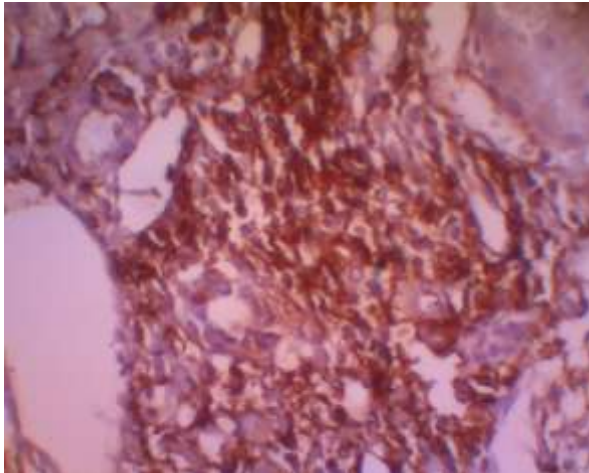


Figure 1. CD4⁺ expression in cell membrane (brown color, 400x magnification).

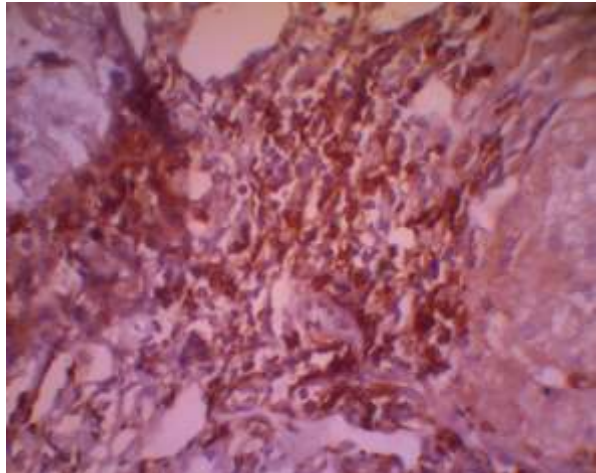


Figure 2. CD8⁺ expression in cell membrane (brown color, 400x magnification).

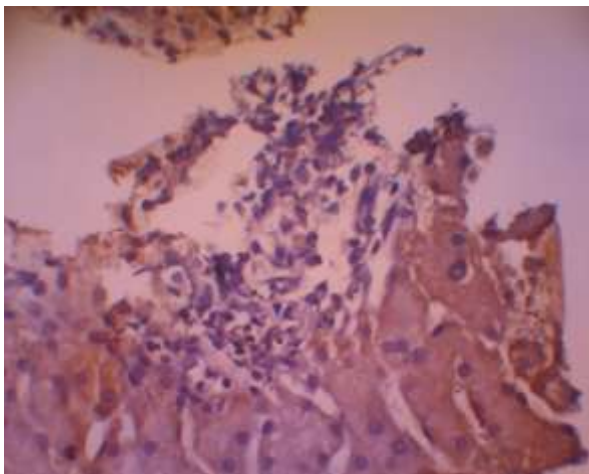


Figure 3. CD25⁺ expression in cell membrane (brown color, 400x magnification).

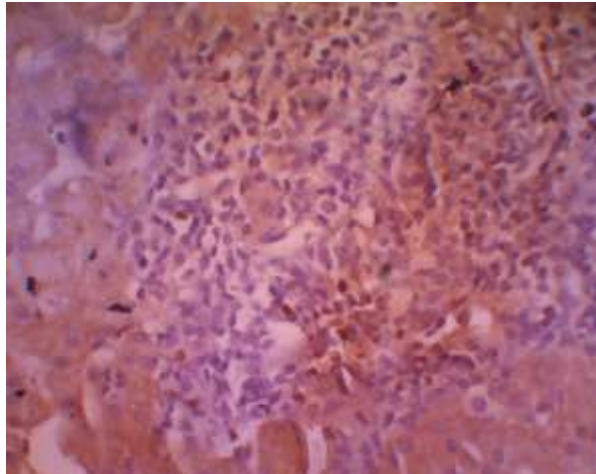


Figure 4. Foxp3⁺ expression within the nucleus (brown color, 400x magnification).

4. Discussion

Infection with HBV and HCV is usually subclinical in which the manifestations are often not recognized until the liver cells damage has occurred for years. Therefore, early infection is often misdiagnosed and the treatment is delayed. Moreover, chimpanzees and squirrels are the only animals could have natural HBV and HCV infection, thus limiting animal models to study the viruses [14, 15].

It has been known that HBV and HCV are hepatotropic and can induce chronic and persistent liver inflammation that leads to immune-mediated liver damage. The inability of host immune system to eliminate the virus is considered as an important cause of chronic persistent infection. Our study results show that viral hepatitis infection cause proliferation and activation of T lymphocytes in liver, mainly T helper (CD4⁺) and T cytotoxic (CD8⁺). A small proportion of the CD4⁺ and CD8⁺ T cells have a regulatory role, indicated by the expression of CD25⁺ and FoxP3⁺. Whilst most studies evaluating immune responses in HBV and HCV infection are conducted during chronic phase and the results are inconsistent, the frequency of Tregs in our study shows similar trends amongst samples evaluated.

The exact mechanism responsible for the proliferation and activation of intrahepatic Tregs is not fully understood. A hypothesis that could be proposed is that perpetual exposures to HBV and HCV

antigen and cytokines lead to the production of induced Tregs. Naturally occurring and inducible Tregs show suppressive effects via intercellular contact or in a contact-independent manner through the release of cytokines. One possible explanation for the increase of intrahepatic Tregs is that during chronic HBV infection, hepatic stellate cells produce tumor growth factor beta (TGF- β) that promote the differentiation of conventional CD4⁺ into induced Tregs [1]. Interleukin-6 also has an important role in the pathogenesis of chronic viral hepatitis and associated liver diseases. An increase in IL-6 production influences Th17 and Tregs response which is correlated with a higher risk of HBV acquisition and liver inflammation [16].

In HBV infection, Tregs serve as a strong immune modulator and appears to play a major role in the development of hepatocellular carcinoma. In addition, a previous study showed that increased secretion of IL-10 and TGF- β in HBV-associated hepatocellular carcinoma [17]. Interleukin 10 and TGF- β are responsible for suppressing anti-tumor immune response, thus facilitating tumor escape. Other causes or risk factors for hepatocellular carcinoma include autoimmune hepatitis, severe alcohol consumption, aflatoxin B1, obesity, excessive iron, age, and gender, by which male is more vulnerable than female [18]. Since we do not yet have samples from patients with hepatocellular carcinoma, we could not evaluate the extent of immune cells, particularly Treg, in these patients. A further effort for recruiting participants with different stages of hepatitis is required to complete our understanding about intrahepatic immune response during hepatitis.

5. Conclusions

In the course of HBV and HCV infection, Tregs suppress T cell response against the virus which is detrimental to the host but at the same time prevent immune-mediated liver injury which is beneficial to the host. It is not clear whether those opposite roles are facilitated by an identical Tregs population or by different T regs population. It is also unclear what mechanisms employed in such events. A more detailed information is needed to broaden our knowledge about immunopathology of hepatitis and may serve as a therapeutic target for clinical application. This study provides evidence of Tregs hepatic involvement during the course of HBV and HCV infection.

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