The Prevalence of T Cells Population in the Liver of Patients with Viral Hepatitis

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ABSTRACT

Background: *It has been widely known that viral hepatitis is a major cause of liver disease that can cause chronic inflammation and carcinoma. This study aimed to describe the frequencies of $CD4^+$ and $CD8^+$ T cells, as well as regulatory T cells ($CD25^+$ and $Foxp3^+$ T cells) in the liver of patients with viral hepatitis in order to understand the comprehensive role of T lymphocytes in the progression of liver diseases attributed to viral hepatitis.*

Method: *Liver biopsies were performed on adult patients presenting to a tertiary hospital in Surakarta, Indonesia with viral hepatitis from 2017 to 2018. Immunohistochemical staining was performed to identify cells expressing $CD4^+$, $CD8^+$, $CD25^+$ and $Foxp3^+$ which represent T helper, T cytotoxic, and T regulatory cells, respectively. Additional data were retrieved from the patients' medical records, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, viral load, and results of ultrasonography and fibroscan.*

Results: *A total of 25 liver samples were collected from patients with chronic HBV infection (n = 21), chronic HCV infection (n = 2), acute HBV infection (n = 1), and from a with multiple liver nodules. The liver injury is minimum in all patients. The study found that $CD8^+$ and $CD4^+$ T cells were predominant whilst the frequency of T regulatory cells is generally low.*

Conclusions: *The findings indicate the involvement of intrahepatic T helper and T cytotoxic in the pathogenesis of viral hepatitis. These liver infiltrating T cell subsets may be readily differentiated into regulatory T cells expressing $CD25^+$ and $Foxp3^+$ in order to prevent severe inflammation and maintain disease chronicity.*

Keywords: *T-lymphocytes, viral hepatitis, immune response*

ABSTRAK

Latar belakang: *Hepatitis virus merupakan penyebab utama penyakit hati yang dapat menimbulkan peradangan kronik dan karsinoma. Penelitian ini bertujuan untuk mendeskripsikan frekuensi sel T $CD4^+$, $CD8^+$ dan sel T regulatory ($CD25^+$ and $Foxp3^+$) pada hati pasien dengan hepatitis virus untuk memahami peran menyeluruh dari limfosit T pada perjalanan penyakit hati yang disebabkan oleh hepatitis virus.*

Metode: *Biopsi hati dikerjakan pada pasien dewasa dengan hepatitis virus yang berkunjung ke sebuah rumah sakit tersier di Surakarta, Indonesia pada tahun 2017 hingga 2018. Pengecatan imunohistokimia dilakukan untuk mengidentifikasi sel-sel yang mengekspresikan $CD4^+$, $CD8^+$, $CD25^+$ and $Foxp3^+$ dimana sel-sel tersebut merepresentasikan sel-sel T helper, T cytotoxic, dan T regulatory. Data tambahan berupa nilai alanine aminotransferase (ALT) dan aspartate aminotransferase (AST), viral load, dan hasil ultrasonografi serta fibroscan diambil dari rekam medis pasien.*

Hasil: *Sejumlah 25 sampel hati diperoleh dari pasien dengan infeksi HBV kronik (21 orang), infeksi HCV kronik (2 orang), infeksi HBV akut (1 orang) dan 1 pasien lain dengan nodul-nodul di hati. Seluruh pasien menderita kerusakan hati minimal. Tiga sampel dieksklusi dari analisis sel T karena ukuran spesimen yang terlalu kecil. Peneliti menemukan bahwa sel T yang mendominasi pada sampel adalah sel-sel $CD8^+$ dan $CD4^+$ sedangkan frekuensi sel T regulatory umumnya rendah.*

Kesimpulan: *Hasil penelitian ini mengindikasikan bahwa sel T helper dan sel T sitotoksik di dalam hati terlibat dalam patogenesis hepatitis virus. Subset dari sel T ini mungkin siap berdiferensiasi menjadi sel T regulatory yang mengekspresikan $CD25^+$ dan $Foxp3^+$ untuk mencegah peradangan yang berat dan menjaga kronisitas penyakit.*

Kata kunci: *limfosit T, hepatitis virus, respon imun*

INTRODUCTION

Viral hepatitis causes systemic infection with liver as the main target. The infection causes liver inflammation and necrotic of hepatocytes accompanied with mononuclear cells infiltration. Hepatitis A and E viruses (HAV and HEV) are transmitted through the fecal-oral route and often being acute self-limiting diseases. In contrast, hepatitis B, C, and D viruses (HBV, HCV, and HDV) are transmitted parenterally and often cause chronic liver diseases that may develop into liver cirrhosis and hepatocellular carcinoma. Although chronic hepatitis are widely studied, there is a lack of knowledge in regards of the immune mechanisms leading to chronicity and irreversible liver damage.[1,2]

Previous studies have reported the importance of T cells in the course of viral hepatitis as well as their involvement in response to treatment, particularly in HBV and HCV infection.[3-7] T cells are subpopulation of lymphocytes that are derived from bone marrow, and develop into several types in the thymic gland and continue to differentiate in peripheral sites and blood circulation under certain conditions. Each group of specific T cells provides distinctive immune responses to internal and external stimuli. For example, $CD8^+$ T cells contain cytotoxic properties that enable them to directly destroy virus infected cells and cancer cells. In contrast, $CD4^+$ T cells also known as T helper cells, perform indirect killing of foreign or infected cells by influencing other cells of immune systems to exhibit appropriate immune responses. Further, $CD4^+$ T helper cells are divided into several types: Th1, Th2, Th17, Th9 and Tfh with different roles in immune defence. Another subset of T cells includes regulatory T cells. These cells are involved in immune tolerance mechanisms thus preventing excessive immune responses that may provoke autoimmunity and self-destruction due to severe inflammation. In this study, we sought to determine the prevalence of intrahepatic T cells in order to figure out the underlying mechanisms of chronicity and immune tolerance in viral hepatitis.

METHOD

The study recruited patients of ≥18 years old with viral hepatitis who presented to Dr. Moewardi Hospital in Surakarta, Central Java, Indonesia from 2017 to 2018. The participation in this study was voluntarily and all the study subjects were given informed consent. We employed total-sampling technique by approaching all the potential participants who met our criteria; i.e., adult, had a clinical diagnosis of viral hepatitis, presented as in- and out-patients at Dr. Moewardi hospital during the study period (2017-2018), and being able to give individual consent. Demographic data, pathology and radiology results were retrieved from medical records. The procedure of liver biopsy was performed by or under supervision of a gastroenterohepatology consultant (TYP), Department of Internal Medicine, Dr. Moewardi Hospital. A total of 25 patients were recruited into this study and following biopsy procedure, the liver samples were sent to both Pathology Anatomy Laboratories at Dr. Moewardi hospital and Faculty of Medicine, Universitas Sebelas Maret (UNS) for the assessment of disease severity and the evaluation of subpopulation of T cells in the area of portal triad using a standard procedure for immunohistochemistry staining with anti-CD4, anti-

CD8, anti-CD25, and anti-Foxp3 antibodies (abcam). Three specimens were excluded from the analysis of T cell population because the specimens were too small and did not represent the area of portal triad.

The assessment of disease severity was performed and reported by a pathologist at Dr Moewardi hospital and the results were categorized into no fibrosis (METAVIR score=F0), mild fibrosis (METAVIR score=F1), medium fibrosis (METAVIR score=F2), and severe fibrosis (METAVIR score=F3 or F4). The enumeration of T cells in portal triad was performed by another pathologist (BW) at Faculty of Medicine, UNS by using quantification and scoring system established by a previous study.[8] The frequencies of T cell subsets were categorized into low (0-5%), medium (6-25%), high (26-50%), and very high (>50%). Data were incorporated into a Microsoft Excel spread sheet in which we performed a heat map to visualize each subpopulation of T cells presented in the samples. This study was part of a larger research project studying the molecular epidemiology of blood-borne viruses conducted by A-IGIC research group of UNS.[9-11] The protocol of this particular study has been approved by the Human Research Ethics Committee at Dr Moewardi hospital (No. 548/ VIII/ HREC/ 2017).

RESULTS

A total of 25 liver samples were collected from 10 males and 15 females with their age ranged from 18 to 70 years old. The majority of samples were taken from patients with chronic hepatitis B (n = 21), followed by chronic hepatitis C (n = 2), and acute hepatitis B (n = 1). A 62 year old female patient with multiple nodule in her liver (the patient ID number is 7) had a clinical diagnosis of viral hepatitis but upon further laboratory investigation, a specific diagnosis was not ascertained in this patient. All patients with chronic hepatitis B and C had mild liver injury determined either by ultrasonography, fibroscan or histologic evaluation.

The HBV DNA viral load ranged from $1.68x10^3$ to $1.1x10^8$ IU/mL and the HCV RNA ranged from $1.55x10^5$ IU/mL to $7.07x10^5$ IU/mL. Chronic hepatitis B is characterized by a detectable HBsAg for at least 6 month period with or without the presence of HBeAg. In our study, 28.6% (6/21) of patients with chronic hepatitis B also had reactive HBeAg. Both HBsAg and HBeAg were detected in the subject with acute hepatitis B, indicating the high level of viral replication so that the patient is highly infectious. In fact, this patient's HBV DNA was $4.22x10^7$ IU/mL.

The frequencies of T helper ($CD4^+$) and T cytotoxic ($CD8^+$) vary considerably among the samples (Table 1). In general, most samples had medium and high level of T helper and T cytotoxic cells. In contrast, the frequencies of regulatory T cells ($CD25^+$ and $Foxp3^+$) were minimum except in a sample taken from a 29 year old female patient with chronic hepatitis B (the patient ID number is 6). In this particular patient, the level of alanine aminotransferase (ALT) and (aspartate aminotransferase) AST were just above the upper normal limit (54 U/l and 39 U/l, respectively).

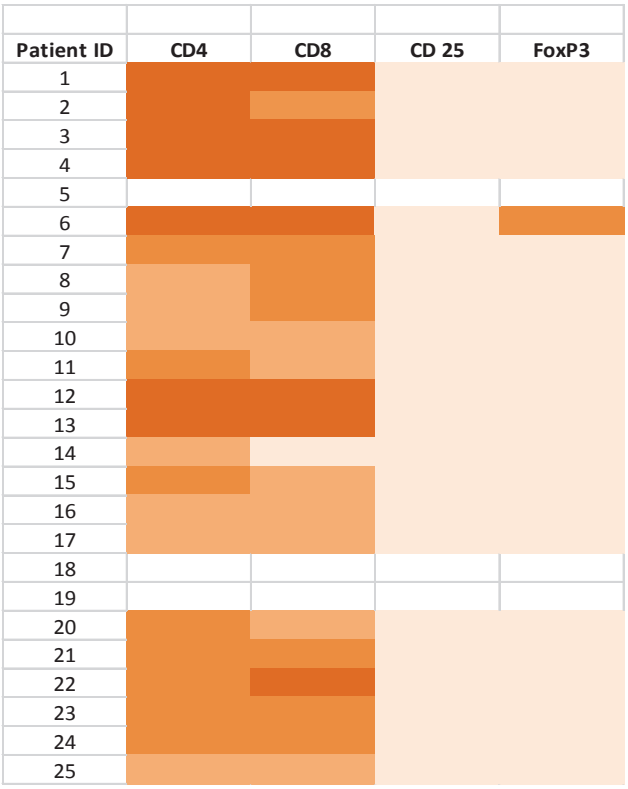

Figure 1. Table 1. T-cell frequencies analyzed by immunohistochemistry staining

The proportion of T cell subsets is shown by graded colour where darker colour indicates a higher frequency. Samples ID number 5, 18 and 19 were excluded from T cells analysis because the specimens were too small and not being representative, of which did not contain portal triad.

DISCUSSION

Viral hepatitis is a major health problem in the world, especially in endemics area of low and middle income countries such as Indonesia. Therefore, control measures of viral hepatitis are needed to suppress morbidities and mortalities as well as other impacts on the country's socio-economic. According to the etiologies, viral hepatitis can be

classified into hepatitis A, B, C, D and E. Hepatitis A dan E often present as outbreaks and the primary control measures include healthy behaviour and high quality environment. Hepatitis B, C and D can be transmitted perinatally so that the prevention can only be achieved through avoiding the source of infection and vaccination. Hepatitis B vaccine has proven to be effective in preventing hepatitis B (and hepatitis D) but unfortunately, there is no available vaccine for preventing hepatitis C.

This study is looking at immunological aspect of viral hepatitis which specifically involve T cells in the host's liver. We found that T helper and T cytotoxic are the predominant subsets of T cells in the liver of patients with HBV and HCV infection. A small proportion of regulatory T cells is also found, indicating the role of these cells in suppressing effector function of T helper and T cytotoxic. In general, liver infiltration of various subsets of T cells indicates the balance between the effort of immune responses to eliminate the virus and the effort of immune responses to minimize tissue and organ damage. As the results, this contradictory events contribute in the disease chronicity and prevention of immune-associated liver injury.[12,13]

The exact mechanism of proliferation, activation, and differentiation of T cells is not fully understood. The continuing exposures to the viral antigens and cytokines may lead to the production and activation of T cells. As for the differentiation of T cells, it has been proposed that during chronic HBV infection, hepatic stellate cells produce tumor growth factor beta (TGF-β) that promotes the differentiation of T helper cells into regulatory T cells.[14] Another study reported that interleukin 6 is associated with a higher risk of HBV acquisition and liver inflammation.[15]

The weakness of this study is that we were only able to recruit a few participants. This is because liver biopsy is a high-risk medical procedure and costly. Another weakness is that we had only a few variety of cases. Most of the study subjects are patients with chronic hepatitis B. Despite the weaknesses, this study provide evidence of hepatic infiltration of various subsets of T cells. Future studies need to involve more study subjects and more variety of cases so that more information can be obtained with and a robust conclusion can be achieved.

CONCLUSION

In viral hepatitis, an adequate response of T cells is required to facilitate spontaneous resolution as well as minimizing inflammation in liver tissues. The identification of different subsets of T cells in this study provides evidence of the involvement of intrahepatic T cells in those two important roles: eliminating the virus as well as preventing immune-associated liver injury. Further studies are needed to understand the comprehensive mechanism of immune responses during the course of viral hepatitis.

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