A Systematic Review of the Frequency of Regulatory T Cells in Hepatitis B and Hepatitis C

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ABSTRACT

Background: *Regulatory T cells (Tregs) play an important role in sustaining the hepatitis B and C viruses (HBV and HCV) persistence and protecting the liver tissues from cytokine-associated detrimental effects through unclear mechanisms. This paper aims to review the frequency of Tregs during the course of HBV and HCV infection.*

Method: *Electronic databases were searched to identify studies investigated the frequency of intrahepatic and peripheral Tregs of the patients infected with HBV and/ or HCV.*

Results: *The majority of studies reported the increase of intrahepatic and peripheral Tregs in acute and chronic infection of HBV and HCV. The decrease of peripheral Tregs occurred in patients with chronic hepatitis B who respond to interferon α or nucleos(t)ide analogues treatment as well as those with chronic hepatitis C who were treated with interferon, ribavirin or liver transplantation.*

Conclusion: *Infection with HBV and HCV appears to induce the production of Tregs in blood and hepatocytes whereas treatment may decrease Tregs levels. As the optimum balance between regulatory and effector T during HBV and HCV infection is crucial for preventing liver damage, further studies should be directed on the development of Tregs during HBV and HCV infection as well as their involvement in immunomodulatory strategies for combating HBV and HCV.*

Keywords: *T-lymphocytes, regulatory, hepatitis B, hepatitis C*

ABSTRAK

Latar belakang: *Regulatory T cells (Tregs) memainkan peranan penting dalam menjaga keberlangsungan infeksi virus hepatitis B dan C (HBV dan HCV) serta melindungi jaringan hati dari kerusakan yang diakibatkan oleh sitokin melalui mekanisme yang belum diketahui secara pasti. Artikel ini bertujuan untuk menelaah frekuensi Tregs selama perjalanan infeksi HBV dan HCV.*

Metode: *Penulis mencari artikel penelitian di basis data elektronik. Artikel yang diidentifikasi adalah studi yang menginvestigasi frekuensi Treg di hati dan di sirkulasi darah dari pasien-pasien yang terinfeksi HBV dan/ atau HCV.*

Hasil: *Sebagian besar artikel melaporkan adanya peningkatan Tregs intrahepatik dan perifer pada infeksi HBV dan HCV, baik akut maupun kronik. Penurunan frekuensi Treg di perifer terjadi pada pasien dengan hepatitis B kronik yang berespon terhadap pengobatan interferon α atau analog nucleos(t)ida, demikian pula pada penderita hepatitis C yang diterapi dengan interferon, ribavirin atau transplantasi hati.*

Simpulan: *Infeksi HBV dan HCV tampaknya menginduksi produksi Treg di darah dan sel-sel hati sedangkan pengobatan dapat menurunkan jumlah Treg. Keseimbangan yang optimal antara Treg dan T efektor selama infeksi HBV dan HCV penting untuk mencegah kerusakan hati. Oleh karena itu, diperlukan penelitian-penelitian lanjutan terkait perkembangan Treg selama infeksi HBV dan HCV serta keterlibatannya dalam strategi imunomodulasi untuk melawan HBV dan HCV.*

Kata kunci: *limfosit T, regulatory, hepatitis B, hepatitis C*

INTRODUCTION

Hepatitis B and hepatitis C are important public health issues with increasing numbers of patients despite the immense efforts to control the transmission. The World Health Organization (WHO) estimated that in 2015, there were 257 million people have chronic HBV infection and 71 million cases of chronic HCV infection.[1] Whilst antiviral and immunological treatments are available and on trials, none can eliminate the viruses and prevent the chronic sequel of HBV and HCV infection. Thus, it poses a substantial threat to the chronically infected patients as the infection cannot be totally cured, leading to terminal illness due to liver failure and carcinoma. The long-term morbidity in hepatitis B and C cases are clearly associated with significant clinical and economic burden.[2]

The rules of cellular and humoral immunity in hepatitis B and C have been widely studied but limited data exist regarding the role of regulatory T cells (Tregs) in the course of these illnesses. The majority of studies on hepatitis B immunity have frequently reported Natural Killer (NK), $CD4^+$ cells, $CD8^+$ cells, and antibodies to HBV. As for hepatitis C, previous reports have primarily focused on interferon response, antibodies HCV and cytotoxic T lymphocytes. Jung and Shin suggested that Tregs are involved in the disease chronicity by maintaining viral persistence and protecting the liver tissues from cytokine-associated detrimental effects.[3]

This paper presents a review of the available literature reporting studies that measure the frequency of Tregs in various states of HBV and HCV infection.

METHOD

On 14 August 2017, literature were searched in PubMed, Scopus, Web of Science and Medline databases using the following terms: "regulatory T cell [Title/Abstract]" AND "hepatitis [Title/Abstract]" AND "infection". The results were reviewed systematically according to PRISMA guidance.[4] Inclusion criteria included journal articles published in English that described Tregs in HBV and HCV research. Review articles were excluded as well as case reports and studies that focused on children, malignancy, inflammatory or autoimmune diseases and those including other pathogens such as co-infection with human immunodeficiency virus (HIV). The articles identified were reviewed systematically and references of the included papers were also examined.

RESULTS

The initial search identified 224 articles. After removing duplicate articles, 129 articles remained, of which 35 were removed after reading their titles and/or abstracts. The entire texts of the 94 remaining articles were reviewed, of which 37 passed all exclusion criteria and met all inclusion criteria. After evaluating the references, listed in 37 articles above, 18 articles were added to the review resulting in the total of 55 articles being analysed. (Figure 1)

Table 1 summarizes the 55 studies evaluating Tregs levels at different stages of HBV or HCV infection. The majority of studies involved chronically infected patients. Only two studies reported Tregs level in acute hepatitis B (AHB) and the other two studied acute hepatitis C (AHC).[5,6,31,32] Five studies reported Tregs level in patients with HBV-related acute-on-chronic liver failure (ACLF).[9,10,21-23]

During the acute stage of HBV infection, the frequency of peripheral Tregs remains normal whilst HCV caused an immediate increase of Tregs level in circulation. Most patients with chronic HBV and HCV infection had an increase of Tregs level in their blood circulation and liver. The treatment of chronic hepatitis B could reduce Tregs frequency but in patients with chronic hepatitis C, the results were not consistent.

DISCUSSION

Overal, there are 20 longitudinal studies and 34 cross-sectional studies. One study performed both longitudinal and cross-sectional approaches.[47] For the

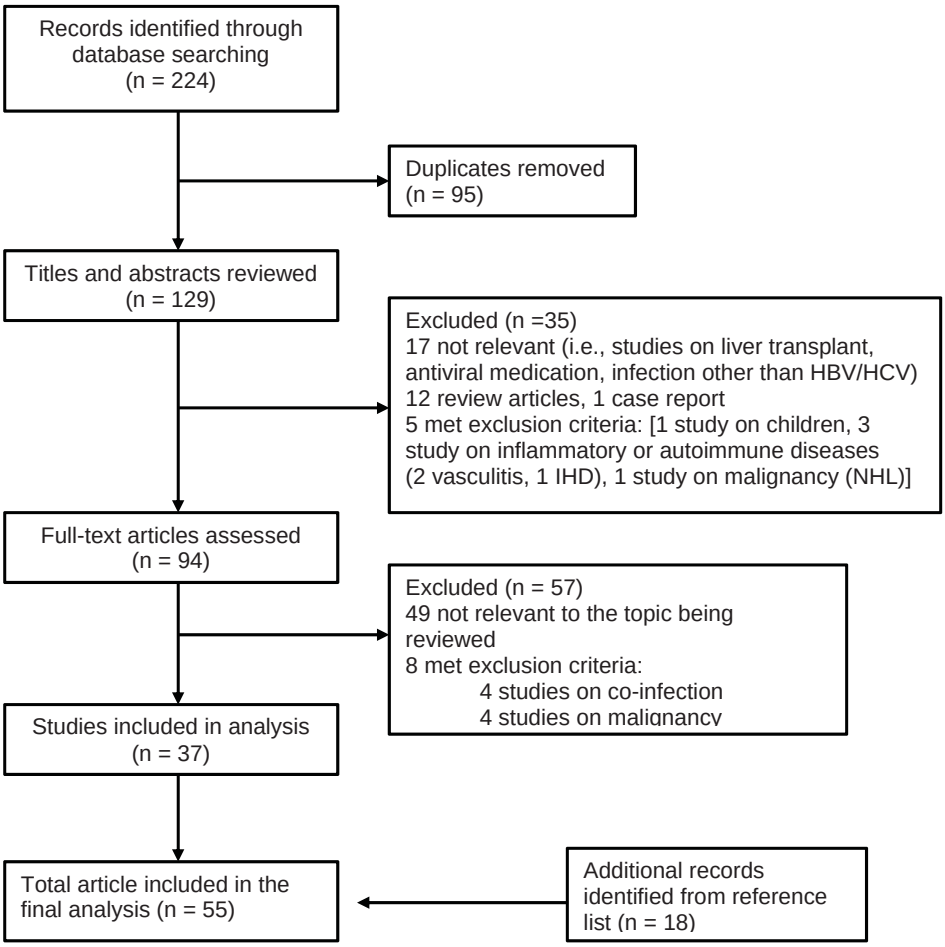
Table 1. The study of Tregs frequency in HBV and HCV infection

No.	Author (year), ref.	Study design	Study subjects (numbers)	Frequency of intrahepatic (I) and peripheral (P) Tregs in patients and other findings*
1.	Peng et al (2008)[5]	CS	AHB (12)	P: =
			CHB (79)	P: +
2.	Xu et al (2006)[6]	L	AHB (16 blood samples)	P: initially low, + in the convalescent phase, normal upon resolution
			CHB (76 blood samples, 13 liver tissues)	I: +; P: +
			CSHB (29 blood samples, 9 liver tissues)	I: ++; P:++
3.	Wang et al (2017)[7]	CS	CHB (43)	I: +
4.	Stoop et al (2008)[8]	CS	CHB (32)	I: +, a higher proportion of Tregs in atients with a high viral load
5.	Shen et al (2015)[9]	CS	CHB (44)	I: +; P: +
			HBV-related ACLF (32)	I: ++; P: ++
6.	Niu et al (2011)[10]	CS	CHB liver failure (26)	I: +
			HBV-related ACLF (31)	I: +, higher IL-17/FoxP3 ratio than the CHB liver failure group
7.	El-Badawy et al (2012)[11]	CS	CHB (59)	P: +
8.	Feng et al (2015)[12]	CS	CHB (96)	P: +
9.	Lin et al (2007)[13]	CS	CHB (79)	P: +
10.	Nan et al (2010)[14]	CS	CHB (39)	P: +, correlated positively with HBV DNA load
11.	Su et al (2013)[15]	CS	CHB (70)	P: +
12.	Yu et al (2014)[16]	CS	CHB (70)	P: ++
			HBV-associated liver chirrosis (28)	P:+
13.	Li et al (2014)[17]	CS	CHB (26)	P: +
14.	Yang et al (2007)[18]	CS	CHB (65)	P: +
15.	Stoop et al (2005)[19]	CS	CHB (50)	P: +
16.	Li et al (2012)[20]	CS	CHB (77)	P: +, a lower Treg/Th17 ratio indicated more liver injury and fibrosis progression
17.	Liang et al (2014)[21]	CS	HBV-related ACLF (18), CHB (18)	P: +, an increased Treg/Th17 ratio was associated with the survival of ACLF patients
18.	Zhai et al (2011)[22]	CS	HBV-related ACLF (30), CHB (30)	P: +, the ratio of TH17/Treg was associated inversely with the survival of ACLF patients
19.	Zhang et al (2013)[23]	CS	HBV-related ACLF (14), CHB (30)	P: +, Treg frequency was significantly lower in the remission stage of ACLF when compared with the progression stage of the CHB group
20.	Nan et al (2012)[24]	L	CHB, treated with telbivudine (28)	P: –, Tregs decline was in parallel with the decline in viral load and serum ALT normalization
21.	Stoop et al (2007)[25]	L	CHB, treated with adefovir dipivoxil (12)	P: –, adefovir induced viral load reduction results in a decline of Tregs
22.	Yu et al (2013)[26]	L	CHB, treated with entecavir, lamivudine and adefovir (44)	P: –, Treg/Th17 ratio was dramatically declined in complete-responders
23.	Xu et al (2012)[27]	L	CHB, treated with IFN-α (47)	P: –
24.	Yang et al (2017)[28]	L	CHB, treated with telbivudine (27)	P: –, Tregs frequency was positively correlated with HBeAg level
25.	Zhang et al (2010)[29]	L	Patients seropositive for HBeAg, treated with entecavir	P: –, significant reduction of Treg/Th17 ratio
26.	Sprengers et al (2007)[30]	CS	Patients positive for HBeAg, treated with Peg-IFN-α	P: + in non responders, – in responders
27.	Heeg et al (2009)[31]	CS	AHC (10), some patients received IFN+RBV	P: + (short-lived)
			CHC (15)	P: +
28.	Smyk-Pearson et al (2008)[32]	L	AHC, treatment naive (27)	P: +, the resolution of disease was associated with a relative loss of functional suppression
29.	Accapezzato et al (2004)[33]	CS	CHC (47)	I: +
30.	Claassen et al (2010)[34]	CS	CHC (8)	I: +, Treg were more numerous in livers with imited fibrosis
31.	Amoroso et al (2012)[35]	CS	CHC (30)	I: +, intrahepatic Tregs was associated with high levels of effector T cells in the peripheral blood and lower activity of hepatitis
32.	Sturm et al (2010)[37]	CS	CHC (20)	I: +, accumulation of $CD4^+FoxP3^+$ Treg cells in necro-inflammatory areas, in contact with $CD8^+$ T cells
33.	Ward et al (2007)[34]	CS	CHC (28)	I: +
34.	Hashempoor et al (2010)[38]	CS	CHC (20)	P: +
35.	Wang et al (2010)[39]	CS	CHC (31)	P: +, the increased Tregs were associated with HCV genotype 1b overexpression of TLR 2 and 4
36.	Bolacchi et al (2006)[40]	CS	CHC, treatment naive patients without liver chirrosis (37); 16 had persistently normal ALT, 21 had persistently abnormal ALT	P: +, no significant differences of Tregs level in patients with persistently normal and elevated ALT

Table 1. The study of Tregs frequency in HBV and HCV infection (continued)

No.	Author (year), ref.	Study design	Study subjects (numbers)	Frequency of intrahepatic (I) and peripheral (P) Tregs in patients and other findings*
37.	Itose et al (2009)[41]	CS	CHC (36)	P: +
38.	Tseng et al (2012)[42]	CS	CHC (57)	P: +, CHC patients with inflammation showed enhanced immunosuppressive function of Tregs
39.	Yoshizawa et al (2010)[43]	CS	HCV-infected patients (173); 76 CHC, 40 liver cirrhosis, 57 HCC	P: +, Tregs were not increased with the progression of fibrosis or the grade of inflammations
40.	Ferri et al (2011)[44]	CS	CHC (51 blood samples, 16 liver tissues)	I:+ in half of liver samples, not detected in another half
				P: +, circulating Tregs did not correlate with intrahepatic Foxp3
			HCC (23 blood samples)	P:++
			ESLD (16 blood samples)	P:++
41.	Ebinuma et al (2008)[45]	CS	CHC (14)	P: +
42.	Cabrera et al (2004)[46]	CS	CHC (30)	P: +
43.	Keoshkerian et al (2016)[47]	L	Injecting drug users were followed from uninfected status to incident infection (17)	Subjects who cleared the virus had HCV-specific CD4+ T-cell responses dominated by effector T cells and produced higher levels of IFN-γ
		CS	CHC (15)	P: +
44.	Manigold et al (2006)[48]	L	Chimpanzees (16); HCV-naive (2), HCV-recovered (8), persistently HCV-infected (6)	P: +, the frequency of Tregs and the extent of suppression was as high in spontaneously HCV-recovered chimpanzees as in persistently HCV-infected chimpanzees
45.	Akiyama et al (2010)[49]	L	CHC, treated with Peg-IFN- α and ribavirin (20)	I: +, Treg ratio was inversely proportional to viral decline in the SVR group
46.	Claassen et al (2011)[50]	L	CHC, treated with IFN-□α and ^{2b}ribavirin (22)	I: + upon treatment administration in about 50% of CHC patients. After cessation of therapy, Treg remained above baseline
				P: =
47.	Carpentier et al (2009)[51]	L	CHC, had undergone liver transplantation (30); 10 had stable HCV-negative and normal histological findings, 20 had recurrent CHC	I: + at 5 years after liver transplantation, Tr1 cells were enhanced in patients with CHC recurrence
48.	Chalupa et al (2016)[52]	L	CHC, treated with IFN-α-based therapy (31)	P: +
49.	Hao et al (2014)[53]	L	CHC, treated with Peg-IFN-α2a and ribavirin (114)	P: – in patients with RVR and EVR, = in patients with NR
50.	Kanto et al (2012)[54]	L	CHC, treated with Peg-IFN- α2b and ribavirin (67)	P: + in SVR patients
51.	Li et al (2016)[55]	L	CHC, treated with IFN- α and RBV in vitro (105)	P: –
52.	Tseng et al (2017)[56]	L	CHC, treated with Peg-IFN-α2a plus RBV or Peg-IFN-α2b plus RBV (30)	P: – regardless of the treatment response
53.	Burton et al (2008)[57]	L	CHC, treated with Peg-IFN- α2a plus RBV (62)	P:=
54.	Soldevila et al (2011)[58]	L	CHC, treated with Peg-IFN-α2a and ribavirin (35)	P: NR patients had a higher Tregs percentage than SVR patients
55.	Langhans et al (2017)[59]	L	CHC, treated with DAA only or DAA plus IFN/RBV (26)	P: + in both treatment groups

* +: the level of Tregs in study subjects increased or higher than that in healthy subjects (basal rate); –: the level of Tregs in study subjects decreased or lower than that in healthy subjects (basal rate); =: the level of Tregs in study subjects is comparabe to that in healthy subjects (basal rate); ACLF: acute-on-chronic liver failure; AHB: acute hepatitis B; AHC: acute hepatitis C; ALT: alanine transaminase; CHB: chronic hepatitis B; CHC: chronic hepatitis; CS: cross-sectional; CSHB: Chronic severe hepatitis B; ESLD: end-stage liver disease; EVR: early virological response; HCC: hepatocellular carcinoma; IFN: interferon; L: longitudinal; NR: no response; RBV: ribavirin; RVR: rapid virological response; SVR: sustained virological response, TLR: Toll-like receptor.



HBV: hepatitis B virus; HCV: hepatitis c virus; NHL: non-Hodgkin lymphoma; IHD: inflammatory heart disease

Figure 1. The workflow of systematic search and analysis

longitudinal studies, the follow up time is considered sufficient. All the studies clearly defined the criteria for inclusion. Study subjects and setting were described in detail and the measurements were performed according to the standards methods. The accurate measurements together with the appropriate use of statistical analysis resulting in valid and reliable results of the reviewed studies. Unfortunately, confounding factors and strategies to deal with them were not elaborated in detail, perhaps due to the complexity of immunological responses in HBV and HBC infection as well as the extent of biological variations amongst study subjects.

Chronic infection with HBV and HCV may cause immune-mediated liver injury that leads to liver cirrhosis, hepatocellular carcinoma, and liver failure. Until now, not all of chronic hepatitis B and C patients can be cured. Currently available treatments may slow down the progressivity of the illness, decrease the viral load and ease the symptoms but the viral clearance may not be completely achieved. The available direct-acting antiviral drugs are well tolerated and cause sustained virological response in most patients with chronic hepatitis C. However, treatment failure may happen in some patients due to the lack of adherence and drug-to-drug interactions. If the infection persist, a constant exposure to the virus antigens will provoke the immune response and sooner or later, liver transplantation is the only solution for patients with irreversible liver damage. The limited liver donors and the continuing increase of demands cause more patients left untreated. Therefore, understanding immunological aspects of HBV and HCV infection is important to find a way to improve patient care.

The main immunological response to HBV and HCV infection is performed by a subset of white blood cells called T lymphocytes (T cells). An adequate response of T cells is necessary for spontaneous resolution during acute infection and for preventing permanent chronic infection. Even though T cells frequency correlates inversely with the viral load, T cells may contribute to liver damage through unknown mechanisms.[60] It has been argued that liver damage in chronic hepatitis patients was due to proinflammatory cytokines produced by non-specific T cells during the

viral clearance from liver cells. Therefore, it can be assumed that the key to cure chronic hepatitis B and C is by restoring an effective T cells response.

The roles of Tregs in chronicity of HBV infection have been confirmed. Increased Tregs correlate with poor short-term outcomes in HBV-related acute-on-chronic liver failure patients.[9] While there is clinical evidence of a relevant role for Tregs in chronic HCV-infected patients, based on their increased number and function; mechanisms underlying such phenomena are still poorly understood.[61] This present review shows that the frequency of Tregs is associated with the chronicity of HBV and HCV infection. The increase of intrahepatic Tregs during chronic infection may be directed to protecting liver tissue from immune-mediated injury whereas the decrease of Tregs frequency may be influenced by the treatment given; i.e. the type and duration of treatment. Thus, analyzing Tregs frequency during the course of hepatitis B and C may give a valuable information to clinicians with regards to disease chronicity, the severity of the ilness, and response to treatment.

CONCLUSION

The role of Tregs for suppression of immune responses during HBV and HCV infection is vital to prevent immune-mediated liver injury. On the other hand, the suppression of immune responses in fighting viral replication poses a detrimental effect as this causes persistent infection. These contrary effects of Tregs may influence the clinical course of HBV and HCV-infected patients. This review shows that infection with HBV and HCV promotes the increase of Tregs in patients' blood and hepatocytes whereas the treatments may decrease Tregs levels. Further studies are needed to examine the dynamics of Tregs during the course of infection and its relation with clinical outcomes. Such studies are important to understand the immunologic properties of Tregs and may be developed as a novel strategy to combat hepatitis B and C.

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