

Cytokine and chemokine profiling in chronic hepatitis- C and -B virus infections with high viral load.

Research Article

Yutaka Kishida M.D., Ph.D. ¹

1. Division of Gastroenterology and Hepatology, Department of Internal Medicine, Osaka Kaisei Hospital. 1-6-10 Miyahara, Yodogawa-Ku, Osaka City, Osaka, 5320003 Japan.

Corresponding author

Yutaka Kishida M.D., Ph.D.,

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Osaka Kaisei Hospital. 1-6-10 Miyahara, Yodogawa-Ku, Osaka City, Osaka, 532-0003 Japan.

Phone : +81-6-6393-6234,

Fax : +81-6-6393-6234

Email : y-kishida@muse.ocn.ne.jp

Received Date : July 14, 2024

Accepted Date : July 15, 2024

Published Date : August 14, 2024

DOI : 10.52338/immunology.2024.4046

Brief title: Cytokine profiling in CHC and CHB infections

ABSTRACT

Background: Primary defects in innate immunity in chronic hepatitis-C and -B may shift towards dysregulated adaptive immunity. Cytokines are critical immune regulators.

Aim: To clarify cytokines and chemokines in chronic hepatitis-C and -B to investigate the innate and adaptive immunity.

Methods: Twenty-seven serum cytokines were evaluated in 27 patients with chronic hepatitis-C (genotype 1 [n=20], genotype 2 [n=7], hepatitis-C virus. RNA 5.72 ± 3.17 Log IU/mL), 12 patients with chronic hepatitis-B (e-antigen [+] [n=5], e-Ag [-] [n=7], genotype B [n=2], genotype C [n=9], hepatitis-B virus. DNA 6.19 ± 1.31 Log-copies/mL) and controls (n=5).

Results: Th1 and Th2 cytokines levels were significantly higher ($p < 0.05$) in chronic hepatitis-B than those in chronic hepatitis-C. Interleukin-12 and -15 levels were significantly higher ($p < 0.05$) in chronic hepatitis-B than those in chronic hepatitis C. CXCL8 and -10 were significantly higher ($p < 0.05$) in chronic hepatitis-C and -B than those

in controls. Immune responses were stronger in patients with chronic hepatitis-C (genotype 2) than in those with chronic hepatitis- C (genotype1). The results showed a distinct state of immunity among chronic hepatitis-B and -C. **Conclusions:** Skewing immunity was observed in chronic hepatitis-B and -C as well as impairments to induce innate immunity linked to adaptive immunity. Augmenting immunological strategies are necessary for the treatment and management of chronic hepatitis-B and -C with a high viral load.

Keywords : chronic hepatitis C, chronic hepatitis B, innate immune response, adaptive immune response, cytokine, chemokine.

INTRODUCTION

Viral infections are controlled by tightly regulated immune networks. In addition to cellular components of the immune system, various soluble mediators are essential for virus elimination. Whether any of these soluble immune mediators can serve as biomarkers for predicting disease severity or the response to antiviral treatment is unclear. Chronic viral infections cause major changes in the inflammatory cytokines and chemokine milieu [1]. Microbial infections are recognized by the innate immune system to elicit immediate defense and generate long-lasting adaptive immunity [2, 3]. Innate immunity acts immediately following infection, directing the production of pro-inflammatory cytokines and orchestrating adaptive immunity [4, 5]. Cytokines that activate natural killer (NK) cell responses and their consorted effects in providing an endogenous milieu promote downstream adaptive immune responses [6]. Th1 and Th2 cytokines play prominent roles in viral infections and dysregulation of these cytokines may account for viral resistance and the evolution of chronic disease [7]. Primary defects in innate immunity may shift the balance of immune responses towards dysregulated adaptive immunity, while insufficient or excessive activation of the innate immune response results in disease [8]. The multiple mechanisms involved in the induction of immune responses are variations of a common principle, in which cells that detect infections produce one set of cytokines to induce lymphocytes to produce another set of cytokines, which in turn activate effector responses [9]. Cytokines play an important role in the pathogenesis of cirrhosis and hepatocellular carcinoma (HCC),

most of which are related to the hepatitis B virus (HBV) or hepatitis C virus (HCV). Immune interactions differ between HBV and HCV infections, and these differences are reflected in serum cytokine and chemokine levels [10].

The outcome of chronic HBV infection is determined by virus-host interactions. HBV is a non-cytopathic virus and liver damage is mainly attributed to the host immune response. During HBV infection, the host immune response acts as a double-edged sword; it provides defense against infection by destroying virusinfected cells while inducing inflammation and aggravating liver injury. The role of classical CD4 and CD8 T cells have been found to be crucial for HBV clearance. Both CD4 and CD8 T cells synergistically controlled HBV infection. HBV can suppress the functions of innate immune cells [11, 12]. Furthermore, HBV is readily suppressed by the innate immune components. The actual elimination of HBV infection requires adaptive immune responses [13, 14]. Upon exposure to high viral titers of HBV, human macrophages are activated by the increased expression of inflammatory cytokines [15]. HCV resistance can be attributed to the ability of the virus to evade immune surveillance by various means such as viral mutation which is a major evasion strategy used by HCV and inhibition of innate immune cells by HCV, as well as by an alteration of the innate and adaptive immune responses [16]. HCV interferes with cytokines and escapes immune responses by inducing Th2/T cytotoxic 2 cytokines [6, 17].

Recent advances in our understanding of the immunological mechanisms of virus host interactions, protective immunity and disease pathogenesis will help us develop vaccines against HCV infection and immunotherapies that cure patients with resistant HBV and/or HCV infection [18]. Patients with HBV or HCV infections have distinctly different cytokine profiles, suggesting potential differences in disease pathogenesis and/or disease characteristics [19]. The expression profiles of cytokines and chemokines have not been completely elucidated in patients with chronic hepatitis B (CHB) and chronic hepatitis C (CHC) compared with healthy controls [20]. A better understanding of cytokine and chemokine profiles is crucial for broadening our knowledge of immune responses in the pathogenesis of HBV and HCV and for developing novel and effective immunotherapies for HBV and HCV [21, 22].

Pretreatment with a high viral load in CHC and CHB is a significant factor contributing to the efficacy of antiviral therapy. Low baseline HBV DNA levels were positively associated with the virologic response. The viral load of HCV RNA has a major effect on antiviral treatment. HCV treatment failure and disease progression are likely to result in high RNA-HCV viral loads. It is crucial to study the components of immune responses, viral strategies for immune evasion, and mechanisms of disease pathogenesis. The present study aimed to clarify the expression patterns of serum cytokines and chemokines to investigate innate and adaptive immune

responses in difficult-to-treat CHB and CHC patients with a high viral load at baseline before treatment and against controls.

METHODS

Patients

We performed a prospective study on 27 patients with CHC (12 men and 15 women, 57.74 ± 12.00 years of age, genotype 1 [n=20] and genotype 2 [n=7], HCV.RNA 5.72 ± 3.17 Log-IU/mL, platelet count $17.1 \pm 10.0 \times 10^4 /\mu\text{L}$, FIB-4 indexes 3.20 ± 2.35 , aspartate aminotransferase to platelet ratio index [APRI] scores 1.27 ± 1.35 , and F1~2) and 12 patients with CHB (seven men and five women, 47.5 ± 13.50 years of age, HBeAg [+] [n=5] and HBeAg [-] [n=7], genotype B [n=2], genotype C [n=9] and the genotype of one patient was not elucidated, HBV.DNA 6.19 ± 1.31 Log-copies/mL, platelet counts $18.6 \pm 6.6 \times 10^4 /\mu\text{L}$, FIB-4 indexes 2.17 ± 1.78 , APRI score 0.76 ± 0.48 and F1~2). The patients were enrolled before treatment between October 2004 and February 2010. The patient had not received any antiviral treatment in the preceding 12 months. Patient characteristics are shown in Tables 1 (A) and 1 (B). Serum samples were collected from 27 patients with CHC, 12 patients with CHB, and five healthy controls and stored at -20°C . All patients who donated samples provided written informed consent, and all the samples obtained were approved for collection. The study protocol conformed to the ethical guideline of the 1975 Declaration of Helsinki and was approved by the institutional review board and ethical committee of Osaka Kaisei Hospital.

Table 1 (A). Patients Characteristics of Chronic Hepatitis C

Characteristics Baseline	All patients (n=27)	genotype 1 (n=20)	genotype 2 (n=7)	p value
Age, years	57.74 ±12.00	57.20 ±12.10	59.30±12.40	0.701
Male, n (%)	12 (44.4%)	8 (40.0%)	4 (57.1%)	0.432
BMI, kg/m ²	22.56 ±3.59	23.0 ±3.40	21.42 ±4.0	0.303
Treatment History				
previous IFN	8 (29.6%)	7 (35.9%)	1 (14.3%)	
Laboratory results				
HCV.RNA				
(LogIU/mL	6.26 ± 0.80	6.10 ± 1.50	6.20± 0.70	0.857
AST (IU/mL	67.6 ± 48.8	58.5 ± 43.2	73.4± 35.3	0.419
ALT (IU/mL)	79.1 ± 60.9	62.5± 53.4	89.4 ± 49.7	0.254
Platelets (104/μl)	17.1 ± 10.0	18.2± 9.1	16.7 ± 3.7	0.654
FIB 4 index	3.20 ± 2.35	3.28 ± 2.62	3.01 ±1.50	0.804
APRI score	1.27 ± 1.35	1.20 ± 1.54	1.24 ± 0.76	0.949

Table 1 (B). Patients Characteristics of Chronic Hepatitis B

Characteristics Baseline	All patients (n=12)	HBe-antigen positive (n=5)	HBe-antigen negative (n=7)	p value
Age, years	47.5±13.5	54.8 ±15.1	42.3 ±10.4	0.117
Male, n (%)	7 (58.3%)	1 (20.0%)	6 (85.7%)	0.023*
BMI, kg/m ²	22.56 ±3.59	21.7 ± 2.7	26.8 ±5.6	0.091
HBV-genotype				
genotype B	2 (16.7%)	1 (20.0%)	1 (14.3%)	
genotype C	9 (75%)	4 (80.0%)	5 (71.4%)	
not determined	1 (8.3%)	0 (0.0%)	1 (14.3%)	
Laboratory results				
HCV.RNA				
(Log copies/mL)	6.1± 1.31	6.80± 0.50	5.80 ± 1.60	0.181
AST (IU/mL)	46.7 ± 20.2	60.8 ± 10.2	36.6 ± 19.8	0.032
ALT (IU/mL)	55.6 ± 30.0	66.4 ± 26.1	47.9 ± 32.0	0.313
Platelet (104/μl)	18.6 ± 6.6	14.2 ± 6.1	21.7 ± 5.4	0.048*
FIB 4 index	2.17 ± 1.78	3.65 ± 1.88	1.11 ±0.58	0.007*
APRI score	0.76 ± 0.48	1.18 ± 0.34	0.471 ± 0.343	0.005*

ALT: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; AST: aspartate aminotransferase; BMI: body mass index; FIB-4: fibrosis index based on four factors; HBV: hepatitis-B virus; HCV: hepatitis -C virus; IFN: interferon

Measurements

HCV RNA was measured at baseline using the Quantitative COBAS AMPLICORE HCV MONITOR test ver. 2.0 (Roche Diagnostic System, Tokyo, Japan; sensitivity < 50 log IU/mL). HCV sero-groups were assessed using the HCV sero- grouping assay (HCV Gr; Sysmex International Reagents, Kobe, Japan), which can subgroup patients into HCV sero-groups 1 and 2, corresponding to HCV genotypes 1 and 2, respectively, with HCV group-specific anti-nonstructural region 4 antibodies [23]. This assay is available not only for patients with chronic HCV infection, but also for those with resolved HCV infection. HBV DNA was measured using the quantitative COBAS TaqMan polymerase chain reaction (PCR) HBV-auto ver. 2.0 (Roche Diagnostic System, Tokyo, Japan, sensitivity < 2.1 Log-copies). HBV genotypes were identified in the serum using the restriction fragment length polymorphism (RFLP) method on the S-gene sequence amplified by PCR with nested primers.

Multiplex cytokine assay

The serum cytokine and chemokine levels (multiple cytokine assays) were measured before treatment. A multiplex enzyme-linked immunosorbent assay (ELISA)- based immunoassay with dyed microspheres conjugated to a monoclonal antibody specific to the target PLEX human cytokine assay (Bio-Rad Inc., Tokyo, Japan) was used. The cytokines measured were Th1 cytokines (interferon [IFN]- γ , tumor necrosis factor [TNF]- α , interleukin [IL]-1 α , IL-1 β , IL-2, IL-7, IL-12 [p70], IL-15 and IL17), Th2 cytokines (IL-4, IL-6, IL-9, IL-10, and IL-13), CXC chemokines (CXCL-8 [L-8] and CXCL-10 [IP-10]), CC chemokines (CCL-2 [MCP-1], CCL-3 [MIP-1 α], CCL-4 [MIP-1 β], CCL-5 [regulated upon activation, normal T-cell expressed and secreted (RANTES)] and CCL-11 [Eotaxin]), granulocyte colony-stimulating factor (G-CSF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).

Statistical Analysis

Data are expressed as mean \pm standard deviation. Relationships between variables were tested using Student's t-test and a p-value of < 0.05 was statistically significant.

RESULTS

Serum cytokine and chemokine levels (multiple cytokine assay) at baseline before treatment were measured in 27 patients with CHC, 12 patients with CHB and five controls. IL-1 α in CHB (all patients with CHB, CHB with HBeAg [+], and CHB with HBeAg [-]) was significantly lower ($p < 0.05$) than that in controls (Figure 1). IFN- γ levels in CHC (genotype 2) were significantly lower ($p < 0.05$) than those in controls. Th1 cytokines (IL-12 and IL-15) levels were significantly higher ($p < 0.05$) in CHB (all patients CHB) than in CHC (all patients CHC). IL-12 levels were higher in CHC (genotype 2) than in

CHC (genotype 1). IL-12 levels were higher in CHB patients with HBeAg (+) than in CHB patients with HBeAg (-). IL-12 levels were significantly higher ($p < 0.05$) in CHB patients with HBeAg (-) and CHC (genotype 2) than in controls. IL-15 levels were higher in CHB patients with HBe Ag(+) status than in those with CHB patients with HBe Ag (-). IL-15 levels were significantly higher ($p < 0.05$) in CHB (all patients CHB) than in CHC (all patients CHC). IL-7 in CHC (all patients with CHC) was significantly lower ($p < 0.05$) than that in the controls. IL-17 levels in CHB with HBe Ag (+) were significantly lower ($p < 0.05$) than those in controls. IL-17 levels were significantly higher ($p < 0.05$) in CHC (all patients with CHC) than in CHB (all patients with CHB) (Figure 2A, 2B, 2C, 2D). IL-10 levels in CHC (all patients with CHC, genotype 1 and genotype 2) were significantly lower ($p < 0.05$) than those in the controls. IL-10 levels were significantly lower in CHB patients with HBeAg (-) than in controls. IL-10 levels were higher in HBeAg (+) CHB patients than in HBeAg (-) CHB patients. IL-13 levels in CHC (all patients with CHC, genotype 1, and genotype 2) were significantly lower ($p < 0.05$) than those in the controls. (Figure 3A and 3B). CCL-2 (MCP1) levels in CHC (all patients with CHC, CHC [genotype 1], and CHC [genotype 2]) and CHB (HBeAg [-]) were significantly higher ($p < 0.05$) than those in the controls. CCL-2 was significantly higher ($p < 0.05$) in CHC (genotype 2) than in CHC (genotype 1). CCL-4 (MIP 1 β) levels in CHC (all patients with CHC, CHC [genotype 1] and CHC [genotype 2]) and CHB (all patients with CHB and CHB with HBeAg [+]) were significantly higher ($p < 0.05$) than those in controls. CCL-4 levels were significantly higher ($p < 0.05$) in CHC (genotype 2) than in CHC (genotype 1). CCL- 5 (RANTES) levels in CHC (all patients with CHC and CHC [genotype 2]) and CHB (all patients with CHB, CHB with HBeAg [+], and CHB with HBeAg [-]) were significantly higher ($p < 0.05$) than those in controls. CCL- 5 was higher in CHC (genotype 2) than in CHC (genotype 1) (Figure 4A, 4B and 4C). CXCL8 (IL-8) levels in CHC (all patients with CHC, CHC [genotype 1]), CHC [genotype 2]), and CHB (all patients with CHB, CHB with HBeAg [+], and CHB with HBeAg [-]) were significantly higher ($p < 0.05$) than those in controls. CXCL-10 (IP10) levels were significantly higher ($p < 0.05$) in CHB (all patients with CHB, CHB with HBeAg [+], and CHB with HBeAg [-]) than in controls. CXCL-10 levels were significantly higher ($p < 0.05$) in CHC (all patients with CHC) than in CHB (all patients CHB) (Figure 5A and 5B). Granulocyte-colony stimulating factor (G-CSF) levels in CHC (all patients with CHC and CHC [genotype 2]) and CHB (all patients with CHB and CHB with HBeAg [-]) was significantly lower ($p < 0.05$) than those in the controls (Figure 6A). Vascular endothelial growth factor (VEGF) levels in CHC (all patients with CHC, genotype 1 and genotype 2) and CHB (all patients with CHB, CHB with HBeAg [+], and CHB with HBeAg [-]) were significantly lower ($p < 0.05$) than those in the controls (Figure 6B). All relevant data are within the paper and its supporting information files.

Figure 1

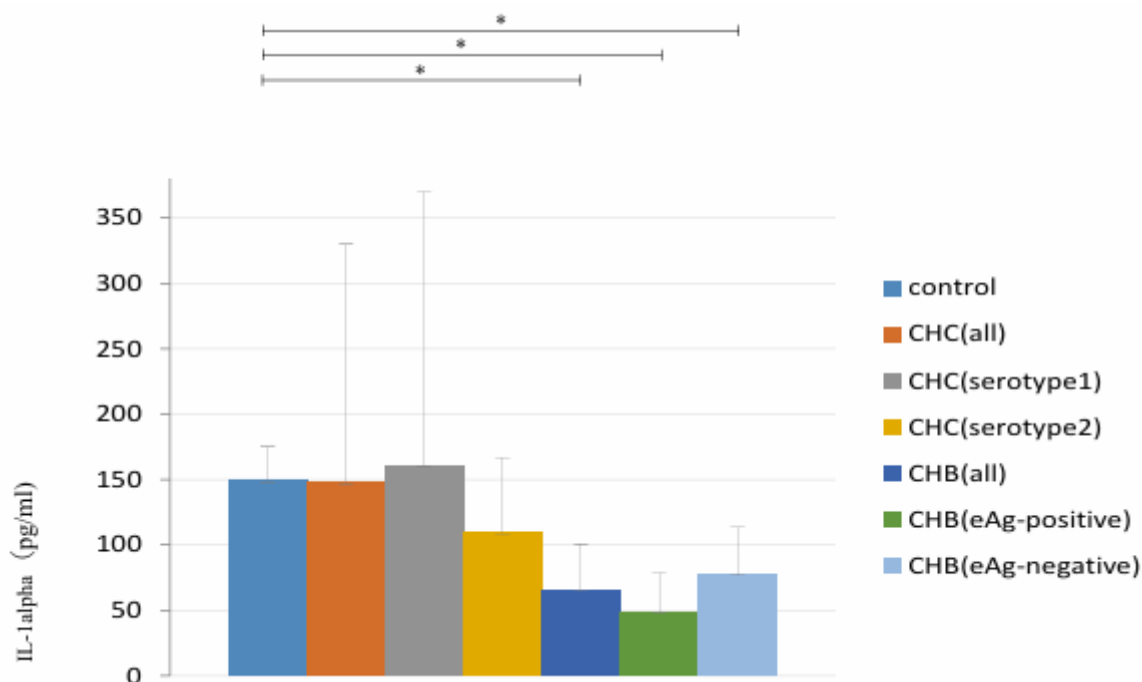
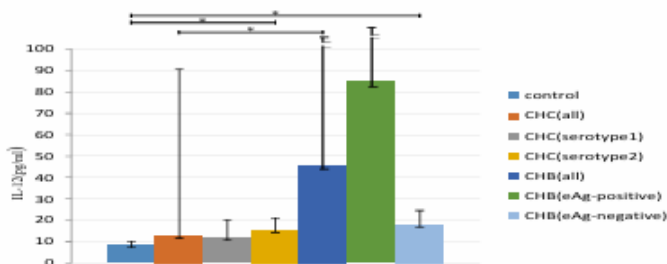


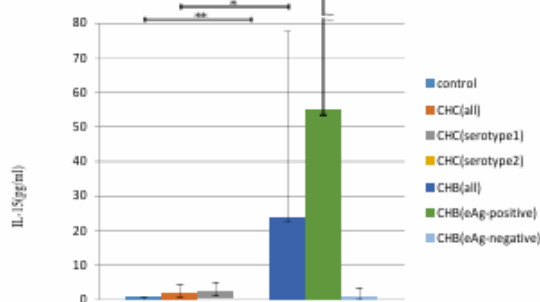
Figure 1. Serum Th1 cytokines (IL-1α) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum Th1 cytokine (IL-1α) expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection, and HBV e-antigen positive and e-antigen negative infection, and healthy controls (mean ± S.D.). All the samples were tested for baseline levels. * P<0.05, ** P < 0.01.

Figure 2

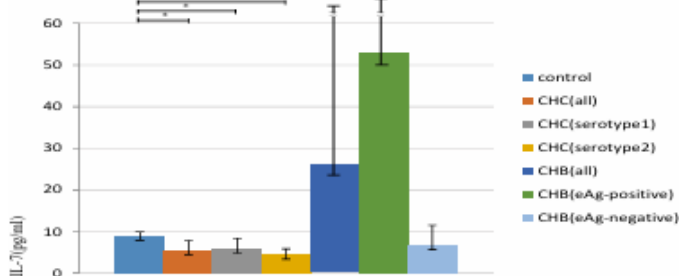
2A. Serum IL-12 expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline



2B. Serum IL-15 expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline



2C. Serum IL-7 expression in chronic hepatitis C and chronic hepatitis B with high viral load



2D. Serum IL-17 expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline

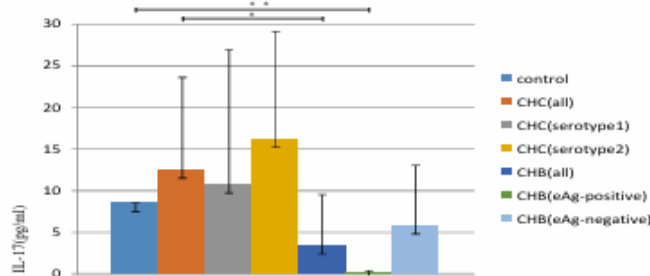
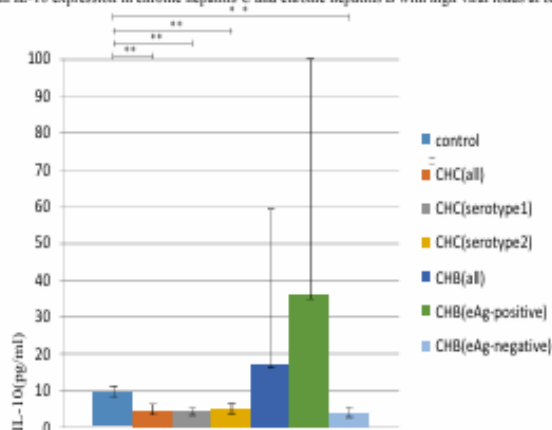


Figure 2. Serum Th1 cytokines (IL-12, IL-15, IL-7 and IL-17) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum Th1 cytokine (IL-12 [2A], IL-15 [2B], IL-7 [2C] and IL-17 [2D]) expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection, and HBV e-antigen positive and e-antigen negative infection, and healthy controls (mean ± S.D.). All the samples were tested for baseline levels. * P<0.05, ** P < 0.01

Figure 3

3A. Serum IL-10 expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline



3B. Serum IL-13 expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline

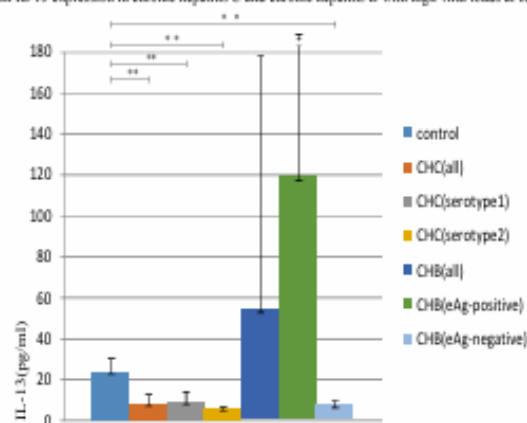
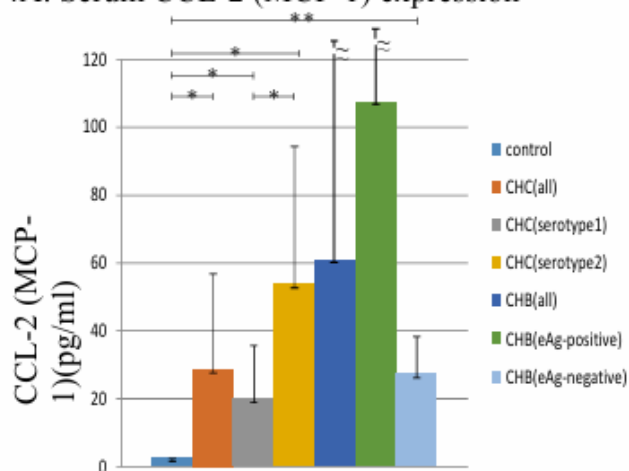
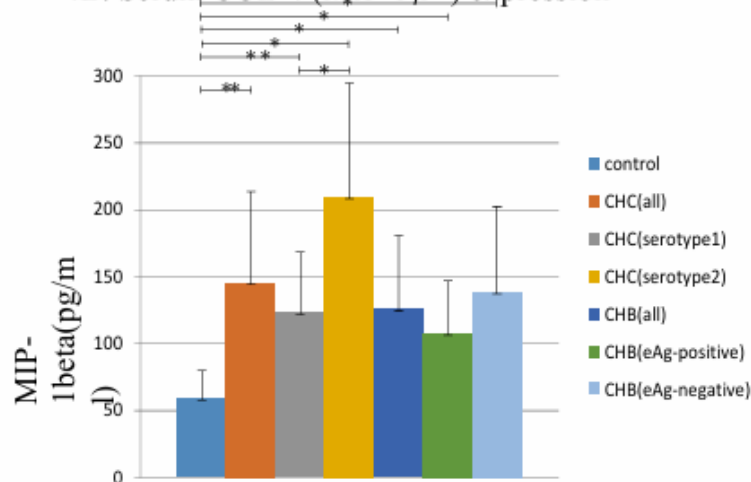


Figure 3. Serum Th2 cytokines (IL-10 and IL-13) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum Th2 cytokine (IL-10 [3A] and IL-13 [3B]) expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection with high loads, and HBV e antigen positive and e-antigen negative infection, and healthy controls (mean \pm S.D.). All the samples were tested for baseline levels. * $P < 0.05$, ** $P < 0.01$

Figure 4

4A. Serum CCL-2 (MCP-1) expression

4B. Serum CCL-4 (MIP-1 β) expression

4C. Serum CCL-5 (RANTES) expression

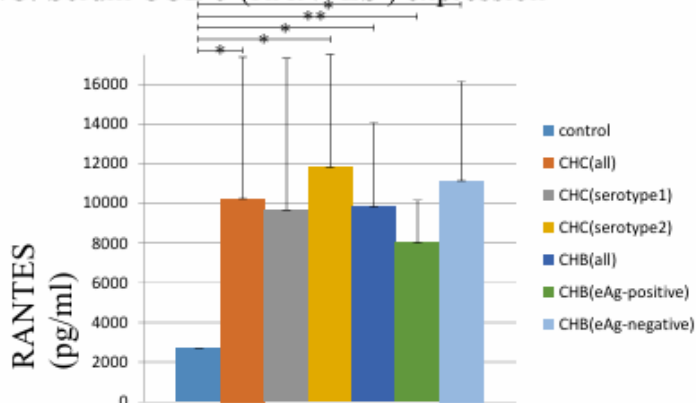
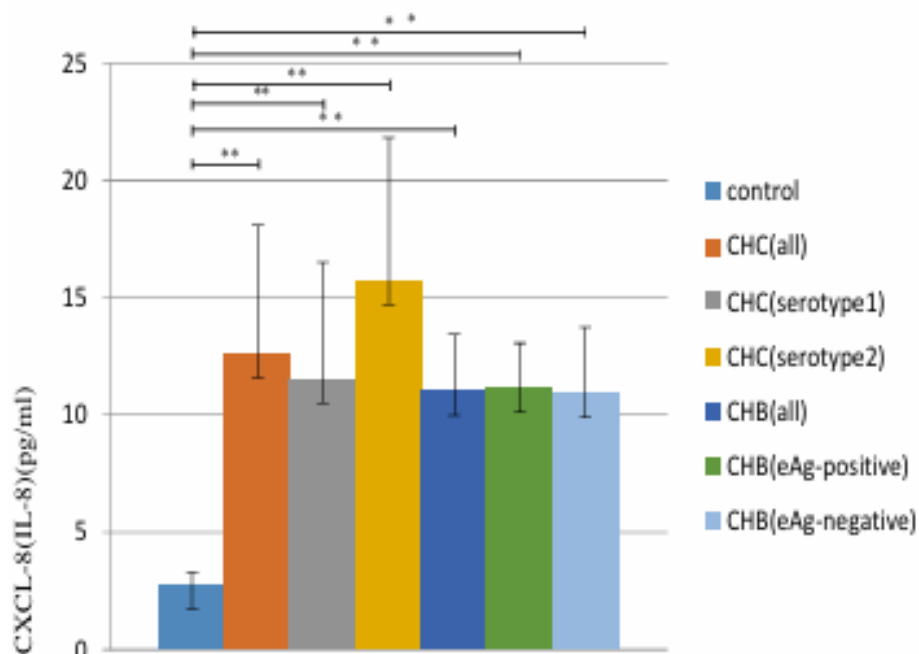


Figure 4. Serum CC-chemokines (CCL-2 [MCP-1], CCL-4 [MIP-1 β] and CCL-5 [RANTES]) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum CC-chemokine (CCL-2 [MCP-1] [4A], CCL-4 [MIP-1 β] [4B]) and CCL-5 [RANTES] [4C] expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection, and HBV e-antigen positive and e-antigen negative infection, and healthy controls (mean \pm S.D.). All the samples were tested for baseline levels. * $P < 0.05$, ** $p < 0.01$

Figure 5

5A. Serum CXCL-8 (IL-8) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline



5B. Serum CXCL-10 (IP-10) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline

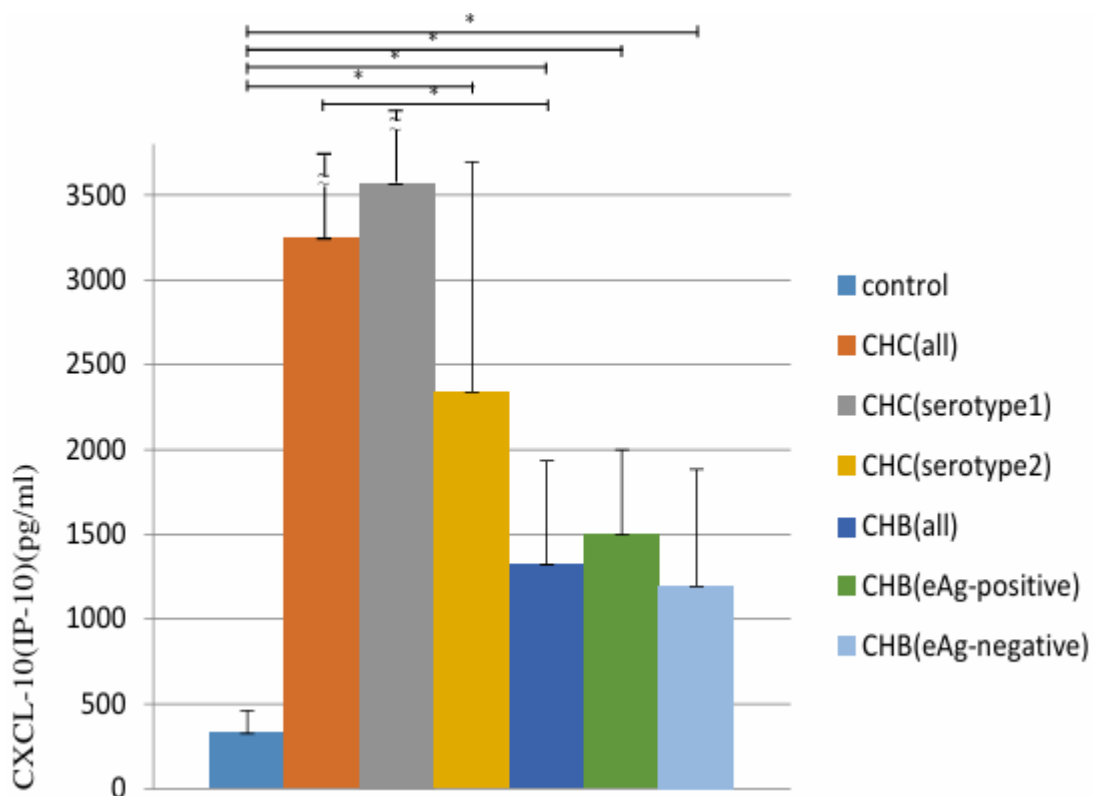
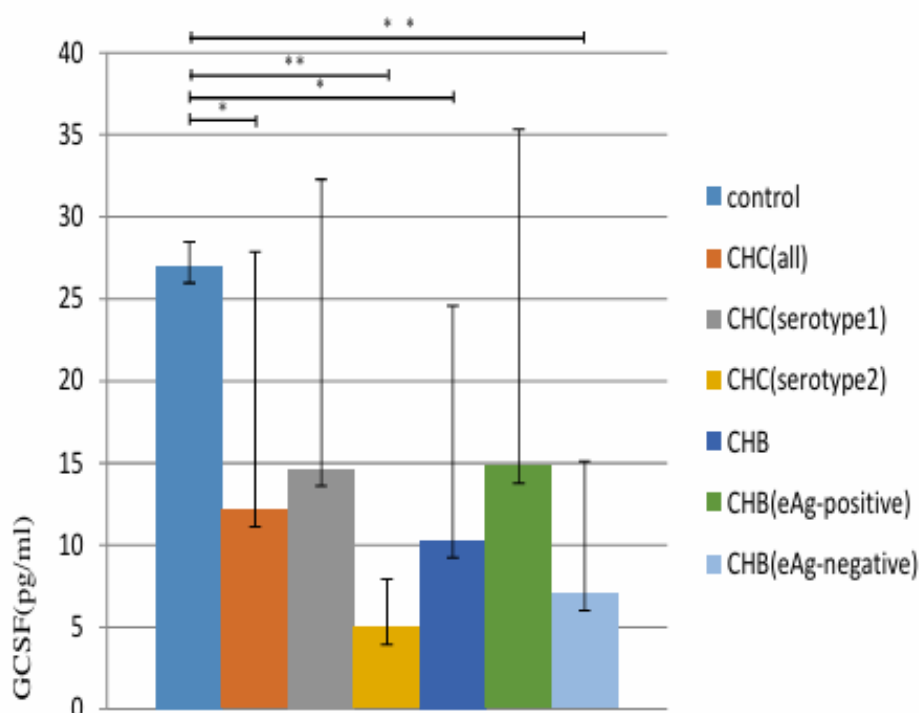


Figure 5. Serum CXCL-chemokines (CXCL-8 [IL-8] and CXCL-10 [IP-10]) expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum CXCL-chemokine (CXCL-8 [L-8] [5A] and CXCL-10 [IP-10] [5B]) expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection, and HBV e-antigen positive and e-antigen negative infection, and healthy controls (mean \pm S.D.). All the samples were tested for baseline levels. * $P < 0.05$, ** $p < 0.01$.

Figure 6

6A. Serum GCSF expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline



6B. Serum VEGF expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline

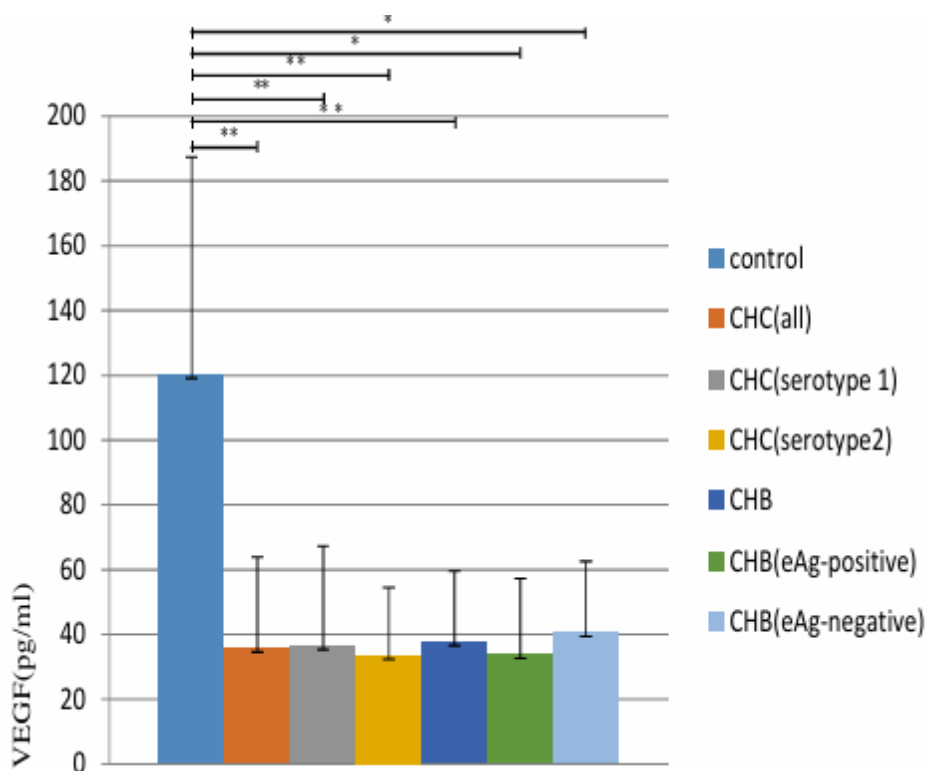


Figure 6. Serum G-CSF, VEGF expression in chronic hepatitis C and chronic hepatitis B with high viral loads at baseline. Histograms represent serum G-CSF (6A) and VEGF (6B) expression in patients with HCV serotype 1 (genotype 1) and serotype 2 (genotype 2) infection, and HBV e-antigen positive and e-antigen negative infection, and healthy controls (mean \pm S.D.). All the samples were tested for baseline levels. * $P < 0.05$, ** $p < 0.01$

DISCUSSION

Primary defects in innate immunity in CHC and CHB may shift towards dysregulated adaptive immunity. Cytokines and chemokines are critical immune regulators. This study aimed to identify the cytokines involved in innate and adaptive immunity. The results revealed a distinct state of immunity in patients with CHB and CHC. Skewing immunity has been observed in patients with CHB and CHC as well as impairments in innate immunity, which are linked to adaptive immunity. Augmenting immunological strategies are necessary for the treatment and management of CHB and CHC with high viral loads. Persistent infection with HCV and HBV causes profound alterations in the cytokine and chemokine milieu in peripheral blood [1]. HCV+ and HBV+ patients have higher levels of Th1 cytokines, particularly during the course of CHB [24]. A comparison of the cytokine profiles of patients with CHB and CHC showed significant differences [10]. Innate and adaptive immune responses were weaker in patients with CHC than in those with CHB. Innate and adaptive immune responses were stronger in CHC (genotype 2) than in CHC (genotype 1).

IL-12 may play an important role in inducing Th1 cytokine responses, HBeAg seroconversion, and viral clearance in CHB [25]. Cellular immune responses are deficient in HCV-infected patients, and IL-12 may enhance these responses [26]. A switch to Th1-type responses would be beneficial and would promote essential cellular immune mechanisms for viral clearance. In transgenic mice expressing HBeAg, systemic application of recombinant IL-12 resulted in a shift from Th2 mediated responses towards a predominance of the Th1 cytokine profile [27]. IL-12 upregulation restored normal function of exhausted CD8+ T cells. IFN- γ is a major cytokine that is secreted by NK cells and a critical factor in the inhibition of viral replication. IL-12 may contribute to the preferential development of Th1 cells that produce IFN- γ over Th2 cells producing IL-5 [28]. IL-17 acts as a link between the innate and adaptive immune responses [29]. IL-15 is an important cytokine involved in the innate and adaptive immune responses against HCV infection. IL-15 may function as a NK cell growth and maturation factor that drives the proliferation of NK cells and memory T-cells [6]. NK cells constitute a major cellular arm of the innate immune system and act as a bridge between the innate and adaptive immune responses [30, 31]. IL-10 negatively regulates of NK cells during the innate immune response. HBeAg induces NKG2A+ NK cell dysfunction via regulatory T-cell (Treg) derived IL-10. HCV can induce myeloid-derived suppressor cells and cytokines such as IL-10 and TGF- β resulting in the promotion of Treg development and suppression of CD4+T cell function [32, 33,34]. IL-6 is critical for the production of Th2 cell phenotype [35]. Th1/Th2 (IL-1/IL-10) ratio in CHC was higher than Th1/Th2 (IL-1/

IL-10) ratio in CHB at baseline. This suggests that the direction of changes in the Th1/Th2 balance is related to a more effective treatment of CHB. The present results on the serum levels of chemokines (CXCL-8 and CXCL-10) demonstrated that innate and adaptive immune responses were inhibited in CHB and CHC patients with high viral loads. A dysfunctional CD26-CXCL10 axis inhibits the development of favorable innate and adaptive host immunity to HCV, and favors the evolution of viral resistance [5]. The present results of serum levels of chemokines (CCL2, CCL4, and CCL5) demonstrated that antiviral immunity increased in CHB and CHC compared to controls, and antiviral immunity increased in CHC (genotype 2) compared to that in CHC (genotype 1). CCL2 influences macrophage, monocyte, NK-cell, basophil, and T lymphocyte infiltration. CCL5 has strong chemotactic activity towards multiple immune cells. CCL4 is a chemoattractant for NK cells, monocytes and various other immune cells. The recruitment of antiviral immune cells to the liver is primarily dependent on the release of specific chemokines. They are released by many different cell types and guide cells of the innate and adaptive immune systems. Interference with the expression of chemokines or chemokine receptors allows HCV to evade the host immunity and establish viral resistance. VEGF and G-CSF are modulators of innate immune responses with suppressive effects [36, 37]. The present results demonstrated that innate immune responses increased in CHB and CHC with a high viral load; however, immune responses were impaired inducing adaptive immune responses to eliminate viruses.

CHB shows a defective early innate immune response, which is essential for further induction of HBV-specific adaptive immunity and may contribute to the persistence of CHB or a weakened capacity to clear HBV [25]. Viral load has previously been shown to affect the quality of the anti-HBV immune responses and outcomes of viral infections [38, 39]. The elimination of HBV infection requires adaptive immune responses [13, 15, 40, 41]. However, upon exposure to high levels of HBV, human macrophages are activated by increased inflammatory cytokine expression. However, quantification of cytokines indicated that HBV infection did not elicit the production of IFNs and IL-15 but induced the production of IL-10 [42-43]. Changes in cytokine levels may result in persistence of HBV infection [15]. HBeAg (+) CHB patients with high viral loads were more strongly associated with the activation of Th1- and Th2 type responses than HBeAg (-) CHB patients. Thus, preferential activation and commitment towards Th1- or Th2-cell subsets may influence the clinical consequences of HBV infection.

During HCV infection, chronic inflammation, regeneration, and fibrosis can lead to liver dysfunction. Cytokines and chemokines are major regulators of these processes. Therefore, the outcome of HCV infection depends in part on a complex network of cytokine and chemokine interactions

that orchestrate the innate and adaptive immune responses to HCV infection. Thus, patients persistently infected with HCV have a disrupted milieu of cytokines and chemokines compared with healthy individuals [1]. Cytokines in the host and innate immune response play a principal role in controlling HCV infection [44]. Cytokines are intracellular mediators secreted by both innate and adaptive immune cells and are involved in viral control. After HBV infection, activation of different immune cells triggers a complex cytokine cascade and generates a protective immune response. Cytokines are required for cellular activation, intracellular signaling, and cell-cell communication [45]. Cytokines which are signaling molecules involved in the immune response against pathogens are likely to play a key role in the pathogenesis of hepatitis, cirrhosis, and HCC. Falasca et al. found that HBV-infected patients had higher plasma levels of IFN- γ , TNF- α , and IL-2 than did HCV-infected patients and healthy controls. They also found that IL-6 and IL-18 levels were higher in both the HBV and HCV groups than in controls. Another study found reported that liver-infiltrating T-cells from CHC patients produced IFN- γ but not IL-4 or IL-5, whereas T-cells from CHB patients were able to produce IFN- γ , IL-4, and IL-5. A comparison of the cytokine profiles of patients with CHB and CHC showed a significant difference [10]. In HCV+ and HBV+ patients, Th1 cytokine levels are high, particularly during the course of CHB, and IL-18 and IL-6 levels may play important roles as markers of both inflammation and hepatic injury, particularly during the course of hepatitis C. Chemokines and their receptors play key roles in leukocyte recirculation in inflamed liver. Furthermore, chemokines may be involved in liver regeneration, fibrosis, and malignant transformation induced by persistent inflammation. Accumulating data indicate that distinct chemokines and chemokine receptors may be associated with different stages of CHC virus infection-associated liver disease.

This study has some limitations. The sample size was relatively small, and the results of the present study should be further validated by well-designed studies with larger sample sizes. Future follow-up studies are required to confirm these findings in light of the limitations of this study.

Future therapeutic strategy targeting immune modulation for CHB and CHC

We also need to identify steps to augment the immune response. A clearer understanding of immunological mechanisms is crucial to broaden our knowledge of immune responses in the pathogenesis of HCV and HBV infections and the development of novel and effective immunotherapies [43].

Immune modulators to activate and restore immune response to HBV

The study of the interaction between HBV and innate immune cells is instrumental in designing novel immunotherapeutic concepts based on the restoration of innate cell function and/or innate effectors [46]. Because a vigorous and multi-specific host immune response against HBV is the major determinant of spontaneous clearance following acute infection, several approaches that are considered to activate antiviral immunity against HBV through the stimulation of HBV infection have been evaluated; antiviral effector cells, generation of "new" T-cells (therapeutic vaccines), or the recovery of exhausted T-cells. This hypothesis is supported by the widespread use of Pegylated (PEG)-IFN- α as a potent immunomodulator for HBV treatment [47].

Cytokine levels before and during treatment may represent potential biomarkers for the selection of CHB patients who can respond to PEG-IFN. Therefore, cytokines can be used as indicators of antiviral drug selection before CHB treatment [48]. A certain pattern in cytokines levels during treatment with PEG-IFN and IP-10 may be a potential biomarker for treatment response [49]. Changes in cytokine/chemokine levels following entecavir(ETV)therapy are associated with response to antiviral therapy in patients with CHB [50]. Dynamic changes in cytokine levels were observed during ETV antiviral therapy. Low baseline HBV DNA load and HBeAg and IL-10 levels were significantly associated with ALT normalization after 48 weeks of ETV treatment [51].

The exhaustion of the adaptive immune response is a major cause of HBV chronicity. In the course of chronic HBV infection, T cells exhibit a weak and dysfunctional response against HBV due to the overexpression of inhibitory receptors including programmed cell death 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and T-cell immunoglobulin and mucin protein 3 (TIM3). Blocking these immune checkpoints may promote improvement by promoting the proliferation of HBV-specific T cells and restoring the function of exhausted T cells. Recently, a synergistic effect of OX40 stimulation combined with PD-L1 blockade was shown to functionally augment HBV-specific CD4 T cells. Therapeutic vaccination aims to stimulate or boost the host immune response to restore immune control, which should lead to the sustained suppression of HBV replication and HBsAg loss. These include combinations with checkpoint inhibitors, Toll-like receptor (TLR) or retinoic acid-inducible gene-I (RIG-I) agonists, and/or other strategies to decrease viral antigen load. Multiple potential immunotherapeutic approaches have been suggested to achieve a cure by restoring the immune competence against HBV- and HBV-infected hepatocytes. A deficit that allows CHB persistence to be resolved and immune restoration may allow a similar clinical cure for CHB. Immunotherapy in clinical development

includes TLR7 agonist [52] and TLR8 agonist [53], checkpoint inhibitors (e.g., anti-PD-1), RIG-I agonists and therapeutic vaccines [54]. A combination of boosting innate immunity and stimulation of adaptive immunity may also be required [55]. Immunization of monoclonal T-cell receptors induce a broad cytokine response [56]. The best future strategy against HBV should combine drugs targeting several steps of the HBV replication cycle with immunomodulators [57]. The combination of three complementary strategies, that is, inhibition of viral replication, inhibition of antigen production/release and immunostimulation, could increase the goal of functional cure [47].

Immune modulators to activate and restore immune response to HCV

Escape due to genetic variations, suppression of immune responses by HCV proteins, inhibition of innate immune responses during chronic HCV infection, dysfunction of T lymphocytes, and involvement of Tregs in CHC infection contributes to an impaired or altered immune response against HCV. NK cells secrete TNF- α and IFN- γ , which inhibit HCV replication as well as cytolytic enzymes that destroy HCV-infected host cells. IFN- γ is a major cytokine secreted by NK cells and a critical factor in the inhibition of viral replication. In addition, dendritic cells (DCs) secrete IL-12, IL-15, and IL-18 which activate NK cells. We previously confirmed the restoration of innate and adaptive immune responses with a decline in viral load with an induction approach using natural IFN- β followed by treatment with PEG-IFN- α and ribavirin (RBV) in CHC as indicated by the upregulation of IL-12 and IL-15 and downregulation of CXCL-8, CCL4, and CXCL10. Restoration of innate immune responses may be a novel therapeutic strategy for CHC [58].

Significantly lower concentrations of IL-10, IL-13, IL-4, IL-5, IL-9 and TNF- α and higher levels of Angiopoietin-2 (Ang-2), Hepatic Growth Factor (HGF) and Stem Cell Factor (SCF) were observed in patients with CHC before and after direct-acting antivirals (DAAs) treatment than in healthy individuals. Patients with severe fibrosis exhibited higher levels of Ang-2 and lower levels of epidermal growth factor (EGF), PDGF-AA and VEGF. Furthermore, IL-4, IL-5, and SCF have been identified as potential biomarkers of DAAs treatment. Inhibition of pro-inflammatory processes and promotion of liver regeneration in CHC patients during DAAs treatment have been suggested [59]. DCs activated by HCV produce high levels of IL-12, which are associated with HCV clearance. Baseline IL-12 levels were significantly higher in patients who achieved sustained virological response (SVR) than in those who did not respond to combination treatment. Pre-treatment IL-12 levels seem to predict in patients who achieved SVR after treatment. Patients with increased IL-12 serum levels are more likely to achieve SVR [60]. Upon treatment, CXCL-10 (IP-10) and

CCL4 (MIP-1 β) levels were significantly higher in the patients who achieved SVR. Logistic regression analyses examining treatment response in all patients demonstrated significant associations between higher baseline MIP-1 β levels and smaller decreases in MIP-1 β early in treatment and SVR. IP-10 is an indicator of innate immune viral recognition. MIP-1 β levels remain elevated in genotype (GT) 2/3 patients who achieve SVR, suggesting differential immune activation in those who respond to sofosbuvir (SOF) and RBV therapy and a potential role in predicting treatment responses [61].

The synthesizing enzyme of 25HC, cholesterol 25-hydroxylase (CH25H), efficiently inhibits HCV infection. CH25H constitutes the primary innate immune response against HCV infection by regulating host lipid metabolism. Manipulation of CH25H expression and function is a novel strategy in anti-HCV therapy. The introduction of CH25H indicated an important host innate response to viral infection. An increasing number of microRNAs (miRNAs) have been reported to control HCV replication and infection by directly interacting with the HCV genome or controlling host innate immunity to build a non-specific antiviral state within cells. The potential application of miRNAs as therapeutic choices for the treatment of HCV infection has been developed [44].

CONCLUSION

HBV and HCV may both employ specific mechanisms to inhibit cytokine production, highlighting the critical roles of these molecules in recovery from viral infections. Cytokine levels differed between CHB and CHC patients at baseline. Skewed cytokine and chemokine expression at baseline was observed in CHB with HBeAg (+), CHB with HBeAg (-), CHC (genotype 1), and CHC (genotype 2) and may play an important role in persistent HCV and HBV infections. Innate and adaptive immune responses were impaired in CHB and CHC and appeared to reflect the distinct state of virus-host immune interactions among CHB with HBeAg (+), CHB with HBeAg (-), CHC (genotype-1) and CHC (genotype-2). In CHC and CHB patients with a high viral load, the restoration of innate immune responses upon a decline in viral load is crucial and treatments that trigger adaptive immune responses is needed to control HBV and HCV infections. Augmenting immunological strategies are necessary for the treatment and management of chronic hepatitis-B and -C with a high viral load. Different effective immunological strategies are necessary to treat and manage CHB and CHC with high viral loads.

Abbreviations

HCC, hepatocellular carcinoma; HBV, hepatitis-B virus; HCV, hepatitis-C virus; CHB, chronic hepatitis-B; CHC, chronic hepatitis-C; APRI, aspartate aminotransferase to platelet

ratio index; FIB-4, fibrosis index based on four factors; PCR, polymerase chain reaction; TNF, tumor necrosing factor; TLR, Tolllike receptors; IFN, interferon; IL, interleukin; G-CSF, granulocyte-colony stimulating factor; VEGF, vascular endothelial growth factor; PDGF, plateletderived growth factor

Acknowledgements

I thank the personnel of the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Osaka Kaisei Hospital for their cooperation during this observational study. This manuscript, including related data, figures, and tables has not been previously published and is not under consideration elsewhere.

Conflict of interest and Source of Funding

None of declared.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, Y.K., upon reasonable request. All data generated or analyzed during this study are included in this published article.

Author contribution

Author performed and analyzed the data and wrote the article. The author had full access to all data and read and approved the final manuscript.

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