

World Journal of *Gastroenterology*

World J Gastroenterol 2020 May 28; 26(20): 2464-2681



OPINION REVIEW

- 2464 Percutaneous endoscopic gastrostomy – Too often? Too late? Who are the right patients for gastrostomy?
Dietrich CG, Schoppmeyer K
- 2472 Can adiponectin have an additional effect on the regulation of food intake by inducing gastric motor changes?
Idrizaj E, Garella R, Squecco R, Baccari MC

REVIEW

- 2479 Diet in neurogenic bowel management: A viewpoint on spinal cord injury
Bernardi M, Fedullo AL, Bernardi E, Munzi D, Peluso I, Myers J, Lista FR, Sciarra T
- 2498 Role of gut microbiota-immunity axis in patients undergoing surgery for colorectal cancer: Focus on short and long-term outcomes
Bartolini I, Risaliti M, Ringressi MN, Melli F, Nannini G, Amedei A, Muiesan P, Taddei A
- 2514 Metabolomics profile in gastrointestinal cancers: Update and future perspectives
Nannini G, Meoni G, Amedei A, Tenori L
- 2533 Nervous mechanisms of restraint water-immersion stress-induced gastric mucosal lesion
Zhao DQ, Xue H, Sun HJ

MINIREVIEWS

- 2550 Treatment of gastrointestinal bleeding in left ventricular assist devices: A comprehensive review
Vedachalam S, Balasubramanian G, Haas GJ, Krishna SG
- 2559 Role of $\gamma\delta$ T cells in liver diseases and its relationship with intestinal microbiota
Zhou QH, Wu FT, Pang LT, Zhang TB, Chen Z

ORIGINAL ARTICLE**Basic Study**

- 2570 Serum outperforms plasma in small extracellular vesicle microRNA biomarker studies of adenocarcinoma of the esophagus
Chiam K, Mayne GC, Wang T, Watson DI, Irvine TS, Bright T, Smith LT, Ball IA, Bowen JM, Keefe DM, Thompson SK, Hussey DJ
- 2584 Conservation and variability of hepatitis B core at different chronic hepatitis stages
Yll M, Cortese MF, Guerrero-Murillo M, Orriols G, Gregori J, Casillas R, González C, Sopena S, Godoy C, Vila M, Taberner D, Quer J, Rando A, Lopez-Martinez R, Esteban R, Riveiro-Barciela M, Buti M, Rodríguez-Frías F

- 2599 Sleeve gastrectomy ameliorates endothelial function and prevents lung cancer by normalizing endothelin-1 axis in obese and diabetic rats
Ruze R, Xiong YC, Li JW, Zhong MW, Xu Q, Yan ZB, Zhu JK, Cheng YG, Hu SY, Zhang GY

Clinical and Translational Research

- 2618 Clinicopathological features of early gastric cancers arising in *Helicobacter pylori* uninfected patients
Sato C, Hirasawa K, Tateishi Y, Ozeki Y, Sawada A, Ikeda R, Fukuchi T, Nishio M, Kobayashi R, Makazu M, Kaneko H, Inayama Y, Maeda S

Case Control Study

- 2632 Anhedonia and functional dyspepsia in obese patients: Relationship with binge eating behaviour
Santonicola A, Gagliardi M, Asparago G, Carpinelli L, Angrisani L, Iovino P

Retrospective Study

- 2645 Decreased of BAFF-R expression and B cells maturation in patients with hepatitis B virus-related hepatocellular carcinoma
Khlaiphuengsin A, Chuaypen N, Sodsai P, Buranapraditkun S, Boonpiyathad T, Hirankarn N, Tangkijvanich P

Prospective Study

- 2657 Role of dynamic perfusion magnetic resonance imaging in patients with local advanced rectal cancer
Ippolito D, Drago SG, Pecorelli A, Maino C, Querques G, Mariani I, Franzesi CT, Sironi S

SYSTEMATIC REVIEWS

- 2669 Association between non-alcoholic fatty liver disease and obstructive sleep apnea
Umbro I, Fabiani V, Fabiani M, Angelico F, Del Ben M

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Vito Annese, MD, PhD, Associate Professor, Chief Doctor, Department of Medical and Surgical Sciences, University Hospital Careggi, Florence 50139, Italy

AIMS AND SCOPE

The primary aim of *World Journal of Gastroenterology* (*WJG*, *World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJG mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

INDEXING/ABSTRACTING

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2019 edition of Journal Citation Report® cites the 2018 impact factor for *WJG* as 3.411 (5-year impact factor: 3.579), ranking *WJG* as 35th among 84 journals in gastroenterology and hepatology (quartile in category Q2). CiteScore (2018): 3.43.

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yan-Liang Zhang*

Proofing Production Department Director: *Yun-Xiaojuan Wu*

Responsible Editorial Office Director: *Ze-Mao Gong*

NAME OF JOURNAL

World Journal of Gastroenterology

ISSN

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE

October 1, 1995

FREQUENCY

Weekly

EDITORS-IN-CHIEF

Subrata Ghosh, Andrzej S Tarnawski

EDITORIAL BOARD MEMBERS

<http://www.wjgnet.com/1007-9327/editorialboard.htm>

PUBLICATION DATE

May 28, 2020

COPYRIGHT

© 2020 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Retrospective Study

Decreased of BAFF-R expression and B cells maturation in patients with hepatitis B virus-related hepatocellular carcinoma

Apichaya Khlaiphuengsin, Natthaya Chuaypen, Pimpayao Sodsai, Supranee Buranapraditkun, Tadech Boonpiyathad, Nattiya Hirankarn, Pisit Tangkijvanich

ORCID number: Apichaya Khlaiphuengsin (0000-0001-5797-5747); Natthaya Chuaypen (0000-0002-5415-510X); Pimpayao Sodsai (0000-0003-0368-9416); Supranee Buranapraditkun (0000-0001-9971-4318); Tadech Boonpiyathad (0000-0001-8690-7647); Nattiya Hirankarn (0000-0003-2224-6856); Pisit Tangkijvanich (0000-0002-2926-8671).

Author contributions:

Khlaiphuengsin A designed the research, performed the research and drafted the manuscript; Chuaypen N, Sodsai P and Buranapraditkun S supervised the report and contributed to the analysis; Boonpiyathad T and Hirankarn N supervised the report; Tangkijvanich P provided clinical advice, drafted and approved the final version of the manuscript.

Supported by the Thailand Research Fund, No. RTA6280004; the Grant for Chula Research Scholar, No. CU-GRS-61-07-30-02; Second Century Fund (C2F), Chulalongkorn University; and the Center of Excellence in Hepatitis and Liver Cancer, Faculty of Medicine, Chulalongkorn University.

Institutional review board

statement: The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB no. 438/60).

Apichaya Khlaiphuengsin, Natthaya Chuaypen, Pisit Tangkijvanich, Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Pimpayao Sodsai, Nattiya Hirankarn, Center of Excellence in Immunology and Immune-mediated Diseases, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Supranee Buranapraditkun, Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Supranee Buranapraditkun, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand

Supranee Buranapraditkun, Center of Excellence in Vaccine Research and Development (Chula Vaccine Research Center), Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Tadech Boonpiyathad, Department of Medicine, Phramongkutklao Hospital, Bangkok 10400, Thailand

Corresponding author: Pisit Tangkijvanich, MD, Doctor, Professor, Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, 1873 Rama 4 Road, Pathumwan, Bangkok 10330, Thailand. pisittkvn@yahoo.com

Abstract**BACKGROUND**

Recent evidence has indicated the role of B cells and B cell-activating factor (BAFF) in the development of hepatocellular carcinoma (HCC).

AIM

To characterize circulating BAFF receptor expression and B cell subpopulations in patients with hepatitis B virus (HBV)-related HCC.

METHODS

Peripheral blood samples collected from 41 patients with chronic HBV infection (25 patients without HCC and 16 patients with HCC) and 9 healthy controls were assessed for BAFF receptors [BAFF-R(B cell-activating factor receptor), transmembrane activator and cyclophilin ligand interactor, B-cell maturation antigen] and B cell subpopulations by multicolor flow cytometry.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Received: January 27, 2020

Peer-review started: February 03, 2020

First decision: February 29, 2020

Revised: March 27, 2020

Accepted: May 13, 2020

Article in press: May 13, 2020

Published online: May 28, 2020

P-Reviewer: Hann HW, Kai K

S-Editor: Zhang H

L-Editor: A

E-Editor: Zhang YL



RESULTS

The frequency of BAFF-R expressing B cells to total B cells was significantly lower in patients with HCC ($3.39\% \pm 2.12\%$) compared with the non-HCC group ($5.37\% \pm 1.90\%$) and healthy controls ($6.23\% \pm 2.32\%$), whereas there was no difference in transmembrane activator and cyclophilin ligand interactor and B-cell maturation antigen. The frequencies of CD27⁺IgD⁺ memory B cells, CD27⁺IgD⁻ class-switched memory B cells and plasmablasts were significantly lower in the patients with HCC compared to patients without HCC (1.23 ± 1.17 vs 3.09 ± 1.55 , $P = 0.001$, 0.60 ± 0.44 vs 1.69 ± 0.86 , $P < 0.0001$ and 0.16 ± 0.12 vs 0.37 ± 0.30 , $P = 0.014$, respectively). However, the ratio of naïve and transitional B cell did not differ significantly between the three groups. In addition, decreased BAFF-R expression on B cells was significantly correlated with large tumor size and advanced tumor stage.

CONCLUSION

Our data demonstrated BAFF-R expression was reduced in B cells that involved with the frequencies of B cells maturation in patients with HCC. The depletion of BAFF-R might play an important role in the development of HCC in patients with chronic HBV infection.

Key words: B cells; Hepatitis B virus; Hepatocellular carcinoma; B cell-activating factor receptor

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This study explored the expression of B cell-activating factor receptor (BAFF-R) and B cell subpopulations in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Our data showed that BAFF-R expression was significantly lower in patient with HCC compared with non-HCC and healthy controls. In addition, the frequencies of CD27⁺IgD⁺ memory B cells, CD27⁺IgD⁻ class-switched memory B cells and plasmablasts were significantly lower in the patients with HCC compared to patients without HCC. In addition, decreased BAFF-R expression was significantly correlated with tumor size and tumor stage. This study is the first report suggesting that the depletion of BAFF-R in B cells might be responsible for B cell maturation in patients with HBV-related HCC.

Citation: Khlaiphuengsin A, Chuaypen N, Sodsai P, Buranapraditkun S, Boonpiyathad T, Hirankarn N, Tangkijvanich P. Decreased of BAFF-R expression and B cells maturation in patients with hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2020; 26(20): 2645-2656

URL: <https://www.wjgnet.com/1007-9327/full/v26/i20/2645.htm>

DOI: <https://dx.doi.org/10.3748/wjg.v26.i20.2645>

INTRODUCTION

Hepatocellular carcinoma (HCC) represents one of the most common malignant tumors worldwide, especially in East and Southeast Asian countries, where hepatitis B virus (HBV) infection is highly prevalent^[1]. In Thailand, the incidence of liver cancer is approximately 38.6 and 17.2 per 100000 person-years, respectively and at least 50%-60% of all HCC cases are attributable to chronic HBV infection^[2]. Hepatocarcinogenesis is a complex multistep process involving the persistence of liver damage and inflammation, which results in a distinctive sequence of chronic hepatitis, cirrhosis and finally HCC development^[3]. As chronic inflammation is a crucial driver of disease progression, accumulative evidence has indicated that immune-mediated host-virus interactions play an essential role in the development of HBV-related HCC^[4]. While the importance of T-cell immunity has been well documented, the role of B cells in HCC progression is not yet completely understood and needs further investigation.

B cells are classically known to stimulate immune response *via* the activation of T cells and antibody production^[5]. The activation of specific B cells results in the

proliferation and development of memory B cells and antibody-producing plasma cells, which are beneficial to neutralize and control active viral replication. The survival of B cells depends on the expression of a functional B cell receptor and signals from B cell-activating factor (BAFF), a member of the tumor necrosis factor (TNF) superfamily^[6]. This cytokine binds to three receptors expressed on B cells including BAFF receptor (BAFF-R), transmembrane activator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA). In general, BAFF-R activates downstream pathways that regulate survival and maturation of B cells, TACI induces immunoglobulin (Ig) class switching, whereas BCMA promotes plasma B cells survival^[7]. Human B cells are comprised of distinct phenotypic and functional subpopulations characterized based on different developmental stages such as transitional, naïve, memory B cells and plasmablasts. Since BAFF receptors and B cell subsets play diverse but crucial roles in modulating B cell function, analysis of their expression and subpopulation frequencies could provide more insights into the immunological characteristics of B cell selection in patients with HCC.

Recent evidence has suggested that B cells exhibit dual biological effects in promoting and inhibiting the development and progression of several cancers^[8]. Regarding HCC, a previous study showed that increased percentage of circulating B cells was found in individuals with advanced HCC compared with early tumor staging^[9]. Recently, it was also demonstrated that tumor infiltrating B cells was associated with disease prognosis in patients with HBV-related HCC^[10]. Moreover, the close proximity and interaction of tumor-infiltrating T cells and B cells suggested an increased immune activation that might contribute to better prognosis in patients with HCC^[11]. In addition, we recently reported that plasma BAFF levels significantly increased in patients with HBV-related HCC compared with the non-HCC group and healthy controls^[12]. Together, these data suggest that B cells and BAFF may play an important role in HCC development and progression in patients with chronic HBV infection. So far, the phenotypes of circulating B-cell subtypes in patients with HBV-related HCC have not been well characterized.

In the present study, we aimed to compare the expression of BAFF receptors and the distribution of B cell subsets in the peripheral blood of patients with HBV-related HCC compared to individuals without HCC and healthy controls. Our data showed that BAFF-R expression was significantly reduced in B cells that involved with the frequencies of B cells maturation in patients with HCC. Interestingly, decreased BAFF-R expression was significantly associated with progressive HCC including large tumor size and advanced cancer stage.

MATERIALS AND METHODS

Patients

A total of 50 participants including 41 patients with chronic HBV infection (25 without HCC and 16 with HCC) and 9 healthy individuals were recruited from King Chulalongkorn Memorial Hospital and blood donors at National Blood Centre Thai Red Cross Society, Bangkok, Thailand, respectively. The diagnosis of chronic HBV infection was confirmed by the presence of serum hepatitis B s antigen (HBsAg) at least 6 mo. Patients with co-infection with hepatitis C virus (HCV) and/or human immunodeficiency virus (HIV) were not included in this study. In addition, patients with evidence of other malignancies or autoimmune disorders were excluded.

Patients in the HCC group were diagnosed on the basis of typical imaging studies and/or histopathology (fine needle aspiration, core liver biopsy or surgical resection) according to the standard guideline^[13]. Diagnostic criteria of HCC by imaging studies were based on findings of focal hepatic lesions with hyperattenuation at the arterial phase, hypoattenuation at the portal phase in dynamic CT or MRI. The clinical parameters of patients with HCC at initial diagnosis were collected, which included sex, age, liver function tests, serum alpha-fetoprotein (AFP) level and HCC staging classified by the Barcelona Clinic Liver Cancer (BCLC) system^[14].

The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB no. 438/60) and participants had provided written informed consent. The study followed the Helsinki Declaration and Good Clinical Practice guidelines.

Flow cytometry staining

All blood samples were collected in sodium heparin tube and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation using Percoll PLUS density gradient media (GE Healthcare, Philadelphia, Pennsylvania, PA, USA) at 1500 rpm for 30 min at room temperature.

PBMCs were washed twice with Hank's balanced salt solution (HBSS) (GIBCO, New York, NY, USA) with 5% fetal bovine serum (FBS) and 100 U/mL penicillin and 100 µg/mL streptomycin. Total lymphocytes were counted and stored at liquid nitrogen before analysis.

Cryopreserved PBMCs from each patient were defrosted and added in complete in RPMI1640 Medium (GIBCO, New York, NY, USA) supplement with 10% FBS. At least 200000 cells were stained with combinations of antibodies including anti-CD19-PE, anti-CD38-FITC, anti-IgD-PerCP-Cy5.5, anti-CD27 Alexa Fluor 700, anti-CD24 PE-CyTM7 (BD Biosciences, San Jose, CA, USA), anti-CD268 (BAFFR)-APC, anti-CD267 (TACI)-APC and anti-CD269 (BCMA)-APC (BioLegend, San Diego, CA, USA). The cells were re-suspended in the buffer before performed flow cytometry using BD LSR II Flow Cytometry Analyzer (BD Biosciences, San Jose, CA, USA). Flow cytometry data were analyzed with Flowjo software version 10 (Tree star, Ashland, Oregon, USA).

Assays of plasma BAFF levels and HBV markers

Plasma BAFF levels were determined by ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Qualitative hepatitis B s antigen (HBsAg) was measured by commercially available enzyme-linked immunosorbent assays (Abbott Laboratories, Chicago, IL). Serum HBV DNA levels were quantified by Abbott Real Time HBV assay (Abbott Laboratories).

Statistical Analysis

Statistical analysis was performed by using SPSS version 22 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism v5.0 (GraphPad Software, San Diego, CA). Values were presented as mean \pm standard deviation (SD), and percentages as appropriate. The statistical significance among groups was assessed by using the Kruskal-Wallis test, followed by multiple comparison test. The correlation data were analyzed by Spearman's rank test. The *P* value less than 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of patients

Baseline clinical characteristics of 9 healthy controls and 41 patients with chronic HBV infection (25 patients without HCC and 16 patients with HCC) are summarized in [Table 1](#). Patients in each group were significantly older than healthy controls ($P < 0.001$). However, there was no difference in mean age distribution among groups of patients. Compared with the non-HCC group, patients with HCC had significantly lower mean hematocrit, platelet counts and serum albumin. However, there was no significant difference among groups in terms of other baseline clinical parameters.

Plasma BAFF levels and BAFF receptor expression

Patients with HCC had significantly higher mean plasma BAFF levels compared with those without HCC (1208.8 ± 386.1 vs 914.8 ± 159.3 pg/mL; $P = 0.042$) ([Table 1](#)), which confirmed our recent report^[12]. Percentages of total B cells in peripheral blood among the studied groups were not different, although there was a trend of higher total B cells in the HCC group compared with the non-HCC group ($20.09\% \pm 10.37\%$ vs $15.03\% \pm 4.09\%$, $P = 0.079$, [Supplement Figure S1](#)). The expression of BAFF receptors including BAFF-R, TACI and BCMA were further investigated ([Figure 1](#) and [2](#)). We found that there was a significantly decreased in percentage of BAFF-R expressing B cells in the HCC group ($3.39\% \pm 2.12\%$) compared with the non-HCC group ($5.37\% \pm 1.90\%$; $P = 0.004$) and healthy controls ($6.23\% \pm 2.32\%$; $P = 0.006$). However, the percentage of BAFF-R expressing B cells was similar between the non-HCC group and controls ($P = 0.28$). In addition, the frequencies of TACI and BCMA expressing B cells were not significantly altered among the studied groups.

Analysis of B cell subpopulations

An in-depth analysis of B cell subpopulations was further analyzed in patients with or without HCC and in healthy controls. B cell population characteristics and gating strategy are shown in [Figure 1](#). Overall, the absolute number of CD19+ B cells was not significantly different between groups. However, the frequency of IgD memory B cells (CD27+IgD+) were significant reduced in patients with HCC compared with the non-HCC individuals ($1.23\% \pm 1.17\%$ vs $3.09\% \pm 1.56\%$; $P = 0.001$) ([Figure 3A](#) and [B](#)). A significantly lower number of class-switched memory B cells (CD27+IgD-) in patients with HCC ($0.60\% \pm 0.44\%$) was also observed when compared with patients without HCC ($1.69\% \pm 0.86\%$; $P < 0.001$) and healthy controls ($1.45\% \pm 0.81\%$; $P = 0.019$).

Table 1 Demographic and clinical characteristics of patients and healthy controls

	Healthy controls (n = 9)	Patients without HCC (n = 25)	Patients with HCC (n = 16)	P value
Sex (males)	4 (44.4%)	12 (48.0%)	13 (81.3%)	0.072
Age, yr	27.6 (25-34)	42.8 (20-77)	60.8 (34-77)	< 0.001 ^c
Hemoglobin (g/dL)		13.3 ± 1.5	12.1 ± 1.9	0.082
Hematocrit (%)		41.4 ± 4.1	36.7 ± 5.7	0.008 ^b
Platelets count (10 ³ /μL)		246.4 ± 56.7	180.1 ± 80.8	0.007 ^b
White cell count (10 ³ /μL)		6.5 ± 2.2	6.1 ± 2.0	0.59
INR		1.0 ± 0.1	1.1 ± 0.2	0.34
AST (U/L)		48.9 ± 42.2	80.0 ± 65.0	0.07
ALT (U/L)		75.8 ± 83.1	56.9 ± 32.3	0.407
ALP (mg/L)		59.3 ± 14.3	177.5 ± 118.0	0.066
Total bilirubin (mg/dL)		0.7 ± 0.1	0.8 ± 0.4	0.757
Albumin (g/dL)		4.5 ± 0.3	3.4 ± 0.6	0.001 ^b
AFP (IU/mL)		2.4 ± 2.2	4810.2 ± 10236.1	0.531
log ₁₀ HBV DNA (IU/mL)		5.4 ± 2.6	3.0 ± 1.9	0.066
BAFF (pg/mL)		914.8 ± 159.3	1208.8 ± 386.1	0.042 ^a
BCLC stage				
A-B			12 (75.0%)	
C			4 (25.0%)	

Data expressed as mean ± SD or *n* (%) as appropriate;

^a*P* < 0.05;

^b*P* < 0.01;

^c*P* < 0.001. INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AFP: Alpha-fetoprotein; BAFF: B cell-activating factor; BCLC: Barcelona clinic liver cancer.

(Figure 3A and C). Moreover, the frequency of plasmablasts was significantly decreased in the HCC group compared with the non-HCC group (0.16% ± 0.12% *vs* 0.37% ± 0.30%; *P* = 0.014) (Figure 4A). However, no significant difference between groups was found regarding the frequencies of naïve and transitional B cells (Figure 3A, D and 4B).

Clinical correlation of BAFF-R

The clinical correlations of BAFF-R expression were further investigated in patients with HCC. Our results showed that BAFF-R had positive correlation with hematocrit (*r* = 0.550, *P* = 0.029), white cell count (*r* = 0.518, *P* = 0.042) and serum albumin (*r* = 0.604, *P* = 0.032). In contrast, BAFF-R exhibited negative correlation with patient's age (*r* = -0.533, *P* = 0.033), serum AFP (*r* = -0.557, *P* = 0.034) and tumor size (*r* = -0.542, *P* = 0.032) (Figure 5A-E). BAFF-R did not significantly correlate with other clinical parameters including platelet counts, international normalized ratio (INR), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and HBV DNA level. Regarding tumor staging, BAFF-R expression in patients in advanced BCLC stage (stage C) was significantly lower than that of patients with earlier tumor stages (stages A and B) (1.61% ± 0.71% *vs* 4.73% ± 3.14%, *P* = 0.007) (Figure 5F).

DISCUSSION

Chronic HBV infection is a complex infectious disorder characterized by immune-mediated liver inflammation, which results in repeated destruction and regeneration of hepatocytes, and ultimately leads to the development of HCC^[3]. Overall, HBV-related HCC is a leading cause of cancer death in Thailand and other Asian countries, with a very poor prognosis because of its aggressiveness and high recurrence rates^[15]. As a result, a better understanding regarding the molecular mechanisms and progression of HCC is highly needed. Among several immunological factors associated with hepatocarcinogenesis, it is likely that B cells and BAFF may contribute to disease progression and HCC development in patients with chronic HBV infection^[10]. In this context, we previously demonstrated that higher expression of plasma BAFF was linked to disease severity and overall survival in HBV-related

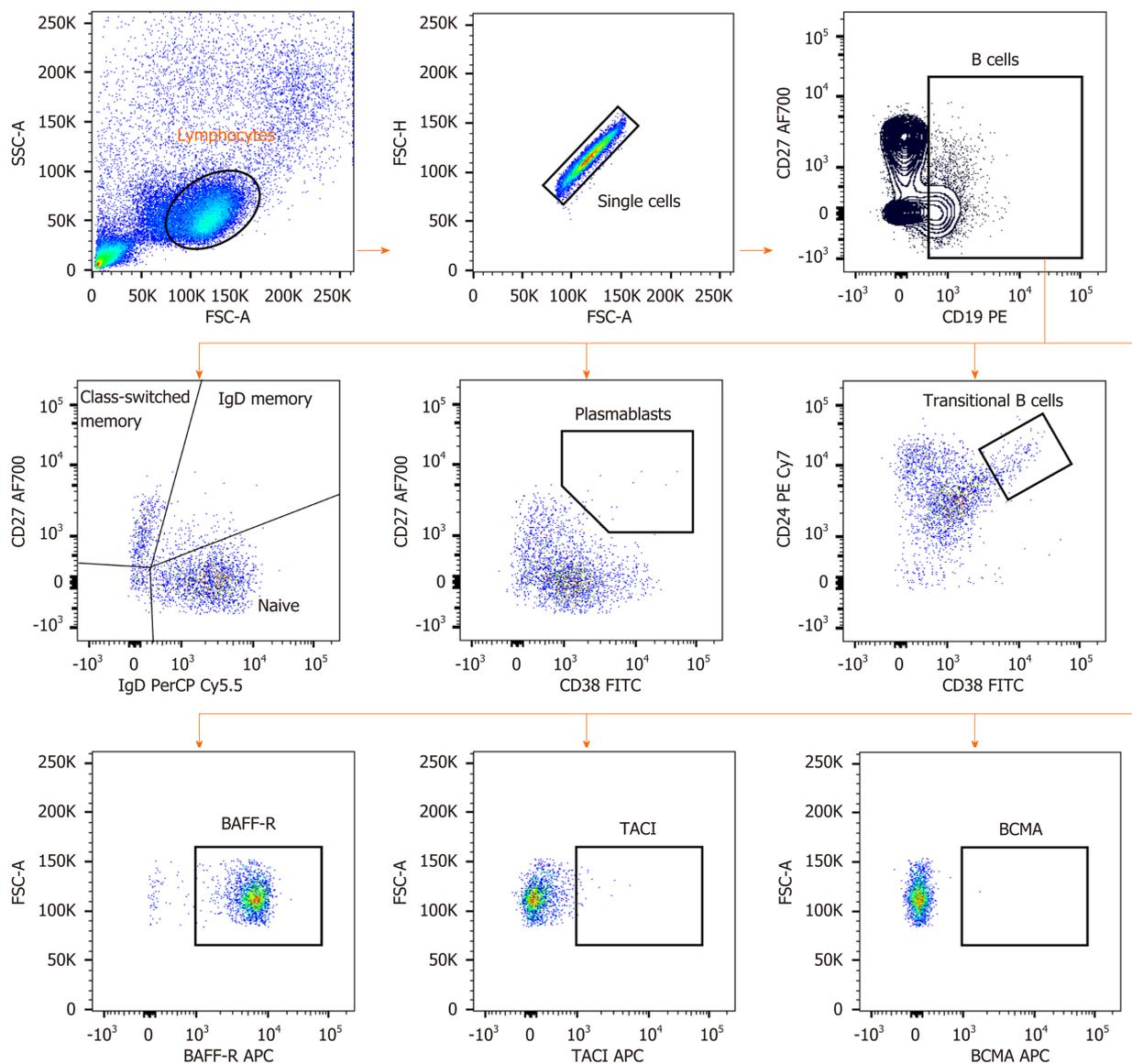


Figure 1 Representative plot shows the gating strategy for identification of B cell subsets. (1) Firstly, lymphocyte population was initially determined from forward scatter and side scatter plot. (2) Positive for CD19 was selected from CD19 vs CD27 plot. (3) Naive B cells, IgD and class-switched memory B cells were then identified from IgD vs CD27 plot. And (4) Plasmablasts and transitional B cells were selected from CD38 vs CD27 and CD38 vs CD24, respectively. Moreover, B cell-activating factor receptors were gated on CD19 positive cells. FSC: Forward scatter; SSC: Side scatter; BAFF-R: B cell-activating factor receptors; BCMA: B-cell maturation antigen; TACI: Transmembrane activator and cyclophilin ligand interactor.

HCC^[12]. However, the role of BAFF receptors and distribution of circulating B-cell subtypes in HCC remains unclear.

In the present study, we explored the expression of BAFF receptors including BAFF-R, TACI and BCMA, which are expressed almost exclusively on B cells^[7]. Interestingly, we found that the percentage of B cells expressing BAFF-R was significantly decreased in patients with HBV-related HCC compared with HBV patients without HCC and healthy controls. However, frequencies of TACI and BCMA on B cells were not significantly altered among groups. Thus, these results might indicate that BAFF-R but not TACI and BCMA play an important role in HCC development in patients with chronic HBV infection. In fact, BAFF-R is clearly a key receptor involved in the successful survival and maturation of B cells^[16]. Previous studies have demonstrated that BAFF-R is important not only in B cell development, but also is the major mediator of BAFF-dependent co-stimulatory responses in peripheral B and T cells^[17]. Similarly to the study of BAFF-R-deficient mice, BAFF-R is crucial for the development of B cells up to the stage of IgM+ immature/transitional B cells but cannot complete maturation in the spleen^[18]. Moreover, BAFF-R is considered to be the most important receptor due to its critical role in regulatory B cells (Bregs) survival^[19]. As previously demonstrated, targeting of BAFF-R in patients with

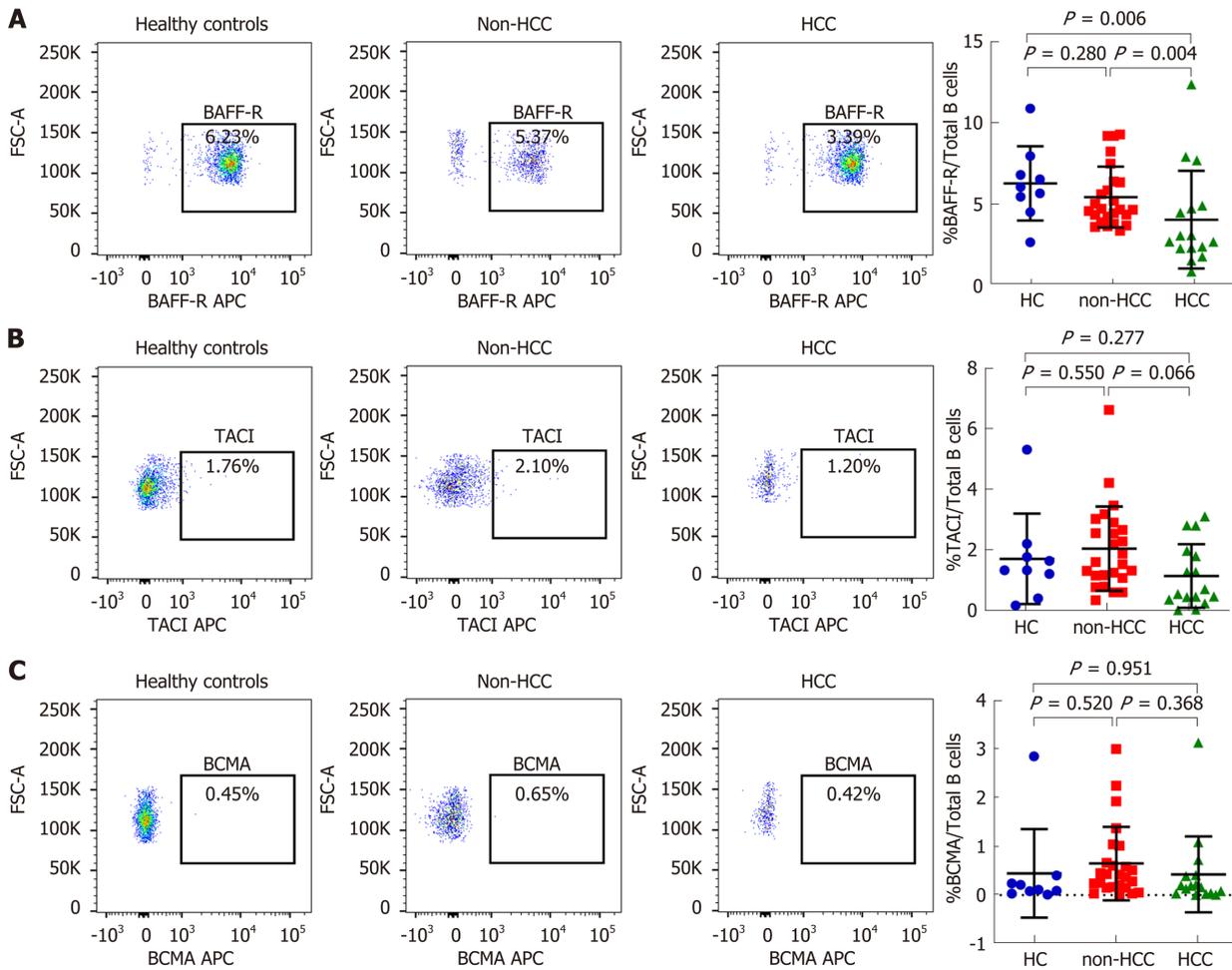


Figure 2 The frequency of B cell-activating factor receptors expression was significantly lower in patients with hepatocellular carcinoma compared with the other groups. Representative fluorescence activated cell sorting and statistic plots of A: B cell-activating factor receptors; B: Transmembrane activator and cyclophilin ligand interactor; C: B-cell maturation antigen. TACI: Transmembrane activator and cyclophilin ligand interactor; BCMA: B-cell maturation antigen; BAFF-R: B cell-activating factor receptors; HCC: Hepatocellular carcinoma; HC: Healthy controls.

precursor B-lineage acute lymphoblastic leukemia (B-ALL) could significantly impact the survival and basal proliferation of leukemia B-cell precursors^[20]. Moreover, downregulation of circulating BAFF-R was shown to be associated with disease activity in patients with autoimmune disorders^[21]. In this study, we also demonstrated that decreased BAFF-R expression on B cells was significantly correlated with tumor size and more advanced BCLC stages, indicating a contribution of BAFF-R to the disease progression of HBV-related HCC.

Normal B cell development and survival can promote an effective immune response to clear pathogens, whereas abnormalities in B cells differentiation and activation leads to the disruption of B cell homeostasis^[5]. Accordingly, circulating B cell subset frequencies were also determined in this study. We found that the frequencies of CD27+IgD+ memory B cells, CD27+IgD- class-switched memory B cells and plasmablasts were significantly lower in patients with HCC compared to the non-HCC group. In contrast, number of naïve B cells did not differ significantly among groups. These findings were consistent with a previous report demonstrating that decreased of CD27+ memory B cells was found in patients with HCC but no differences in CD27- naïve and total B cells^[22]. Moreover, a similar result was observed in patients with HCV-related HCC in which CD27+ memory B cells, and more specifically CD27+IgM+ memory B cells, were markedly less frequent in patients with HCC independent of HCV infection^[23]. Memory B cells are long-lived and could capture and present the antigen to MHC molecules and then modulate CD4+ and CD8+ T cells immune response^[24]. They also could potentially activate other immune cells such as dendritic cells and macrophages by producing pro-inflammatory cytokines. Therefore, it is possible that the decrease in total memory B cells and plasmablast in HBV-related HCC might reflect the defect of generating protective immunity against HBV in these individuals. In a model of lung cancer, other cell

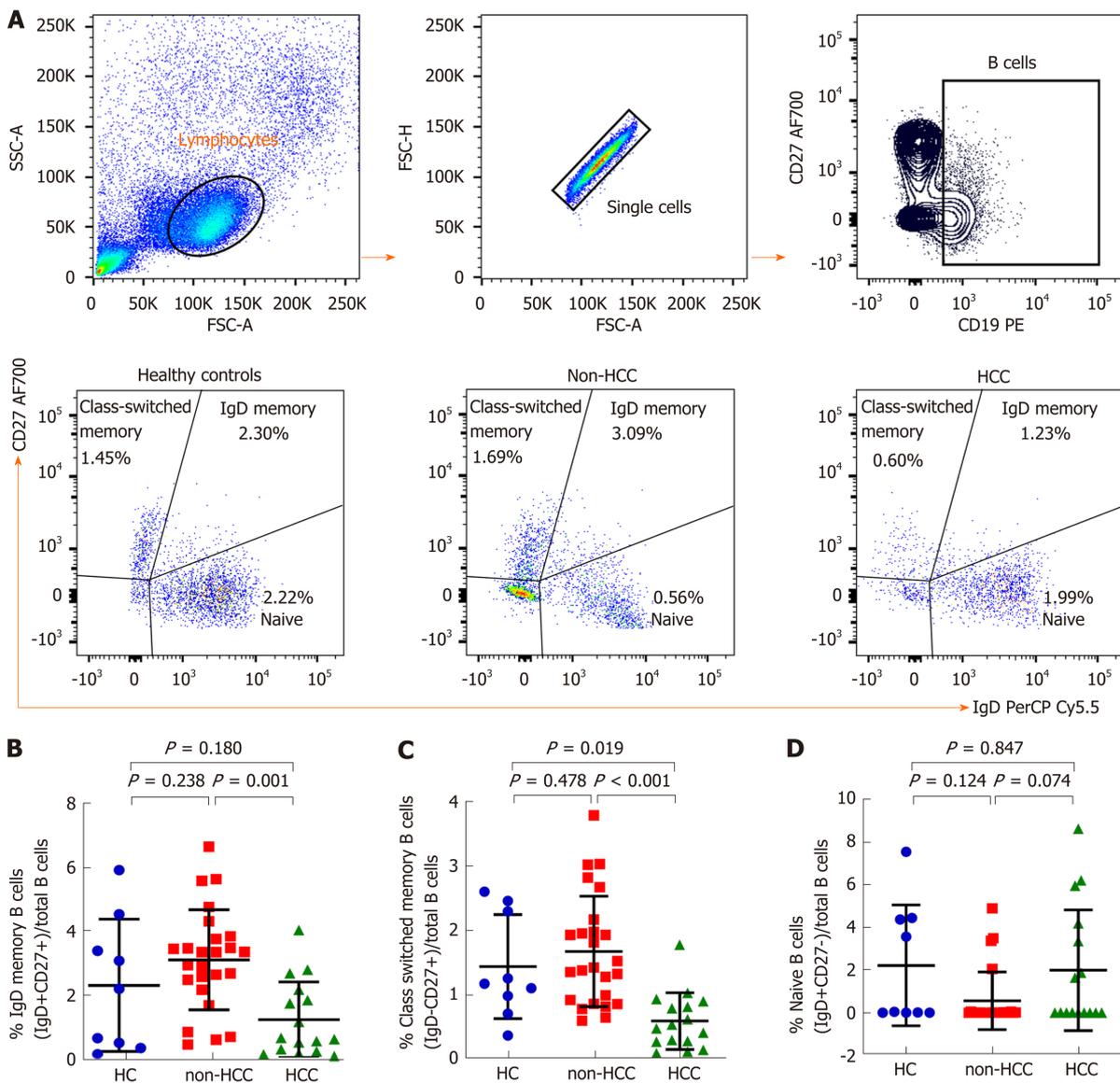


Figure 3 Class-switched and IgD memory B cells were significantly lower in patients with hepatocellular carcinoma compared with the non-hepatocellular carcinoma group. A: Gating strategy and relative of; B: IgD memory B cells; C: Class switched memory B cells; D: Naïve B cells in healthy controls, patients without hepatocellular carcinoma (Non-HCC) and patients with hepatocellular carcinoma. HCC: Hepatocellular carcinoma; HC: Healthy controls.

types, such as myeloid-derived suppressor cells can also impair B cells function through the secretion of IL-17, which is associated with decrease of antibody production^[25]. In addition to anti-tumor capacity, B cells can also modulate T cell immune responses *via* the function of Bregs^[26]. Here, we did not directly measure Bregs population but instead investigated the CD19+CD24^{hi}CD38^{hi} transitional B cells, which have predominant Breg in this subset^[27]. In this context, we did not observe any significant difference of the transitional B cells among the studied groups.

Interestingly, different finding regarding the distribution of B cell subpopulations was observed in patients with colorectal cancer, in which higher percentages of memory and plasma B cells were detected in peripheral blood^[28]. In addition, increased numbers of circulating switched memory cells and plasmablasts were observed in other solid tumors including urinary bladder cancer, malignant melanoma, pancreatic cancer and prostate cancer^[29]. Moreover, there was evidence that antibodies produced by B cells play a role in the cancer progression by initiating chronic inflammation^[30]. Taken together, it is likely that role of B cell subsets might not be similar among different cancer types. Therefore, categorizing B cell subpopulations in each cancer type is important not only for a better understanding the mechanisms by which immune cell subsets affect tumor biology but also for designing a successful treatment using immunotherapeutic approaches.

Overall, our findings indicated that BAFF-R expressing B cells had a decreased dependency on the maturation of B cells in patients with HCC compared with the

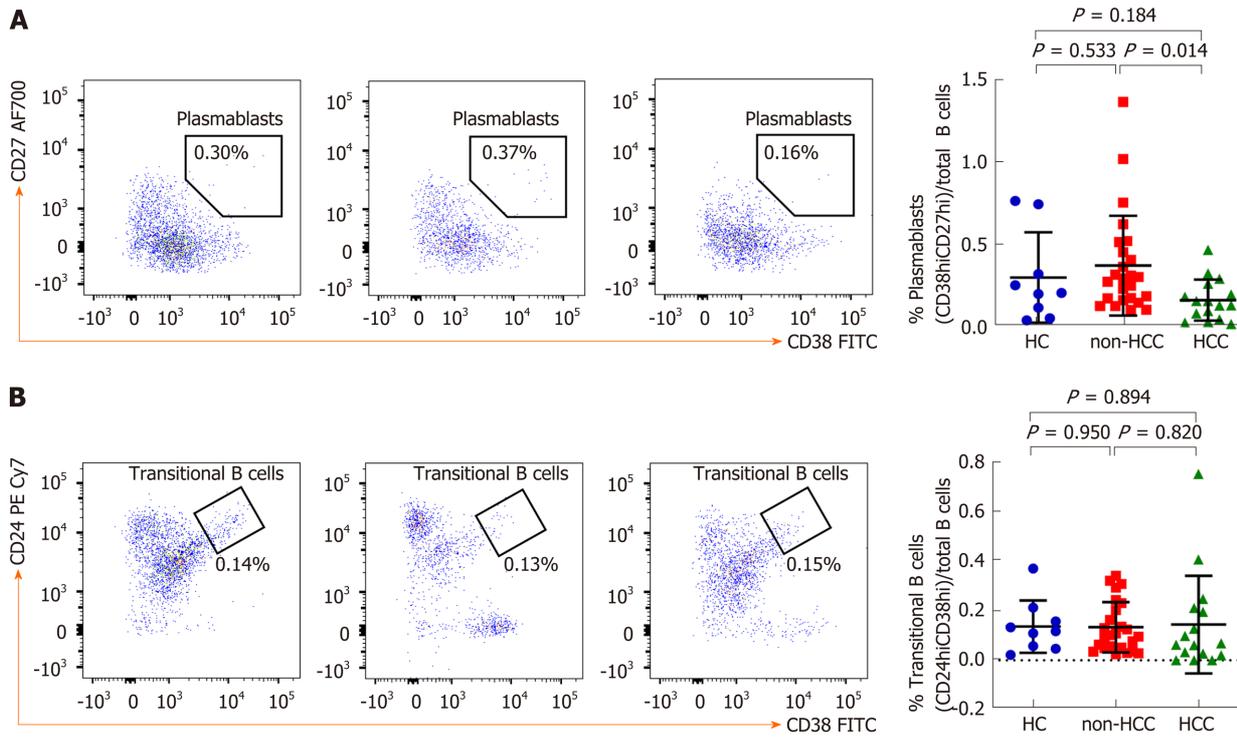


Figure 4 Lower frequency of plasmablasts in patients with hepatocellular carcinoma compared with non-hepatocellular carcinoma. Representative and statistic plots of A: Plasmablasts; B: Transitional B cells in healthy controls, patients without hepatocellular carcinoma (Non-HCC) and patients with hepatocellular carcinoma. HCC: Hepatocellular carcinoma; HC: Healthy controls.

non-HCC groups. It is therefore possible that down-regulation of BAFF-R could constitute resistance to the biological actions of BAFF and as such might, in part, be responsible for the elevated plasma BAFF concentrations in patients with HCC. On the other hand, it might be possible that chronic elevated BAFF levels in patients with HCC could lead to a downregulation of BAFF-R expression, as previously observed in patients with autoimmune disorders^[21]. At this point, however, the underlying mechanism by which BAFF and BAFF receptors involve in HCC development is not clear and needed to be further elucidated. In this perspective, the design of therapeutic target of BAFF or BAFF-R might also be important for patients with HCC in the future.

Despite our interesting findings on the role of B cells and their ligand/receptors in disease progression in patients with HBV-related HCC, there were some limitations in this study. First, the study was retrospective and there might have many possible confounding factors, such as age and sex. Second, the sample sizes of patients with or without HCC were relatively small and a replicate study with a larger number of patients is needed to verify these observations and would provide further insights into the role of BAFF, as well as BAFF receptors and B cell response in HBV-related HCC. Moreover, further studies also need to validate these observations in patients with HCC regardless of underlying etiologies and to elucidate the mechanistic roles of B cell-mediated immune response in hepatocarcinogenesis.

In summary, we found the expression of BAFF-R was reduced in B cells that might reflect the different frequency of B cells maturation in patients with HCC. The memory B cells especially CD27+IgD- class-switched memory B cells and plasmablasts were significantly decreased whereas naïve and transitional B cells were not different in patients with HCC as compared with the non-HCC group and healthy controls. Additionally, decreased BAFF-R expression on B cells was significantly correlated with tumor size and more advanced BCLC stages. Together, these data indicate that abnormalities of B cell development according to the expression of BAFF-R is contributable to the development and progression of HBV-related HCC.

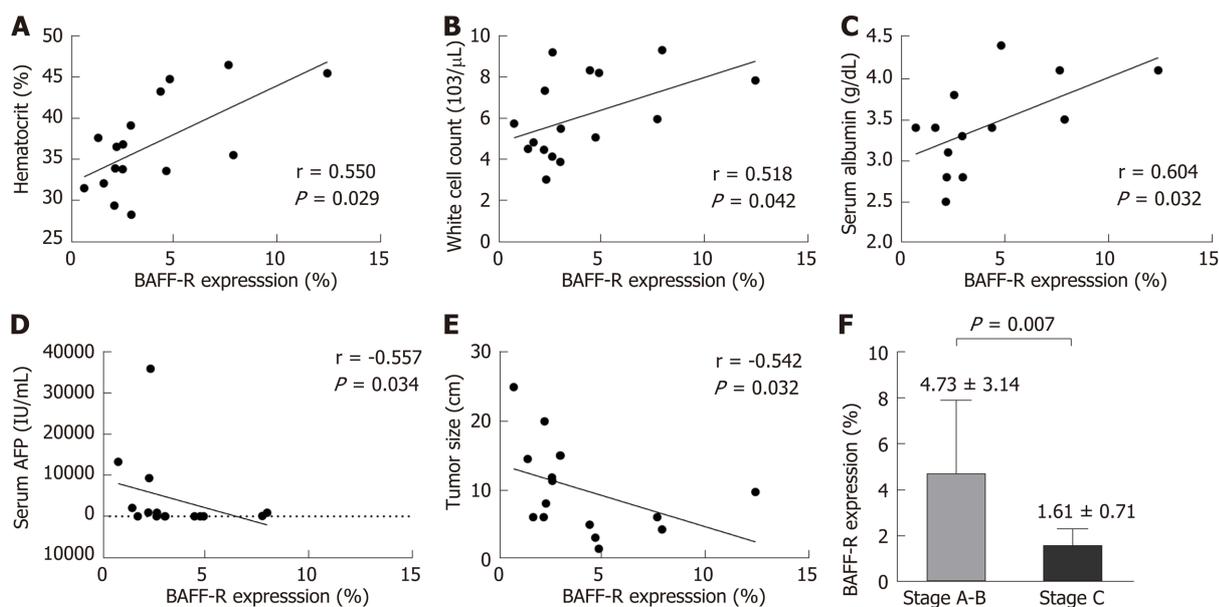


Figure 5 B cell-activating factor expression and clinical correlation in patients with hepatocellular carcinoma. A: Hematocrit; B: White cell count; C: Serum albumin; D: Serum alpha-fetoprotein; E: Tumor size; F: Tumor staging. BAFF-R: B cell-activating factor receptors; AFP: Alpha-fetoprotein.

ARTICLE HIGHLIGHTS

Research background

Recently, B cell-activating factor (BAFF) receptors and B cell subsets play diverse but crucial roles in modulating B cell function. Therefore, analysis of their expression and subpopulation frequencies could provide more insights into the immunological characteristics of B cell selection in patients with hepatocellular carcinoma (HCC).

Research motivation

To evaluate the association of BAFF receptors on B cell subsets according to clinical outcome of patients with chronic HBV infection.

Research objectives

This study aimed to compare the expression of BAFF receptors and the distribution of B cell subsets in the peripheral blood of patients with HBV-related HCC compared to individuals without HCC and healthy controls.

Research methods

Peripheral blood samples collected from chronic HBV infected patients with or without HCC and healthy controls were assessed for BAFF receptors [BAFF-R(B cell-activating factor receptor), transmembrane activator and cyclophilin ligand interactor, B-cell maturation antigen] and B cell subpopulations by multicolor flow cytometry.

Research results

The frequency of BAFF-R expression on B cells was significantly decreased in HBV-related HCC compared with HBV patients without HCC and healthy controls. In addition, the frequency of CD27+IgD+ memory B cells, CD27+IgD- class switched memory B cells and plasmablasts were significantly lower in patients with HCC compared to the non-HCC group. However, the frequency of naïve and transitional B cell did not differ significantly among groups. Moreover, decreased BAFF-R expression on B cells was significantly correlated with tumor size and more advanced Barcelona Clinic Liver Cancer stages.

Research conclusions

The expression of BAFF-R on B cells was significantly decreased that involved in the different frequencies of B cells maturation in patients with HCC.

Research perspectives

Larger samples and the mechanistic roles of B cell-mediated immune response in hepatocarcinogenesis are needed to further validate.

REFERENCES

- 1 **El-Serag HB.** Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
- 2 **Tangkijvanich P,** Mahachai V, Komolmit P, Fongsarun J, Theamboonlers A, Poovorawan Y. Hepatitis B virus genotypes and hepatocellular carcinoma in Thailand. *World J Gastroenterol* 2005; **11**: 2238-2243 [PMID: 15818732 DOI: 10.3748/wjg.v11.i15.2238]
- 3 **Arzumanyan A,** Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]
- 4 **Ringelhan M,** Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol* 2018; **19**: 222-232 [PMID: 29379119 DOI: 10.1038/s41590-018-0044-z]
- 5 **Gitlin AD,** Nussenzweig MC. Immunology: Fifty years of B lymphocytes. *Nature* 2015; **517**: 139-141 [PMID: 25567266 DOI: 10.1038/517139a]
- 6 **Lied GA,** Berstad A. Functional and clinical aspects of the B-cell-activating factor (BAFF): a narrative review. *Scand J Immunol* 2011; **73**: 1-7 [PMID: 21128997 DOI: 10.1111/j.1365-3083.2010.02470.x]
- 7 **Mackay F,** Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. *Annu Rev Immunol* 2003; **21**: 231-264 [PMID: 12427767 DOI: 10.1146/annurev.immunol.21.120601.141152]
- 8 **Liu M,** Sun Q, Wang J, Wei F, Yang L, Ren X. A new perspective: Exploring future therapeutic strategies for cancer by understanding the dual role of B lymphocytes in tumor immunity. *Int J Cancer* 2019; **144**: 2909-2917 [PMID: 30183084 DOI: 10.1002/ijc.31850]
- 9 **Lin JC,** Shih YL, Chien PJ, Liu CL, Lee JJ, Liu TP, Ko WC, Shih CM. Increased percentage of B cells in patients with more advanced hepatocellular carcinoma. *Hum Immunol* 2010; **71**: 58-62 [PMID: 19819282 DOI: 10.1016/j.humimm.2009.10.003]
- 10 **Zhang Z,** Ma L, Goswami S, Ma J, Zheng B, Duan M, Liu L, Zhang L, Shi J, Dong L, Sun Y, Tian L, Gao Q, Zhang X. Landscape of infiltrating B cells and their clinical significance in human hepatocellular carcinoma. *Oncoimmunology* 2019; **8**: e1571388 [PMID: 30906667 DOI: 10.1080/2162402X.2019.1571388]
- 11 **Garnelo M,** Tan A, Her Z, Yeong J, Lim CJ, Chen J, Lim KH, Weber A, Chow P, Chung A, Ooi LL, Toh HC, Heikenwalder M, Ng IO, Nardin A, Chen Q, Abastado JP, Chew V. Interaction between tumour-infiltrating B cells and T cells controls the progression of hepatocellular carcinoma. *Gut* 2017; **66**: 342-351 [PMID: 26669617 DOI: 10.1136/gutjnl-2015-310814]
- 12 **Khlaiphuengsin A,** Chuaypen N, Pinjaroen N, Sirichindakul B, Hirankarn N, Tangkijvanich P. Plasma B-cell activating factor levels and polymorphisms in hepatitis B-related hepatocellular carcinoma: Clinical correlation and prognosis. *Asian Pac J Allergy Immunol* 2019; Epub ahead of print [PMID: 30660167 DOI: 10.12932/AP-250718-0376]
- 13 **Bruix J,** Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 14 **Forner A,** Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 15 **Ogunwobi OO,** Harricharran T, Huaman J, Galuza A, Odumuwagon O, Tan Y, Ma GX, Nguyen MT. Mechanisms of hepatocellular carcinoma progression. *World J Gastroenterol* 2019; **25**: 2279-2293 [PMID: 31148900 DOI: 10.3748/wjg.v25.i19.2279]
- 16 **Smulski CR,** Eibel H. BAFF and BAFF-Receptor in B Cell Selection and Survival. *Front Immunol* 2018; **9**: 2285 [PMID: 30349534 DOI: 10.3389/fimmu.2018.02285]
- 17 **Ng LG,** Sutherland AP, Newton R, Qian F, Cachero TG, Scott ML, Thompson JS, Whewey J, Chtanova T, Groom J, Sutton IJ, Xin C, Tangye SG, Kalled SL, Mackay F, Mackay CR. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. *J Immunol* 2004; **173**: 807-817 [PMID: 15240667 DOI: 10.4049/jimmunol.173.2.807]
- 18 **Batten M,** Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, Browning JL, Mackay F. BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med* 2000; **192**: 1453-1466 [PMID: 11085747 DOI: 10.1084/jem.192.10.1453]
- 19 **Thompson JS,** Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, Hession C, Schneider P, Sizing ID, Mullen C, Strauch K, Zafari M, Benjamin CD, Tschopp J, Browning JL, Ambrose C. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. *Science* 2001; **293**: 2108-2111 [PMID: 11509692 DOI: 10.1126/science.1061965]
- 20 **Maia S,** Pelletier M, Ding J, Hsu YM, Sallan SE, Rao SP, Nadler LM, Cardoso AA. Aberrant expression of functional BAFF-system receptors by malignant B-cell precursors impacts leukemia cell survival. *PLoS One* 2011; **6**: e20787 [PMID: 21687682 DOI: 10.1371/journal.pone.0020787]
- 21 **Sellam J,** Miceli-Richard C, Gottenberg JE, Ittah M, Lavie F, Lacabaratz C, Gesteremann N, Proust A, Lambotte O, Mariette X. Decreased B cell activating factor receptor expression on peripheral lymphocytes associated with increased disease activity in primary Sjögren's syndrome and systemic lupus erythematosus. *Ann Rheum Dis* 2007; **66**: 790-797 [PMID: 17185325 DOI: 10.1136/ard.2006.065656]
- 22 **Wang XD,** Wang L, Ji FJ, Zhu JM, Ayana DA, Fang XD. Decreased CD27 on B lymphocytes in patients with primary hepatocellular carcinoma. *J Int Med Res* 2012; **40**: 307-316 [PMID: 22429370 DOI: 10.1177/147323001204000131]
- 23 **Doi H,** Iyer TK, Carpenter E, Li H, Chang KM, Vonderheide RH, Kaplan DE. Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-positive B-cell population. *Hepatology* 2012; **55**: 709-719 [PMID: 21932384 DOI: 10.1002/hep.24689]
- 24 **Milich DR,** Chen M, Schödel F, Peterson DL, Jones JE, Hughes JL. Role of B cells in antigen presentation of the hepatitis B core. *Proc Natl Acad Sci USA* 1997; **94**: 14648-14653 [PMID: 9405667 DOI: 10.1073/pnas.94.26.14648]
- 25 **Wang Y,** Schafer CC, Hough KP, Tousif S, Duncan SR, Kearney JF, Ponnazhagan S, Hsu HC, Deshane JS. Myeloid-Derived Suppressor Cells Impair B Cell Responses in Lung Cancer through IL-7 and STAT5. *J Immunol* 2018; **201**: 278-295 [PMID: 29752311 DOI: 10.4049/jimmunol.1701069]
- 26 **Balkwill F,** Montfort A, Capasso M. B regulatory cells in cancer. *Trends Immunol* 2013; **34**: 169-173 [PMID: 23206438 DOI: 10.1016/j.it.2012.10.007]
- 27 **Blair PA,** Noreña LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C. CD19(+)/CD24(hi)/CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity* 2010; **32**: 129-140 [PMID: 20079667 DOI: 10.1016/j.immuni.2010.05.011]

- [10.1016/j.immuni.2009.11.009](https://doi.org/10.1016/j.immuni.2009.11.009)]
- 28 **Shimabukuro-Vornhagen A**, Schlöber HA, Gryschock L, Malcher J, Wennhold K, Garcia-Marquez M, Herbold T, Neuhaus LS, Becker HJ, Fiedler A, Scherwitz P, Koslowsky T, Hake R, Stippel DL, Hölscher AH, Eidt S, Hallek M, Theurich S, von Bergwelt-Baildon MS. Characterization of tumor-associated B-cell subsets in patients with colorectal cancer. *Oncotarget* 2014; **5**: 4651-4664 [PMID: [25026291](https://pubmed.ncbi.nlm.nih.gov/25026291/)] DOI: [10.18632/oncotarget.1701](https://doi.org/10.18632/oncotarget.1701)]
- 29 **Zirakzadeh AA**, Marits P, Sherif A, Winqvist O. Multiplex B cell characterization in blood, lymph nodes, and tumors from patients with malignancies. *J Immunol* 2013; **190**: 5847-5855 [PMID: [23630345](https://pubmed.ncbi.nlm.nih.gov/23630345/)] DOI: [10.4049/jimmunol.1203279](https://doi.org/10.4049/jimmunol.1203279)]
- 30 **De Visser KE**, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 2005; **7**: 411-423 [PMID: [15894262](https://pubmed.ncbi.nlm.nih.gov/15894262/)] DOI: [10.1016/j.ccr.2005.04.014](https://doi.org/10.1016/j.ccr.2005.04.014)]



Published by Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

