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Original Article

B cells may play a pathological role in hepatic inflammation in chronic liver diseases

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Abstract: B cells have been proven to promote liver inflammation and fibrotic responses in animal models. However, whether B cells may play a pathological role in patients with chronic liver diseases (CLD) remains unclear. B cells were determined by immunohistochemistry in the liver tissues of 93 CLD patients with different etiologies and 23 normal liver tissue specimens in the present study. We found that the density of CD20 positive B cells was significantly increased in the liver tissues of CLD patients with different etiologies compared to normal liver tissues. CLD patients with higher inflammatory grades had significantly more CD20 positive B cells infiltration in their livers compared to those with lower grades. However, intrahepatic CD20 positive B cells were not positively associated with liver fibrosis stages in these patients. Intrahepatic B cells were positively correlated with serum alanine aminotransferase (r=0.467, P<0.001), aspartate aminotransferase (r=0.310, P=0.003), alkaline phosphatase (r=0.316, P=0.002) and gamma-glutamyl transferase levels (r=0.247, P=0.019) in CLD patients. The results of the present study suggest that B cells may play a pathological role in hepatic inflammation in CLD patients caused by different etiologies. A clear understanding of the phenotype and functional roles of intrahepatic B cell in CLD should be further elucidated.

Keywords: Chronic liver diseases, B cells, inflammation, immunohistochemistry, liver fibrosis

Introduction

Chronic liver diseases (CLD) are major health problems worldwide. The patients with CLD, independently of the underlying etiology, are prone to develop liver fibrosis and cirrhosis, which may result in potentially lethal complications such as hepatic decompensation and hepatocellular carcinoma [1]. Inflammation is considered to be the key factor in the initiation and maintenance of fibrosis processes in the liver [2, 3]. Several cellular components of the immune system such as monocytes/macrophages, NK cells, neutrophils and T cells play major roles in perpetuating and modulating chronic liver inflammation [4-9]. Over recent years, several studies have emphasized the important role of B cells for the liver inflammation and fibrosis [10-13].

Historically, the classical roles of B cells are as antibody-secreting cells and potent antigen-presenting cells (APCs). However, the role of B cells in inflammation is becoming increasingly important. B cells can modulate inflammation through the secretion of proinflammatory or anti-inflammatory cytokines such as interleukin-6 (IL-6), IL-8, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-10 and IL-35 [14-17]. B cells are reported to cluster in the liver tissues and correlated with necroinflammation in patients with chronic hepatitis B (CHB) [13]. Depleting B cell can effectively promote the remission of liver inflammation in a mouse model of type 2 autoimmune hepatitis (AIH) [16].

However, to the best of our knowledge, whether B cells may play a pathological role in chronic

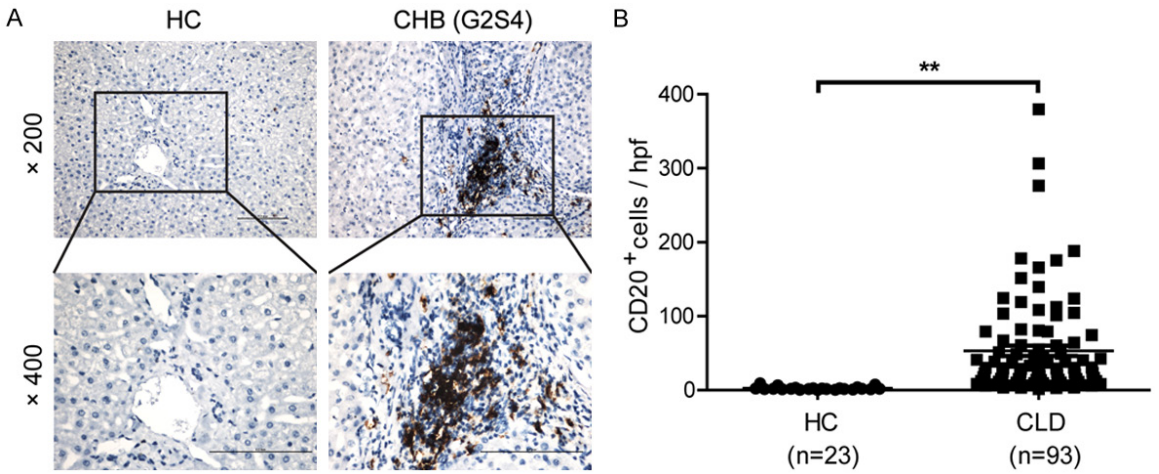

Figure 1. Immunohistochemical analysis of CD20 positive B cell accumulation in chronic liver diseases. Representative images of CD20 marker by imunohistochemistry in the liver tissues of healthy control and a patient with chronic hepatitis B (A). Comparison of hepatic CD20 positive B cells between healthy controls and CLD patients (B). CHB, chronic hepatitis B; CLD, chronic liver diseases; G, inflammatory grades; HC, healthy control; hpf, high-power field; S, fibrotic stages. **P<0.01. Scale bars represent 200 μm.

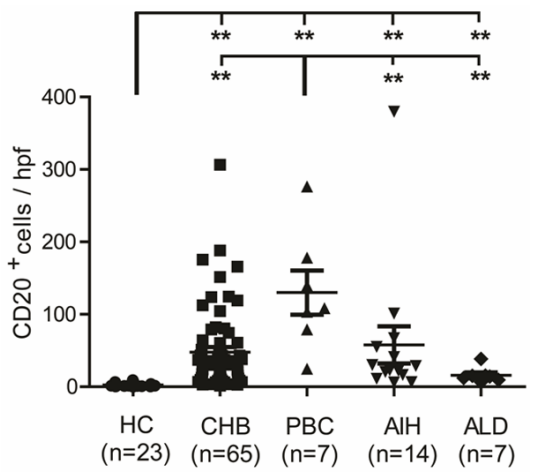

Figure 2. Comparison of CD20 positive B cells in the liver tissues of CLD patients with different etiologies. AIH, autoimmune hepatitis; ALD, alcoholic liver disease; CHB, chronic hepatitis B; HC, healthy control; PBC, primary biliary cirrhosis. **P<0.01.

liver diseases (CLD) remains poorly understood. To elucidate the role of intrahepatic B cells in the pathogenesis of CLD, we investigated the infiltration of B cells in human liver tissues of CLD by immunohistochemistry.

Materials and methods

Patients

Liver biopsy specimens form 93 CLD patients including CHB (n=65), AIH (n=14), alcoholic liver disease (ALD, n=7) and primary biliary cirrhosis (PBC, n=7) were included. Twenty-three specimens of healthy liver tissues obtained from donors whose livers were used for transplantation were used as controls. The clinical data of CLD patients at the time of liver biopsy were collected from their medical records. The protocol for this study was approved by the Ethics Committee of Nanjing Drum Tower Hospital.

Histopathology

The degree of hepatic inflammation and fibrosis of liver fibrosis was evaluated according to the classification proposed by Scheuer on the liver sections stained with hematoxylin-eosin [18]. The histopathological classification was performed by two liver histopathologists who were blinded to the clinical data.

Immunohistochemistry

Immunohistochemical staining was performed on 2 μm paraffin sections of liver biopsies. Sections were deparaffinized and blocked endogenous peroxidase with 3% H_2O_2. The antigen retrieval was performed using a standard protocol. Monoclonal antibody against CD20 (1:800, Dako) was used to stain the sections. The slides were incubated with primary antibodies overnight at 4°C. Immunohistochemical staining was performed with a peroxidase avidin-biotin complex technique. Stained sections

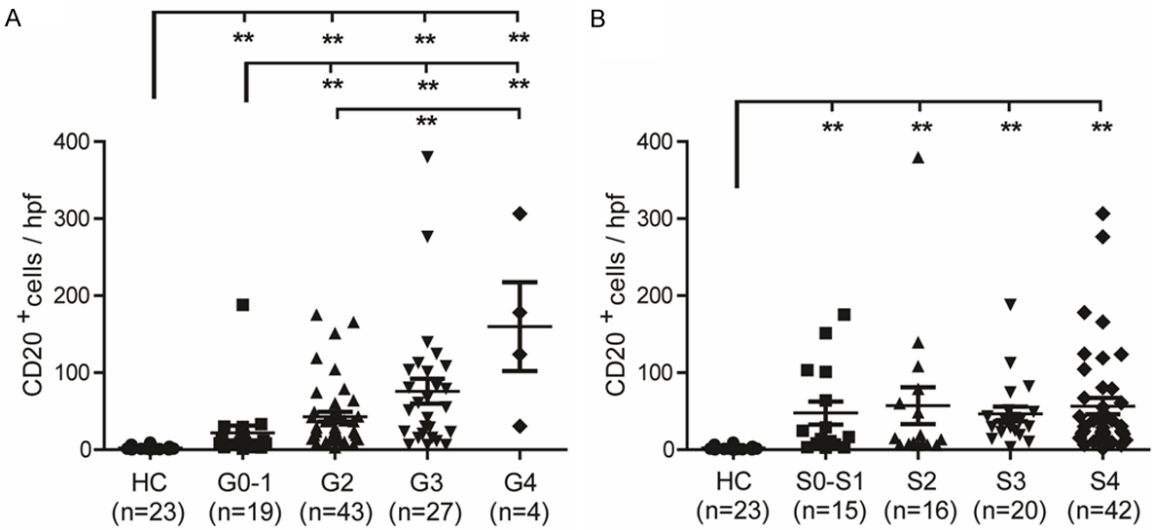

Figure 3. Comparison of CD20 positive B cells in CLD patients with different inflammatory grades (A) and fibrotic stages (B). G, inflammatory grades; S, fibrotic stages. **P<0.01.

were developed with diaminobenzidine and counterstained with hematoxylin.

Measurement of cells

Five visual-fields per slide were analyzed. The photomicrographs were obtained at a magnification of 400× and captured for analysis using Image Pro-Plus 5.0 software (Media Cybernetics, SilverSpring, MD, USA). The mean number of CD20 positive cells per high-power field (HPF) were counted for each slide [19, 20].

Statistical analysis

Data are depicted as bar graphs representing mean and standard error of the mean (SEM). All data analysis was performed using SPSS 22.0 software (IBM Corporation, Somers, NY). Statistical comparisons between two groups were performed by Mann Whitney U test, multiple comparisons by ANOVA test followed by U test for post hoc analysis. Correlation analysis was performed by Spearman rank correlation test. A *P* value <0.05 was considered statistically significant.

Results

Intrahepatic accumulation of CD20 positive B cells in CLD

Only a small amount of CD20 positive B cells was found in the normal liver tissues. However, the density of CD20 positive B cells was significantly increased in the liver tissues of CLD patients compared to normal liver tissues (P<0.01). The CD20 positive B cells were predominantly seen in the periportal area of liver tissues in CLD patients (**Figure 1**).

Intrahepatic accumulation of CD20 positive B cells in CLD patients with different causes

In the liver tissues of CLD patients with different etiologies, the CD20 positive B cells were all significantly increased (P<0.01). CD20 positive B cells were increased more significantly in the liver tissues of PBC patients as compared with CHB, AIH and ALD patients (**Figure 2**).

CD20 positive B cells correlate with inflammatory grades (G) in CLD patients

CLD patients with higher G scores had significantly more CD20 positive B cells in their livers compared to those with lower scores (**Figure 3A**). The CD20 positive B cells were increased in all the fibrotic stages (S) compared to healthy controls (**Figure 3B**). The density of CD20 positive B cells was positively correlated with G scores (r=0.472, P<0.0001), but not correlated with S scores (r=0.158, P=0.131).

Clinical relevance of the CD20 positive B cells

The association between the CD20 positive B cells and the clinical parameters were analyzed (**Table 1**). The numbers of CD20 positive B cells in the liver tissues of CLD patients were posi-

Table 1. The clinical relevance of CD20 positive B cells in CLD patients

	Spearman's correlation coefficient	*P* value
ALT	0.467	<0.001
AST	0.310	0.003
ALP	0.316	0.002
GGT	0.247	0.019
Tbil	0.039	0.716
Alb	0.019	0.859

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; Tbil, total bilirubin.

tively correlated with serum alanine aminotransferase (ALT, r=0.467, P<0.001), aspartate aminotransferase (AST, r=0.310, P=0.003), alkaline phosphatase (ALP, r=0.316, P=0.002) and gamma-glutamyl transferase levels (GGT, r=0.247, P=0.019).

Discussion

In the present study, we investigated the CD20 positive B cells in the liver tissues of CLD patients by immunohistochemistry. We found that the CD20 positive B cells were significantly accumulated in the liver tissues of CLD patients. The CD20 positive B cells mainly clustered in the portal areas of the liver tissues and correlated with inflammatory grades. In the previous studies, a significantly higher frequency of circulating B cells in PBC and CHB patients was reported [21]. B cells are significantly increased in the liver tissues and correlated with necroinflammation in patients with CHB [13]. Our study was consistent with previous studies [13, 22]. Few studies have reported the B cells infiltration in the liver tissues in other CLD such as AIH, PBC and ALD. In the present study, we compared the B cell infiltration in the liver tissues of different etiologies of CLD patients and the CD20 positive B cells were all significantly increased. CD20 positive B cells were increased more significantly in the liver tissues of PBC patients compared with CHB, AIH and ALD patients. However, the sample sizes of PBC, AIH and ALD patients included in the present study were relatively small. The difference of B cells infiltration in the liver tissues among CLD patients with different etiologies should be further investigated. Furthermore, the intrahepatic CD20 positive B cells were positively correlated with ALT, AST, ALP and GGT levels. These results indicate that B cells may play a pathological role in hepatic inflammation in CLD.

However, the mechanism of B cells affect the pathogenesis of CLD remains elusive. B cells are traditionally considered to be involved only in antibody production and antigens presentation. There is growing evidence that B cells can secrete cytokines, thereby acting as positive regulators in immune responses [23, 24]. Upon stimulation, B cells can secret a large amount of proinflamatory cytokines such as IL-6 and TNF-α and thus involve in the perpetuation of inflammatory responses [24]. In the AIH animal model, B cells can secret proinflammatory cytokines including IFN-γ and TNF-α, contributing to a proinflammatory liver environment [16]. Furthermore, the activation of B cells may result in the characteristic hyper-immunoglobulin G (IgG) and circulating autoantibodies participating in the pathogenesis of autoimmune liver diseases including AIH and PBC [16, 25]. Béland K et al reported that depletion of B cells by anti-CD20 antibodies could significantly reduce liver inflammation and effectively ameliorate AIH in mice [16]. Thus, B-cell depleting may ameliorate the liver inflammation.

Over recent years, B cells are considered to be a diverse and multifaceted cell population comprising both regulatory and effector cells. Similarly to CD4+ T cells that can either be T helper cells or regulatory T cells, B cell subsets could either be pathologic (presenting autoantigens and secreting proinflamatory cytokines) or protective (secreting IL-10, IL-35) [26]. A population of suppressor B cells, known as regulatory B (Breg) cells, has been associated with the inhibition of excessive inflammation. Through the production of IL-10, IL-35 and transforming growth factor β (TGF-β), Breg cells suppress immunopathology by prohibiting the expansion of pathogenic T cells and other proinflammatory lymphocytes [27-29]. B cell depletion therapy by anti-CD20 or anti-CD79 monoclonal antibodies may lead to the reduction of IL-10 and exacerbate murine PBC [12]. Thus, B cells may play both positive and negative regulatory roles in the pathogenesis of CLD.

We note that the data herein reflect that the CD20 positive B cell infiltration is not correlated with the stage of fibrosis in CLD patients. However, B cells have been reported to play a role in hepatic fibrosis in mice [11, 14]. In B cell-

deficient mice, markedly reduced collagen deposition was found as compared with wild-type mice following 6 weeks of carbon tetrachloride treatment [11]. The contribution of B cells to liver fibrosis in CLD patients deserves further investigation [10].

In conclusion, B cells may play a pathological role in hepatic inflammation in CLD patients caused by different etiologies. The present study provides additional evidence on the important role of B cells in the pathogenesis of CLD. However, detailed phenotyping and functional studies of intrahepatic B cells in CLD are needed.

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Disclosure of conflict of interest

None.

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