

Alterations of red blood cell immunoadherence function in hepatitis B patients

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Received: October 30, 1995
Revised: November 14, 1995
Accepted: December 11, 1995
Published online: March 25, 1996

Abstract

AIM: To investigate the alterations of red blood cell (RBC) immunoadherence function in patients with hepatitis B.

METHODS: RBCC3bRR, RBCICRR and serum CIC levels were measured in 42 patients with acute and chronic hepatitis B at active and convalescence stages.

RESULTS: RBCC3bRRs at the active/acute stage of hepatitis were decreased, with $13.54\% \pm 5.23\%$ in acute hepatitis, $7.61\% \pm 4.12\%$ in acute fulminant hepatitis, and $16.18\% \pm 6.10\%$ in chronic hepatitis, all of which were lower than the value in normal persons ($18.12\% \pm 3.91\%$). At the quiescent/recovery stage of hepatitis, the RBCC3bRRs were increased significantly. The changes of RBCICRR and serum CIC level were contrary to those of RBCC3bRR.

CONCLUSION: RBC immunoadherence function is decreased in acute and chronic hepatitis, and the decrease is in direct proportion to severity of the diseases.

Key words: Hepatitis, viral, human/immunology; Erythrocytosis/immunology; Antigen-antibody complex/blood

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Sun ZQ, Wang YJ, Quan QZ, Xiao RM, Guo F. Alterations of red blood cell immunoadherence function in hepatitis B patients. *World J Gastroenterol* 1996; 2(1): 20-21 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v2/i1/20.htm> DOI: <http://dx.doi.org/10.3748/wjg.v2.i1.20>

INTRODUCTION

Red blood cells (RBCs) have a multitude of immunological functions, the most important being immunoadherence which has garnered much research attention in recent years. In previous studies^[1,2], we observed that the RBC immunoadherence function as significantly reduced in rats with acute liver damage induced by D-galactosamine. In the present study, the RBC immunoadherence function was detected in patients with acute and chronic hepatitis B by determining the RBC C3b receptor yeast rosette rate (RBCC3bRR) and the RBC immune complexes rosette rate (RBCICRR).

MATERIALS AND METHODS

Materials

Forty-two patients with acute and chronic hepatitis B participated in the study. The diagnosis had been made according to the diagnostic criteria for hepatitis revised by the National Viral Hepatitis Conference held in Shanghai in 1990. Among the patients were 14 with acute hepatitis (AH; including 9 males and 5 females with mean age of 39.5 years), 7 with acute fulminant hepatitis (AFH; including 5 males and 2 females with mean age of 35.4 years), 21 with chronic hepatitis (CH; including 17 males and 4 females with mean age of 36.3 years). All patients had evidence of HBV infection. Blood specimens were obtained from the patients at the active (or acute) stage and the convalescent stage. Fifteen healthy blood donors were recruited for participation as normal controls (NC; mean age: 35.6 years).

Methods

RBCC3bRR test: Heparin-anticoagulated blood specimen (1 mL) was mixed with Ficol liquor and was centrifuged to separate the RBCs. The RBCs were then washed three times with normal saline and resuspended in the same (1.25×10^6 cells/mL). Next, 0.5 mL of complement-sensitized yeast (1×10^6 /mL) was added into 0.5 mL of the RBC suspension, and then incubated at 37 °C in a constant-temperature water bath for 30 min. After being diluted with 1 mL of normal saline, the RBC sample was fixed with 0.3 mL of 0.25% glutaraldehyde. An aliquot of the prepared RBC suspension was smeared on a slide and stained with Wright's stain. The number of RBCs combining with two yeasts or more was calculated among 200 RBCs and represented the RBCC3bRR.

RBCICRR test: This procedure was performed the same as described above, except that the yeast used was not sensitized by complement. The circulating immune complexes (CIC) test was then performed on the serum samples by using the polyethylene glycol precipitation method.

Table 1 Changes in RBCC3bRR, RBCICRR and serum CIC in hepatitis

Group	RBCC3bRR, %		RBCICRR, %		Serum CIC, U/L	
	A stage	C stage	A stage	C stage	A stage	C stage
AH	13.54 ± 5.23	17.47 ± 3.82	6.46 ± 2.44	4.73 ± 2.21	470.5 ± 97.3	285.3 ± 57.7
AFH	7.61 ± 4.12	15.38 ± 3.55	9.10 ± 3.83	5.32 ± 1.68	534.7 ± 173.8	227.1 ± 76.4
CH	13.96 ± 5.01	15.42 ± 5.13	7.23 ± 1.94	5.78 ± 4.88	401.9 ± 137.4	357.2 ± 90.1
NC	18.12 ± 3.91		4.61 ± 1.12		216.6 ± 34.2	

A: Active; C: Convalescent; AH: Acute hepatitis; AFH: Acute fulminant hepatitis; CH: Chronic hepatitis NC: Normal controls.

RESULTS

RBCC3bRR change in hepatitis

The RBCC3bRRs were lower at the active stage of AH and CH, compared to the convalescent stage of each. The more severe the disease, the lower the RBCC3bRR. There was no significant difference between AH and CH. The RBCC3bRR increased gradually as the patients recovered. Of the 7 patients with AFH, 3 died of acute liver failure; RBCC3bRRs in the 4 survivors also significantly increased. In the CH group, however, the increase was much slower.

RBCICRR changes in hepatitis

The RBCICRRs were higher in the patients with hepatitis than in the NCs (in the order of AFH > CH > AH > NC), which was inversely proportional to the changes observed for the RBCC3bRR ($r = -0.863$, $P < 0.05$).

Serum CIC level

The serum level of immune complexes was higher in patients with hepatitis than in the NCs, which was correlated strongly with RBCICRR changes ($r = 0.824$, $P < 0.05$) but negatively with RBCC3bRR changes ($r = -0.959$, $P < 0.01$) (Table 1).

DISCUSSION

The present study demonstrated that RBCC3bRRs in AH and CH are decreased and the degree of decrease is closely related to the severity of liver damage. When the patients recovered or were in the quiescent stage, RBCC3bRR became elevated. Therefore, RBC-C3bRRs can be used to assess the severity and prognosis of hepatitis conditions.

It has been verified that there are type I complement receptors (CR1) on the surface of RBCs, through which the RBCs combine with C3b-opsonized immune complexes and bring them to macrophages in the liver and other organs and finally are eliminated^[3,4]. RBC-C3bRR and RBCICRR tests are reliable and convenient for detecting the RBC immunoadherence function^[5]. The decrease of RBCC3bRR observed in this study suggests that the number of CR1 is reduced and its function is diminished.

In a recent *in vivo* study^[1,6], we observed that the RBC immunoadherence function in acute liver damage was reduced significantly. The degree of the depression was strongly related to the increase of hepatic and serum lipid peroxidate (LPO) level. *In vitro* study also showed that LPO could reduce RBCC3bRRs. Antioxidant therapy with vitamin E protected against the observed reduction. The reason for this finding may be that LPO can combine with amino acids of the CR1 protein, causing intra- and inter-cross linking, and thereby destroying the normal structure of CR1 and leading to the decrease in RBC immunoadherence function.

In the present study, we found that the CIC was increased in relation to the decrease of RBCC3bRR. Because CR1 plays an important role in eliminating CIC, and about 95% of total C3b receptors in circulation are on the RBC surface, the increase of RBCICRR reflects in part the increase of CIC. The mechanism underlying the decrease in RBC immunoadherence function may be a complicated one. Further study will be beneficial to understand the relation of these findings to the prevention and treatment of hepatitis.

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S- Editor: Filipodia L- Editor: Jennifer E- Editor: Liu WX



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ISSN 1007-9327

