Expression of β2-integrin on leukocytes in liver cirrhosis

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Abstract

AIM: To analyze β2-integrin expression on blood leukocytes in liver cirrhosis.

METHODS: In 40 patients with liver cirrhosis and 20 healthy individuals, the evaluation of expression of CD11a (LFA-1α), CD11b (Mac-1α), CD11c (αX) and CD49d (VLA-4α) on peripheral blood leukocytes was performed using flow cytometry. The analysis was carried out in groups of patients divided into B and C according to Child-Pugh's classification.

RESULTS: An increased CD11a, CD11b, CD11c and CD49d integrin expression was observed on peripheral blood leukocytes in liver cirrhosis. The integrin levels were elevated as the advancement of liver failure progressed. The highest expression of integrins occurred predominantly on monocytes. A slight expression of VLA-4 was found on lymphocytes and granulocytes and it increased together with liver failure. A positive correlation was noted between median intensity of fluorescence (MIF) expression on polymorphonuclear cells of CD11a and CD11c and CD49d ($r = 0.42$, $P < 0.01$; $r = 053$, $P < 0.01$, respectively) in liver cirrhosis stage C. However, no correlation was observed between integrin expression on leukocytes. The concentrations of sICAM-1, sVCAM-1, and TNFα, were significantly elevated in liver cirrhosis.

CONCLUSION: β2-integrin expression on leukocytes increases in liver cirrhosis decompensated as the stage of liver failure increases, which is a result of permanent activation of leukocytes circulating through the inflamed liver environment. β2-integrin expression on circulating leukocytes can intensify liver cirrhosis.

Key words: β2-integrin; Liver cirrhosis; Flow cytometry; Leukocytes

INTRODUCTION

Integrins play a role in organ and tissue damage, by the inflammatory and immunological processes, and in auto-immune diseases[1-4]. They participate in the regulation of leukocytes in the procoagulant activity and adhesion to infectious factors[5]. Many integrins mediate transduction of signals that stimulate T cells[6]. β2 integrins participate in the presentation of antigens by antigen-presenting cells (APCs). They mediate the adhesion of T cells to APCs through the formation of immunological synapses with the help of β2/ICAM-1 complexes, known as the supra-molecular activation complex (SMAC). Integrin expression strongly depends on cellular energy metabolism. Activated leukocytes express β1 and β2 that integrins play a key role in leukocytes mobilization to circulate to inflammatory sites[7]. Monocytes and granulocytes are important in organic defense against bacterial and viral infections. LFA-1 and Mac-1 are involved in other functions of neutrophils, such as phagocytosis, degranulation, and apoptosis[2].

Inflammatory and immunological reactions in the liver are accompanied by peripheral blood leukocytes infiltration in the tissue[8]. Leukocyte recruitment takes its course with the participation of adhesive molecules presented by capillary endothelial cells[8,9]. The particular stages of recruitment, such as leukocyte rolling, adhesion, and transmigration, depend on signals released by endothelial cells. Activated leukocytes expressed adhesion molecules β2-integrins CD11a (LFA-1a), CD11b (Mac-1a), CD11c (αX), and β1-integrins CD49d (VLA-4a) on their surfaces[1,3,4]. Integrins mediate in interactions between cells and extra-cellular matrix (ECM) proteins. ICAM-1 (ligand for LFA-1a and Mac-1a), ICAM-2 (ligand for LFA-1a), VCAM-1 (ligand for VLA-4a), extracellular matrix proteins, such as collagen, laminine, vitronectine as well as blood clotting proteins (fibrinogen, factor X, kininogen) are ligands for integrin receptors on leukocytes. Products released by microorganisms, that can facilitate penetration into cells, can be ligands for integrins[5]. Cytokines released in inflammatory sites induce the expression of endothelial ligands that activate circulating leukocytes[10].
Inflammation in liver cirrhotic tissue and in the systemic circulation affects peripheral leukocytes β2-integrin receptors. In this study, the evaluation of β2-integrin expression

on monocytes, lymphocytes, and granulocytes of peripheral blood in liver cirrhosis was carried out. The influence of liver failure stage on the expression of β2-integrin leukocyte receptors was taken into account.

MATERIAL AND METHODS

Forty patients (28 men and 12 women; age 50 ± 13 years) with post-alcoholic liver cirrhosis were enrolled in this study. Liver biopsy of the right lobe established the liver cirrhosis. Clinical characteristics of patients are given in Table 1. According to Child-Pugh's classification[11], patients were divided into 2 groups: B ($n = 21$) and C ($n = 19$). Those overusing alcohol with immunosuppression therapy, hepatotropic viral infections (HBV, HCV, CMV) or fever were excluded from the study. The control group consisted of 20 healthy individuals (12 women and 8 men; age 40 ± 7 years).

Leukocyte population

Peripheral blood leukocyte population (monocytes, lymphocytes, granulocytes) was determined by flow cytometry (Coulter, USA). Leukocytes were identified by their forward and orthogonal light scatter characteristics on immunological gate. The percentage of leukocyte population and median intensity of fluorescence (MIF) of integrin receptors were determined using mononuclear antibodies CD11a (CD11a PE, DAKO Cytomation, Denmark), CD11b (CD11b PE, DAKO Cytomation, Denmark), CD11c (CD11c PE, DAKO Cytomation, Denmark), CD49D (CD11a PE, DAKO Cytomation, Denmark), and CD14 (CD14 FITC, DAKO Cytomation, Denmark). Two millilitres of blood was collected in plastic tubes containing sodium citrate. Five microlitres of mAb was added to 50 μL of blood and incubated for 15 min at room temperature in the dark. Erythrocytes were eliminated by adding diluting-lysing fluid (ImmunoPrep-Reagenziensysem ABC, Coulter). A minimum of 10 000 leukocytes was analysed in each sample.

Tumor necrosis factor alpha (TNFα, R&D, England), sICAM-1 (sICAM, R&D, England), sVCAM-1 (sVCAM-1, R&D, England) concentrations were determined in the blood sera using ELISA methodology. Leukocyte counts was carried out in hematological analyzer Sysmex K-1000 (Japan). Ethical approval for research was obtained from the Local Ethics Committee in the Medical University.

Statistical analysis

The results were expressed as median with range values and mean ± SD. Statistical analysis was performed by nonparametrical Mann-Whitney U-test. Result correlation was calculated with the use of Spearman test. A P value less than 0.05 was considered statistically significant.

RESULTS

Monocytes

The expression of CD11a (LFA-1a) receptors on peripheral blood monocytes was increased in liver cirrhosis

Table 1 Clinical characteristics of patients with liver cirrhosis

Characteristic	Child-pugh B	Child-pugh C
Encephalopathy (n)	2	10
Ascites (n)	9	19
Median bilirubin (range) (mg/L)	34 (9–78)	69 (22–139)
Median albumin (range) (g/L)	29 (22–33)	22 (19–29)
Median prothrombin time (range) (s)	19 (17.2–23.3)	23 (18.2–26.8)

Table 2 β2-integrin expression on peripheral blood leukocytes and sICAM-1 and TNF-α serum concentrations in liver cirrhosis (stages B and C) and in healthy subjects

Leukocytes	Receptors	Healthy subjects ($n = 20$)	Liver cirrhosis	
			Stage B ($n = 21$)	Stage C ($n = 19$)
Monocytes	CD14 (%)	26.2 ± 2.3	26.1 ± 6.3	26.8 ± 3.9
	MIF[1]	2.9 ± 1.5	8.7 ± 6.9	8.1 ± 6.1
	CD11a (%)	99.9 ± 0.09	99.9 ± 0.08	99.9 ± 0.8
	MIF[1]	14.8 ± 2.4	15.1 ± 1.8[a]	16.3 ± 1.7[b]
	CD11b (%)	99.8 ± 0.08	99.5 ± 0.7	99.8 ± 0.2
	MIF[1]	18.4 ± 4.6	18.7 ± 4.8[a]	22.2 ± 4.4[a]
	CD11c (%)	99.5 ± 0.5	97.5 ± 4.5	96.6 ± 7.0
	MIF[1]	6.5 ± 1.3	7.0 ± 1.5	7.4 ± 2.1
	CD49d (%)	63.8 ± 14.7	66.9 ± 22.5	66.2 ± 21.2
	MIF[1]	1.39 ± 0.43	1.85 ± 0.72	1.52 ± 0.68
Granulocytes	CD11a (%)	99.3 ± 0.5	98.0 ± 4.8	94.7 ± 0.5
	MIF[1]	4.4 ± 0.4	4.3 ± 0.5	4.4 ± 0.7
	CD11b (%)	99.5 ± 1.1	99.5 ± 0.7	98.6 ± 2.7
	MIF[1]	10.5 ± 2.4	10.0 ± 2.8[a]	12.1 ± 2.6
	CD11c (%)	87.0 ± 5.0	80.8 ± 11.4	71.9 ± 12.4
	MIF[1]	2.8 ± 0.4	2.7 ± 0.7	3.0 ± 0.9
	CD49d (%)	4.4 ± 2.4	4.6 ± 3.0	6.3 ± 3.9
	MIF[1]	0.13 ± 0.01	0.17 ± 0.05[b]	0.17 ± 0.03[b]
Lymphocytes	CD11a (%)	98.1 ± 0.8	97.2 ± 1.7	97.6 ± 1.3
	MIF[1]	8.0 ± 1.2	9.7 ± 1.6[b]	9.9 ± 2.0[b]
	CD11b (%)	23.7 ± 5.3	24.5 ± 5.2	24.0 ± 8.0
	MIF[1]	0.56 ± 0.12	0.61 ± 0.18[a]	0.75 ± 0.24
	CD11c (%)	25.2 ± 6.6	23.2 ± 5.6	29.4 ± 7.3
	MIF[1]	0.77 ± 0.12	0.91 ± 0.33	1.0 ± 0.25[b]
	CD49d (%)	31.2 ± 12.5	37.9 ± 12.1	38.8 ± 11.8
	MIF[1]	0.48 ± 0.12	0.68 ± 0.34[b]	0.63 ± 0.18[b]
sICAM-1 (μg/L)		254 ± 74	839 ± 34[b]	957 ± 328[b]
TNFα (μg/L)		1.58 ± 0.22	2.89 ± 1.34[b]	2.98 ± 1.42[b]

[1]Medial intensity of fluorescence; [a]$P < 0.05$ vs Child-Pugh C stage, [b]$P < 0.01$ vs healthy subjects (Mann-Whitney U-test).

patients as compared with healthy subjects (Table 2). Progressive liver damage resulted in increased population and number of receptors for this integrin on monocytes. The differences in CD11a MIF values between patients in B and C stages were statistically significant ($P < 0.05$). There was a positive correlation between the expression of CD11a and CD11c receptors on monocytes in the control group and in B and C stages of liver cirrhosis ($r = 0.73$, $P < 0.01$; $r = 0.39$, $P < 0.05$; $r = 0.56$, $P < 0.01$, respectively, Table 3). Moreover, a positive correlation was found between monocyte CD11a MIF and CD11a expression on lymphocytes in patients in stage B. However, no other significant relations were observed between CD11a expression and other integrins on other leukocytes.

Table 3 The correlation of β2-integrin expression on leukocytes and sICAM level in blood sera from liver cirrhosis (stages B and C)

	CD11a	CD11b	CD11c	CD49d
Monocytes CD11a		H[1]: r = 0.6, P < 0.01	**Monocytes** B[2]: r = 0.39, P < 0.05 C[3]: r = 0.56, P < 0.01 H[1]: r = 0.73, P < 0.01	NS[4]
	NS[4]	NS[4]	**Granulocytes** NS[4]	NS[4]
	B[2]: r = 0.56, P < 0.01	NS[4]	**Lymphocytes** NS[4]	NS[4]
Granulocytes CD11a	NS[4]	NS[4]	**Monocytes** C[3]: r = 0.47, P < 0.04	NS[4]
			Granulocyte C[3]: r = 0.42, P < 0.01 H[1]: r = 0.54, P < 0.05	C[3]: r = 0.53, P < 0.01
	NS[4]	NS[4]	**Lymphocytes** NS[4]	NS[4]
Lymphocytes CD11a	NS[4]	NS[4]	**Monocytes** NS[4]	NS[4]
	NS[4]	NS[4]	**Granulocytes** NS[4]	NS[4]
		C[3]: r = 0.51, P < 0.02	**Lymphocytes** NS[4]	C[3]: r = 0.51, P < 0.02

[1]Healthy subjects; [2]B stage of liver cirrhosis; [3]C stage of liver cirrhosis; [4]Not significant. Spearman test.

CD11c MIF increased slightly on monocytes in B and C stages of liver cirrhosis ($P > 0.05$). Furthermore, CD 49d expression in subsequent stages of liver cirrhosis was slightly elevated on monocytes ($P > 0.05$). There was no dependence observed between VLA-1a receptor expression and the serum concentration of soluble forms of ICAM-1, VCAM-1, and TNFα.

Granulocytes

The expression of CD11a on granulocytes in healthy subjects and liver cirrhosis (independently the stages B and C) was comparable. CD11b receptor expression on granulocytes in severe liver cirrhosis was significantly elevated ($P < 0.05$, controls *versus* stage B). In contrast, no significant difference was observed in the expression of CD11c on granulocytes between the liver cirrhosis and control groups. CD49d expression on granulocytes was approximately 10 times lower than that on monocytes, although it increased together with the stage of liver damage ($P < 0.01$ *versus* the controls). A positive correlation was observed between CD11a and CD11c MIF on granulocytes ($r = 0.42$, $P < 0.01$) in liver cirrhosis stage C. Also, a positive correlation between CD11a expression on granulocytes and CD49d in liver disease stage C ($r = 0.53$, $P < 0.01$) was found.

Lymphocytes

LFA-1, among other β2-integrins, had the highest expression on lymphocytes. In liver cirrhosis, higher values of LFA-1 on leukocytes were observed, and were found to be increased with the advancement of liver insufficiency (stage B: 9.7 ± 1.6, stage C: 9.9 ± 2.0, healthy: 8.0 ± 1.2; $P < 0.05$ *vs* controls). A significantly lower expression of Mac-1, aX, and VLA-4 was noted on lymphocytes, while increased expression was detected in liver cirrhosis. The elevation of CD11b and CD11c expression was found to be affected by the stage of liver failure, whereas VLA-4 expression was not influenced by the condition. A positive correlation was noticed between LFA-1 expression and Mac-1 expression (stage C: $r = 0.51$, $P < 0.02$), as well as VLA-4 expression (stage C, $r = 0.51$, $P < 0.02$).

We did not observe any correlation between the concentrations of soluble forms of ICAM-1 and VCAM-1 and the expression of integrins in particular stages of liver cirrhosis and in the controls. Stage B of liver cirrhosis was the only stage, in which a negative correlation was observed between sICAM-1 and CD11a expression on lymphocytes ($r = 0.49$, $P < 0.03$). On contrary, a positive correlation was noted between TNF-α concentration and sICAM-1 level in liver cirrhosis stage B ($r = 0.68$, $P < 0.01$).

DISCUSSION

Studies showed that the stage of liver failure has a significant impact on β2-integrins by the intensification of their expression on leukocytes. Liver cirrhosis was associated with elevated expression of β2-integrins CD11a, CD11b, CD 11c, and CD49d on leukocytes surfaces. The results indicated leukocyte stimulation in liver cirrhosis, and that a cell population of higher function can potentially appear. It was observed that the expression of β2-integrins on monocytes, granulocytes, and lymphocytes increased in patients with liver cirrhosis. The only exception was LFA-1 on granulocytes, whose expression did not differ as compared to the controls. Monocytes had the highest β2-integrin expression on the surface, indicating that monocytes have an important role in the pathogenesis of liver cirrhosis. The population of monocytes with β2-integrin expression increases with the stage of liver failure. It causes the elevation of their activity in relation between cells and ECM proteins. Circulating monocytes

are precursors of dendritic cells and macrophages of the liver. Activated by a liver inflammatory environment, they expose on their surfaces adhesion molecules that enable active participation in the intensification of inflammatory and immunological processes in the liver. Immunological disorders belong to the main phenomena in the pathogenesis of liver cirrhosis. Moreover, it has been shown that liver cirrhosis is accompanied by high concentrations of TNF-α and soluble adhesive molecules sICAM-1 and sVCAM-1, whose concentrations did not correlate with β2-integrin expression on leukocytes in our studies.

The activation of endothelial cells has a crucial role in modulation of leukocyte functions in liver cirrhosis. Endothelial soluble adhesive molecules, induced by proinflammatory cytokines, are the markers of the functional condition of endothelial cells. The role of adhesive molecules in the pathogenesis of liver cirrhosis and portal hypertension has not been fully explained yet. Integrins participate in adhesion and migration of leukocytes in the microcirculation of highly perfused organs (the liver, lungs)[12]. Endothelial cell incubation with antibodies against integrin receptors inhibits monocyte adhesion[7]. The administration of anti-LFA-1 or anti-ICAM-1 antibodies diminishes significantly the stage of hepatocyte damage. It is suggested that T lymphocytes, infiltrating the liver through LFA-1/ICAM-1 interactions, cause the damage of hepatocytes by releasing toxic substances and oxygen radicals[6]. Damaged endothelial cells present adhesive molecules (selectins, ICAM-1, VCAM-1) on their surfaces and influence flowing leukocytes. Activation signals are transmitted directly cell to cell or by the contact of cells with soluble adhesive molecules. Inflammatory and immunological phenomena have common paths, that are frequently paralleled, and leukocytes are main effector cells.

Integrin expression on leukocytes undergoes regulation by pro-inflammatory cytokines such as TNF-α and IL-8. CD11b expression on leukocytes correlates with IL-6 level and the stage of liver failure[13]. Increased LFA-1, LFA-3, and ICAM-1 expression on leukocytes and TNF-α production in post-alcoholic liver cirrhosis has been observed[14]. Peripheral blood monocytes showed activity and elevated expression of TNF-α which correlated with liver disease activity (Child-Pugh stage B and C)[15]. The concentrations of TNF-α and soluble adhesive molecules are significantly higher in the advanced phase of cirrhosis ($P < 0.05$, Child-Pugh class B and C *versus* A) and can reflect hemodynamic alterations in the liver[16]. This is accompanied by the activation of monocytes and increased intracytoplasmatic expression of TNF-α[17]. Bacterial infections in the alimentary tract play an important role in cellular immunological disorders in patients with liver cirrhosis. A correlation between TNF-α level in blood serum and TNF-α contained in monocytes occurs in decompensated liver cirrhosis with a high concentration of LPS in blood. Antibacterial treatment diminishes monocyte activity and TNF-α-production activity[17]. Inflammatory mediators, cytokines (TNF-α), and selectins elevate CD11b/CD18 expression on granulocytes. CD11b/CD18 cell population is increased in inflamed tissues. Accumulation of leukocytes with LFA-1, Mac-1,

and VLA-4 expression occurs in liver parenchyma near metastasis focuses[18].

Patients with liver cirrhosis reveal the immunological system disorders and immunodeficiency, and the activation of certain immunological responses. Luna-Casad *et al*[19] showed an increase in ICAM-1, LFA-3, and Mac-1 expression on lymphocytes and LFA-3 on monocytes. This correlated with the severity of liver cirrhosis and immunological disorders.

Wong *et al*[12] showed that most stimulated leukocytes (approximately 80%) adhere to sinusoids and the rest (20%) to post-sinusoidal venules. The adhesion of stimulated leukocytes is independent of P, E, and L-selectin presence in the liver microcirculation. The adhesion of neutrophils to ICAM-1 is possible due to molecules exposed on CD11a and CD11b surfaces[3]. Bacterial infections are accompanied by activation of peripheral blood leukocytes and their recruitment in the liver. LFA-1 immunoneutralization reduced leukocyte adhesion to a great extent (by 55%), decreased aminotransferase activity by 65%, and reduced apoptosis in the liver by 45%[20]. It has been shown that LFA-1 plays an important role in the development of liver deficiency through leukocytes. LFA-1 expression in inflammatory infiltrates is elevated in PBC, which points to the role of activated lymphocytes in bile duct damage[21]. Hepatotropic virus infections (e.g. HHV-6) also induce LFA-1 and VLA-1 expression on lymphocytes infiltrating the liver[22].

Liver resident macrophages–Kupffer's cells constitutively show high LFA-1, Mac-1, and ICAM-1 expression[23]. The studies performed on transgenic mice (CD11b-DTR) revealed that activated macrophages participate both in tissue damage and repair in inflammatory processes. Macrophages are capable of diminishing liver fibrosis through ECM protein degradation[24].

In summary, β2-integrins play an important role in the development of liver cirrhosis. The stage of disease advancement activates peripheral blood leukocytes, which results in the intensification of liver inflammatory and immunological processes. The activation of leukocytes is a complex phenomenon, influenced by endo- and exogenic factors. The blockade of integrin expression on leukocytes may constitute an important therapeutic aspect of liver cirrhosis.

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