Clinical implication of vascular cell adhesion molecule-1 and very late activation antigen-4 interaction, and matrix metalloproteinase-2 production in patients with liver disease

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OBJECTIVES: To clarify the role of adhesion molecule in liver cell injury.

PATIENTS AND METHODS: The serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), and the expression of VCAM-1 and its ligand, very late activation antigen-4 (VLA-4), were examined in patients with various liver diseases. In addition, the presence of matrix metalloproteinase-2 (MMP-2) was investigated because the release of MMP-2 is thought to be mediated by VLA-4-positive cells. sVCAM-1 and MMP-2 were measured by ELISA assay, and VCAM-1 and VLA-4 were studied by immunohistological methods.

RESULTS: In acute hepatitis (AH) patients, the serum level of sVCAM-1 was significantly elevated compared with that in other cohorts. VCAM-1 was expressed on sinusoidal lining cells but not on hepatocytes. In patients with chronic liver disease, sVCAM-1 levels rose in concert with the progression of chronic hepatitis (CH), and VCAM-1 was also expressed. VLA-4 was detected in both mononuclear cells and Kupffer cells in AH livers, but mainly in Kupffer cells in patients with CH. In AH patients, MMP-2 levels were similar to those in control subjects, but in CH and liver

cirrhosis patients, MMP-2 level was elevated in association with CH progression.

CONCLUSIONS: The immune response through the VCAM-1 and VLA-4 pathways is important in hepatocyte injury, especially in AH patients, to attach VLA-4-positive mononuclear cells to VCAM-1-positive sinusoidal lining cells. The distribution of VLA-4-positive cells differs between AH and CH patients. VLA-4-positive Kupffer cells in chronic liver diseases might be involved in the progression of CH, perhaps through the mechanism of upregulation of MMP-2 production.

Key Words: Liver cell injury; Matrix metalloproteinase; Soluble vascular cell adhesion molecule; Vascular cell adhesion molecule; Very late activation antigen

Implications cliniques de la molécule-1 d'adhérence aux cellules vasculaires et de l'interaction de l'antigène-4 d'activation très tardive ainsi que de la production de la métalloprotéinase-2 matricielle chez les patients atteints d'une maladie du foie

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OBJECTIFS : Éclaircir le rôle de la molécule d'adhésion dans la formation de lésions dans les cellules du foie.

PATIENTS ET MÉTHODES : Les concentrations sériques de la molécule-1 soluble d'adhésion aux cellules vasculaires (sVCAM-1), et l'expression de VCAM-1 et de son ligand, l'antigène-4 d'activation très tardive (VLA-4), ont été étudiées chez des patients atteints de différentes maladies du foie. De plus, la présence de la métalloprotéinase-2 (MMP-2) matricielle a été recherchée parce qu'on pense que la libération de MMP-2 est médiée par les cellules positives pour VLA-4. sVCAM-1 et MMP-2 ont été mesurées par dosage par la méthode ELISA, et VCAM-1 et VLA-4 ont été étudiés à l'aide de méthodes immunohistologiques.

RÉSULTATS : Chez les patients atteints d'hépatite aiguë (HA), la concentration sérique de sVCAM-1 était considérablement élevée comparativement à celle mesurée dans d'autres cohortes. VCAM-1 était exprimée sur les cellules tapissant les sinusoides mais pas sur les hépatocytes. Chez les patients atteints d'une maladie chronique du foie, les concentrations de sVCAM-1 augmentaient de concert avec la progression de l'hépatite

chronique (HC) et VCAM-1 était également exprimée. VLA-4 a été décelé à la fois dans les cellules mononucléées et dans les cellules de Kupffer chez les patients atteints d'HA, mais principalement dans les cellules de Kupffer chez les patients atteints d'HC. Chez les patients atteints d'HA, les concentrations de MMP-2 étaient similaires à ceux des sujets témoins, mais chez les patients atteints d'HC et de cirrhose du foie, la concentration de MMP-2 était élevée et associée à une hépatite chronique évolutive.

CONCLUSIONS : La réponse immunitaire par le biais des voies de VCAM-1 et de VLA-4 est importante dans la lésion des hépatocytes, en particulier chez les patients atteints d'HA, pour lier les cellules mononucléées positives pour VLA-4 aux cellules tapissant les sinusoides positives pour VCAM-1. La distribution des cellules positives pour VLA-4 varie chez les patients atteints d'HA et ceux atteints d'HC. Les cellules de Kupffer positives pour VLA-4 dans les maladies chroniques du foie pourraient jouer un rôle dans l'évolution de l'hépatite chronique, peut-être par le biais d'un mécanisme de régulation positive de la production de MMP-2.

The emigration of leukocytes, including monocytes, from blood into tissues is a prominent feature of acute and chronic inflammation in many diseases (1). Furthermore, the ability of leukocytes to adhere to the endothelium is essential for leukocyte migration into inflammatory sites (2). One of the facilitating molecules for this is intracellular adhesion molecule-1 (ICAM-1). Volpes et al (3) reported that the co-expression of ICAM-1 and human leukocyte antigen-DR antigens by hepatocytes correlated positively with the site and extent of the inflammatory infiltrate, which was composed of lymphocytes expressing lymphocyte function-associated antigen-1 (LFA-1). Another study (4) found that the ICAM-1/LFA-1 pathway was important for hepatocellular injury.

Another important adhesion molecule is the vascular cell adhesion molecule-1 (VCAM-1) (5). VCAM-1 is a cytokine-inducible glycoprotein belonging to the immunoglobulin (Ig) supergene family (6). VCAM-1 is expressed on the surface of the endothelium and mediates the adhesion of lymphocytes and histiocytes to inflammation sites via its ligand, very late antigen-4 (VLA-4). The interaction of the luminal surface of blood vessels is important as a first step of inflammatory reaction. Although the expression of VCAM-1 is very low or absent on resting endothelial cells in culture, it is inducible by stimulation with cytokines such as interleukin (IL) -1 or tumour necrosis factor- α (TNF- α) (7). Adhesion molecules are released in a soluble form, but their biological function is unclear. Measurement of the soluble form of these adhesion molecules has been useful for monitoring several disease activities (8). To clarify the role of other pathways in hepatocyte injury, we studied VCAM-1 and VLA-4 regulation during acute hepatitis (AH) and chronic hepatitis (CH). We also studied matrix metalloproteinase-2 (MMP-2) (72 kD, type IV collagenase or gelatinase), one of the gene families of zinc enzymes capable of degrading type IV collagen (9). The serum level of MMP-2 is thought to be useful for estimating the degree of liver fibrosis in both humans (10) and rats (11). Moreover, T cells and macrophages secrete the gelatinases MMP-2 and

-9, after beta α_1 integrin- or VCAM-1-dependent stimulation by cytokines and inflammatory mediators (9). As for the disease example, the cause of autoimmune encephalomyelitis (EAE) is thought to be mediated by immune response. In the animal model of EAE, VLA-4-positive T cells that adhere to VCAM-1-positive endothelial cells exhibited an induction of MMP-2 mRNA protein and activity (12).

Comparing the serum level of soluble VCAM-1 (sVCAM-1) and the expression of VCAM-1/VLA-4 in liver tissues, we studied the role of the VCAM-1/VLA-4 interaction. Furthermore, we examined the level of MMP-2 in AH and CH patients in relation to the VCAM-1/VLA-4 pathway.

PATIENTS AND METHODS

Patients: One hundred and twenty-one individuals were studied. There were 22 AH patients: age range 24 to 71 years, mean 44.4 years; 14 male and six female; and 10 with hepatitis A virus (HAV), eight with hepatitis B virus (HBV), one with hepatitis C virus (HCV) and three with unknown etiology. There were 31 CH patients: age range 11 to 75 years, mean 47.2 years; 25 male and six female; and 14 with HBV and 17 with HCV. Thirty-two patients had liver cirrhosis (LC): age range 35 to 77 years, mean 56.1 years; 20 male and 12 female; and 14 with HBV, 11 with HCV and seven with alcoholic LC. Eleven healthy control subjects (age range 22 to 73 years, mean 43.4 years) were studied, together with 25 patients with fatty liver (age range 24 to 71 years, mean 44.4 years) as 'disease control' (even though the serum levels of alanine aminotransferase [ALT] were elevated, hepatocyte damage was not due to hepatocyte injury induced by immune response but to fat accumulated in the hepatocyte). They were diagnosed according to their serological tests and ultrasonographic examinations. Patients with fatty liver were negative for hepatitis B surface (HBs) antigen, and HCV antibody and autoantibodies, and they were diagnosed by serological tests and ultrasonographic examinations. All patients were tested for serum HBs antigen (Abbott Laboratories, Chicago, Illinois) and for anti-HCV

TABLE 1
Characteristics of patients and controls

Characteristic	Control (n=11)	Fatty liver (n=25)	Chronic hepatitis (n=31)	Liver cirrhosis (n=32)	Acute hepatitis (n=22)
Mean age, years (range)	43.4 (22–73)	44.4 (24–71)	47.2 (11–75)	56.1 (35–77)	37.8 (19–77)
Sex (male:female)	8:3	19:6	25:6	20:12	14:8
Etiology					
Hepatitis A virus	–	–	–	–	10
Hepatitis B virus	–	–	14	14	8
Hepatitis C virus	–	–	17	11	1
Alcohol	–	–	–	7	–
Unknown	–	–	–	–	3
Aspartate aminotransferase (U/L)	20±6	40±21	63±37*	59±33*	1865±3061*
Alanine aminotransferase (U/L)	19±8	59±37	90±76*	55±37*	2134±2158*
Gamma glutamyl transpeptidase (U/L)	23±15	134±217	78.8±87	96±109	261±261*
Total bilirubin (μmol/L)	5.1±1.7	10.3±3.4	10.3±5.1	20.5±15.4	140.2±102.6*

Data are expressed as mean ± SD. * $P < 0.01$ versus healthy controls

antibodies by third generation ELISA (Ortho Diagnostic Systems Inc, Raritan, New Jersey). In anti-HCV-positive patients, the presence of serum viral HCV RNA was further confirmed using Amplicor HCV amplification kit (Roche Diagnostic Systems Inc, Somerville, New Jersey). Patients with evidence of coinfection of HBV and HCV, and a history of autoimmune diseases and metabolic disorders were excluded from these studies. In addition, none of the patients received interferon therapy, which is thought to be beneficial for hepatic fibrosis in parallel with improvement of aminotransferase activity (13). Characteristics of the patients are briefly summarized in Table 1.

Serum samples from patients were immediately aliquoted and stored at -20°C until assayed.

Measurement of sVCAM-1 and MMP-2: Serum concentrations of sVCAM-1 (Boehringer-Mannheim, Mannheim, Germany) and MMP-2 (Amersham Life Science Buckinghamshire, United Kingdom) were measured by commercially available ELISA kit. The assay principles were based on the quantitative sandwich enzyme-immunoassay method using two monoclonal antibodies directed against different epitopes of either human sVCAM-1 or MMP-2. The analysis was carried out according to the recommendations of the manufacturer.

Immunohistochemical staining for VCAM-1 and VLA-4:

Liver biopsy specimens were stained as previously described (14). Liver specimens were embedded in Tissue-Tek OCT compound (Sakura Finetechnical Co, Ltd, Tokyo, Japan) and immediately frozen with liquid nitrogen and kept at -80°C until used. Frozen sections were sliced into 5 μm thick sections and fixed in acetone for 10 mins. Throughout the immunostaining, 10 μg/mL of monoclonal anti-VCAM-1 (clone 1.G11B1; Ancell, Bayport, Minnesota), anti-VLA-4 (clone 9F10; PharMingen, San Diego, California) and isotype-matched control IgG₁ antibody (clone MOPC-21; PharMingen) were used for primary antibodies. Staining of VCAM-1 and VLA-4 in the human liver specimens was graded semiquantitatively on a four-point scale (–, ±, + and ++).

Serum samples and human liver specimens for all experiments were obtained with the informed consent of each patient, following the agreement of the Research Ethics Committee of Tokyo Women's Medical University, Tokyo, Japan.

Statistical analysis: The values of sVCAM-1 and MMP-2 were expressed as mean ± SD. Analysis was performed using the SAS system (SAS Institute, Cary, California). One way ANOVA was used for the AH, CH, LC, healthy control and fatty liver groups. The Tukey-Karner multiple comparisons method was applied when appropriate. The unpaired *t* test was used to assess the statistical significance among AH, CH, LC, healthy control and fatty liver groups. The paired *t* test was used to compare the peak and remission phases of all patients. Spearman's rank correlation was used to evaluate the relationship between any corresponding two groups. The normality of the distribution and homogeneity of variance were assessed by the Shapiro-Wilks test, Studentized residual and F test of Levene test, respectively. When data violated the mentioned assumption, the variabilities were transformed logarithmically. Two-tailed $P < 0.01$ was considered statistically significant.

RESULTS

Serum level of sVCAM-1 in various hepatitis patients:

The sVCAM-1 level of AH patients (1863 ± 380 ng/mL) was significantly higher than that of CH patients (899 ± 378 ng/mL; $P < 0.01$), LC patients (1442 ± 488 ng/mL; $P < 0.01$), healthy controls (415 ± 217 ng/mL; $P < 0.01$) and fatty liver patients (483 ± 128 ng/mL; $P < 0.01$). No difference in serum levels of sVCAM-1 was observed based on the etiology. In the AH group, no difference in sVCAM-1 level was observed between the group with AH induced by HAV (2293 ± 1060 ng/mL) and the group with AH induced by HBV (1839 ± 470 ng/mL) (not significant). In the CH group, no difference in sVCAM-1 level was observed between the group with CH induced by HBV (825 ± 265 ng/mL) and the group with CH induced by HCV (959 ± 449 ng/mL) (not significant). In the LC group, there was no difference in

Figure 1) In acute hepatitis (AH) patients, vascular cell adhesion molecule-1 (VCAM-1) was mainly expressed on the surface of the sinusoidal endothelium. **Top left** The expression was not observed on the hepatic parenchyma (original magnification $\times 100$). **Middle left** No upregulation of VCAM-1 expression was detected in the control liver (original magnification $\times 100$). **Bottom left** In liver cirrhosis (LC) patients, VCAM-1 was expressed on the surface of the sinusoidal endothelium, but the degree of VCAM-1 expression was weaker than that in AH patients (original magnification $\times 100$). **Top right** Very late activation antigen-4 (VLA-4) was detected on the surface of mononuclear inflammatory cells and Kupffer cells around hepatocytes in AH patients (original magnification $\times 100$), but not in control livers (**Middle right**) (original magnification $\times 100$). **Bottom right** In LC patients, VLA-4-positive cells were highly upregulated on Kupffer cells (original magnification $\times 100$)

sVCAM-1 level between the alcoholic LC group (1178 ± 380 ng/mL) and the group with LC induced by HBV (1394 ± 496 ng/mL) (not significant), between the alcoholic LC and the group with LC induced by HCV (1586 ± 557 ng/mL) (not sig-

nificant), and between the group with LC induced by HBV and the group with LC induced by HCV (not significant).

Follow-up studies were carried out in five AH patients after discharge for the duration of their outpatient status.

TABLE 2
Very late activation antigen-4 expression in the liver

	Kupffer cells			Mononuclear inflammatory cells			Hepatocytes		
Patient	1	2	3	1	2	3	1	2	3
Normal controls (n=2)	—	—		—	—		—	—	
Acute hepatitis (n=3)	+	+	±	++	+ or ++	+	—	—	—
Chronic hepatitis (n=3)	+	+ or ++	+	+	+	±	—	—	—
Liver cirrhosis (n=3)	+ or ++	++	+ or ++	±	±	±	—	—	—

Staining of very late activation antigen-4 in human liver specimens was graded semiquantitatively on a four-point scale (—, ±, + and ++)

Figure 2) Serum concentrations of matrix metalloproteinase-2 (MMP-2) in acute hepatitis (AH), chronic hepatitis (CH), liver cirrhosis (LC) and fatty liver patients, as well as controls. The data are expressed as mean $\pm$ SD. $P < 0.01$ was considered statistically significant (unpaired t test)

Figure 3) Serum concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1) in acute hepatitis (AH), chronic hepatitis (CH), liver cirrhosis (LC) and fatty liver patients, as well as controls. The data are expressed as mean $\pm$ SD. $P < 0.01$ was considered to be statistically significant (unpaired t test)

Their sVCAM-1 levels were compared between peak (maximal ALT level) and remission (ALT returned to normal range) phases. At the maximal ALT period, sVCAM-1 levels in those five patients were elevated (1719 ± 405 ng/mL), and in the remission phase, corresponding with the recovery of hepatocellular damage, sVCAM-1 levels were decreased (442 ± 250 ng/mL) to the same levels as those of healthy control subjects. In patients with chronic liver disease, the serum level of sVCAM-1 was elevated in conjunction with the disease progression of CH. sVCAM-1 levels in CH patients were significantly higher than those of healthy controls ($P < 0.01$) and patients with fatty liver ($P < 0.01$), and levels of sVCAM-1 in LC patients were significantly higher than those of CH patients ($P < 0.01$). On the other hand, there was no significant difference in sVCAM-1 level between healthy control subjects and fatty liver patients.

Immunohistochemical staining: To determine the localization of VCAM-1 and VLA-4, immunohistochemical liver tissues obtained from these hepatitis patients were stained. In AH patients, VCAM-1 was mainly expressed on the surface of the sinusoidal endothelium. The expression was not observed on the hepatic parenchyma (Figure 1, top left). No upregulation of VCAM-1 expression was detected in the control liver (Figure 1, middle left). In LC patients, VCAM-1 was expressed on the surface of the sinusoidal en-

dothelium but to a lesser degree than that in AH patients (Figure 1, bottom left). VLA-4 was detected on the surface of mononuclear inflammatory cells and Kupffer cells around hepatocytes in AH patients (Figure 1, top right) but not in the livers of controls (Figure 1, middle right). In LC patients, VLA-4-positive cells were highly upregulated on Kupffer cells (Figure 1, bottom right). The relative ratio of VLA-4-positive mononuclear inflammatory cells was higher in AH patients, whereas in CH patients, including both CH and LC patients, the ratios of VLA-4-positive Kupffer cells were higher than in AH patients (Table 2).

Serum level of MMP-2 in various hepatitis patients: There was no significant difference between MMP-2 levels of AH patients (969 ± 338 ng/mL) and those of healthy controls (763 ± 145 ng/mL) and fatty liver patients (877 ± 276 ng/mL). However, the serum level of MMP-2 increased together with the disease progression of CH (Figure 2). MMP-2 levels in CH patients (1104 ± 212 ng/mL) were significantly higher than those of controls ($P < 0.01$) and fatty liver patients ($P < 0.01$). MMP-2 levels in LC patients (2053 ± 708 ng/mL) were significantly higher than those of CH patients ($P < 0.01$). No difference in serum MMP-2 levels was observed based on the etiology. In the AH group, no difference in MMP-2 levels was observed between the group with AH induced by HAV (940 ± 247 ng/mL) and the group with AH induced by

HBV (827 ± 159 ng/mL) (not significant). In the CH group, no difference in MMP-2 levels was observed between the group with CH induced by HBV (999 ± 181 ng/mL) and the group with CH induced by HCV (1189 ± 201 ng/mL) (not significant). In the LC group, there was no difference in MMP-2 serum levels between the alcoholic LC group (2003 ± 754 ng/mL) and the group with LC induced by HBV (2261 ± 851 ng/mL) (not significant), between the alcoholic LC group and the group with LC induced by HCV (1819 ± 385 ng/mL) (not significant), and between the group with LC induced by HBV and the group with LC induced by HCV (not significant).

Coefficient of correlation: To determine whether sVCAM-1 and MMP-2 levels were correlated with the progression of chronic liver disease, the coefficient of correlation between sVCAM-1 and MMP-2 was studied. No significant correlation between sVCAM-1 and MMP-2 levels was observed in AH patients, but a significant correlation was found between sVCAM-1 and MMP-2 levels in serum obtained from chronic liver disease patients (CH and LC; $r=0.553$, $P<0.001$).

DISCUSSION

It has been reported that the ICAM-1/LFA-1 pathway is important for the generation of hepatocellular injury (3,15). In the present study, we attempted to analyze the mechanism of hepatocellular injury during AH and CH from the aspect of another adhesion molecule, VCAM-1, and its ligand VLA-4. In addition, we studied the serum level of MMP-2 because it is thought to be a marker of hepatic fibrosis (10) and because the release of MMP-2 may be mediated by VLA-4-positive cells (12).

First, sVCAM-1 levels were significantly higher in AH patients than in CH patients, LC patients, healthy controls and fatty liver patients (Figure 3). Immunological staining showed that, in AH patients, VCAM-1 was mainly expressed on the surface of the sinusoidal endothelium and was not expressed on the hepatic parenchyma (Figure 1, top left). Further, VLA-4 was detected on the surface of mononuclear inflammatory cells and Kupffer cells around hepatocytes in AH patients (Figure 1, top right). VLA-4-positive cells may infiltrate and adhere to VCAM-1-positive sinusoidal lining cells, thereby contributing to the acute phase of hepatocyte injury. However, VCAM-1 itself was not detected on hepatocytes. This suggests that, during the development of hepatocyte injury, the pathway through VCAM-1/VLA-4 may not contribute directly to hepatocyte damage. In contrast, Volpes et al (3) demonstrated that ICAM-1 was upregulated on hepatocytes and that LFA-1 was upregulated on leukocytes in the active phase of CH, suggesting that the pathway through ICAM-1/LFA-1 plays an important and direct role in hepatocyte damage. Considering these results, we speculate that the role of adhesion molecules in AH is as follows. First, acute viral infection stimulates the cell-mediated immune response and several cytokines are upregulated (16). These cytokines may stimulate the expression of adhesion molecules such as ICAM-1

and VCAM-1. Activated T cells expressing VLA-4 may infiltrate sinusoid and adhere to sinusoidal lining cells of liver via the VCAM-1/VLA-4 pathway. Then, infiltrated T cells would attack hepatocytes through the ICAM-1/LFA-1 pathway, combined with the major histocompatibility complex and T cell receptor systems (3). The VCAM-1/VLA-4 and ICAM-1/LFA-1 pathways may play roles in different steps of hepatocellular injury. Second, in the CH group, the sVCAM-1 levels were significantly elevated compared with those of the control group (Figure 3). In accordance with hepatitis progression, CH to LC, the level of sVCAM-1 was seen to increase. This suggests that serum levels of sVCAM-1 may be correlated with chronic liver damage. However, in fatty liver patients, no significant elevation of sVCAM-1 was observed (Figure 3). In fatty liver, fatty deposits rather than cellular infiltrates were the dominant matter in the liver, and hepatocyte damage was not due to hepatocyte injury induced by immune response or cytopathic viruses, so that no significant elevation of sVCAM-1 was observed. There are several reports about increased levels of sICAM-1 in chronic liver diseases, including viral hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, cryptogenic cirrhosis and alcoholic hepatitis (17-21). Our observation revealed that not only sICAM-1 level but also sVCAM-1 level was elevated in relation to chronic liver disease.

Sporadic and weak expression of VLA-4 on Kupffer cells has been observed in murine liver; this expression on Kupffer cells as well as on infiltrating leukocytes was upregulated by IL-12 stimulation (22). In CH and LC patients, the relative ratios of VLA-4-positive mononuclear cells were lower, and the numbers of VLA-4-positive Kupffer cells were much higher than in AH patients. On the other hand, the expression of VCAM-1-positive sinusoidal lining cells was weaker than that of AH patients (Figure 1, bottom left). In chronic liver disease progression, the interaction between VLA-4-positive intrahepatic mononuclear inflammatory cells and VCAM-1-positive sinusoidal lining cells may not be so important compared with acute hepatocyte injury. In addition, Garcia-Monzon et al (23) reported that VCAM-1-positive dendritic cells in the portal tract were upregulated in livers obtained from chronic viral hepatitis patients. VCAM-1-positive dendritic cells contributed to an appropriate activation of portal T lymphocytes to facilitate the proliferation of activated T cells in the portal area and then invasion into the surrounding parenchyma (23). We found that upregulated VLA-4-positive Kupffer cells exist not around the portal or periportal area but rather mainly in the intrahepatic area. The interaction between these VLA-4-positive Kupffer cells and VCAM-1-positive sinusoidal lining cells may be involved in chronic liver disease progression.

We also examined the serum level of MMP-2, a kind of liver fibrosis marker (10). T cells and macrophages secrete the gelatinases MMP-2 and -9, after β_1 integrin- or VCAM-1-dependent stimulation by cytokines and inflammatory mediators (9). In addition, it has been reported that the VLA-4/VCAM-1 pathway and MMP-2 upregulation are

possible causes of a kind of immune-mediated disorder, EAE. In the animal model of EAE, VLA-4-positive T cells adhering to VCAM-1-positive endothelial cells were induced in MMP-2 mRNA protein and activity (12). Our data indicate that the serum level of MMP-2 rose significantly corresponding with the progression of chronic liver diseases. However, in AH patients, MMP-2 levels were similar to those of healthy controls and fatty liver patients (Figure 2). In the chronic liver disease groups with CH and LC, the MMP-2 level correlated with sVCAM-1 level.

Goetzl et al (9) reported that the lower levels of MMPs, such as MMP-2, produced by T cells serve principally to facilitate T cell migration through connective tissues, whereas the higher levels of a broader range of MMPs produced by macrophages also contribute substantially to degradation, removal and remodelling of connective tissue. The pathogenesis of liver fibrosis is closely associated with the ubiquitous protective mechanisms involved in inflammation and repair. Once they become excessive and prolonged, the intended protection becomes a disease entity in itself by inducing a chronic fibroproliferative response (24).

Accordingly, we speculated that the discrepancy between the upregulated levels of sVCAM-1 and MMP-2 in AH but not in CH and LC patients might be explained as follows. In

AH patients, the acute mechanisms involved in the inflammation process mediated by VLA-4-positive mononuclear cells and VCAM-1-positive sinusoidal lining cells may result in acute inflammation in the liver. In this acute process, cytokine profiles and concentrations produced by mononuclear cells would be deeply associated. Actually, in AH patients, TNF- α was greatly upregulated compared with in CH and LC patients (unpublished data, Tokushige et al). In patients with chronic liver diseases, because of the excessive and prolonged stimulation of the liver, perhaps in some part, the mediation by VLA-4-positive Kupffer cells of the VCAM-1 pathway may contribute to the chronic fibroproliferative process. Once the fibroproliferative activity has been in process, the sequential phenomena of degradation, removal and remodelling of connective tissues may occur, and MMP-2 may be upregulated. Clarification of the mechanisms of the regulation between the VCAM-1/VLA-4 interaction and MMP-2 level during acute and chronic hepatocyte damage may provide some clues for furthering the understanding of the generation of AH and CH progression. Further studies are required.

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