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Clinical Significance of Soluble Intercellular Adhesion Molecule-1 Antigen in Sera of Patients with Various Liver Diseases

Kazuhiro ARIMA[1], Mitsuo SUGA[1], Hirofumi SAKAMOTO[2], Masayuki TSUJISAKI[2], Yuji HINODA[2] and Kohzo IMAI[2]

[1]*Department of Internal Medicine, NTT Sapporo Hospital, Sapporo 060, Japan*

[2]*Department of Internal Medicine (Section 1), Sapporo Medical University School of Medicine, Sapporo 060, Japan*

SUMMARY

A double determinant immunoassay (DDIA) to detect soluble intercellular adhesion molecule-1 (sICAM-1) established in our laboratory was used to measure sICAM-1 levels in sera of patients with various liver diseases. Elevated levels were found in 100% of 7 patients with acute phase of acute viral hepatitis (AH), in 44% of 18 chronic persistent hepatitis (CPH), in 66% of 41 chronic active hepatitis (CAH), in 89% of 28 liver cirrhosis (LC), in 86% of 7 primary biliary cirrhosis (PBC), and in 90% of 49 hepatocellular carcinoma (HCC). The mean levels of sICAM-1 in those diseases were significantly higher than in healthy controls. The levels of sICAM-1 correlated positively with serum AST in patients with CPH, CAH, LC, and PBC. Levels were significantly higher in patients with multi-nodule HCC than in those with single-nodule HCC, and the amount of sICAM-1 tended to increase with the size of the tumor. These results suggest that serum levels of sICAM-1 correlate with the degree of inflammation and the host immune response in liver and carcinoma tissues. The measurement of sICAM-1 may be useful for evaluating the disease states of patients with HCC.

Key words: ICAM-1, sICAM-1, Hepatocellular carcinoma

INTRODUCTION

Intercellular adhesion molecule-1 (ICAM-1), a 90-kD cell-surface glycoprotein, is one of the ligands for the lymphocyte function associated antigen-1 (LFA-1) molecule; it promotes cell adhesion in immune and inflammatory reactions(1, 2). ICAM-1 is one of the members of the immunoglobulin supergene family, and is constructed of five extracellular immunoglobulin-like domains (D), D1, D2, D3, D4, and D5(3). D1 contains the primary site of contact for LFA-1,

while D3-D5 contain non-binding sites for LFA-1(4). ICAM-1 is also known to have a receptor for rhinoviruses, members of the picornavirus family(5). ICAM-1 expression is normally present on the surfaces of several cells including lymphocytes, monocytes, endothelial cells and fibroblasts. Its expression *in vivo* is low in normal tissues but can be upregulated by cytokines(6, 7).

In a previous study, we have established a double determinant immunoassay (DDIA) using two monoclonal antibodies (MoAb), HA58 and CL207, and detected soluble form of ICAM-1 (sICAM-1) in serum(8). These MoAbs were identified to recognize different epitopes of ICAM-1; HA58 (IgG1) recognizes D1, which contains a binding site for LFA-1, and CL207 (IgG1) recognizes D4. At almost the same time, other investigators reported that sICAM-1 was detected in normal human serum(9, 10). Using the above DDIA, we have demonstrated that sICAM-1 levels in patients with malignant diseases such as gastric cancer were higher than in those with benign diseases or in healthy controls(8).

Here we examined the levels of sICAM-1 in sera of patients with various liver diseases using the DDIA. The present study provides evidence that its levels were related to the degree of inflammation and immune response of hosts. sICAM-1 may turn out to be a useful marker to diagnose patients with hepatocellular carcinoma.

PATIENTS AND METHODS

Patients

Serum samples were collected from the following groups of patients referred to the Sapporo Medical University Hospital in Japan. There were 7 patients with acute hepatitis (AH; 3 type A and 4 type B), 18 with chronic persistent hepatitis (CPH; 8 type B and 10 type C), 41 with chronic active hepatitis (CAH; 25 type B and 16 type C), 28 with liver cirrhosis (LC; 7 type B and 21 type C), 49 with hepatocellular carcinoma (HCC; 9 type B and 40 type C) and 7 with primary biliary cirrhosis (PBC). The diagnoses of acute hepatitis and liver cirrhosis were based on clinical, biochemical and serological findings, and those of chronic hepatitis (CH) and PBC, on liver biopsy findings. The diagnosis of HCC was confirmed histologically and by imaging procedures (ultrasonography, computed tomography and angiography). Type A hepatitis was confirmed by high titers of IgM antibody to hepatitis A virus, type B by existence of HBsAg and/or IgM antibody to hepatitis B core antigen, and type C by existence of 2nd generation antibody to hepatitis C virus (HCV) (2nd generation anti-HCV ELISA Test, Abbott Laboratories, North Chicago, Ill, USA) and/or by detection of HCV RNA by two-stage reverse transcription-polymerase chain reaction with an amplified 5'-nontranslation region of HCV(11). In addition, serum sICAM-1

levels were measured in 25 healthy adults. Details of the patients studied are shown in Table 1.

Monoclonal antibodies (MoAb)

MoAb HA58 (IgG1) against ICAM-1 was developed in our laboratories(10). Briefly, HA58 is secreted by a hybridoma constructed with myeloma cells and splenocytes from a BALB/c mouse that was immunized with colonic carcinoma BM314 cells treated with interferon-gamma (IFN-γ). MoAb CL207 (IgG1) against ICAM-1 was developed as described elsewhere(12). MoAbs are purified from ascitic fluid either by affinity chromatography on protein A Sepharose, by ion exchange chromatography on DEAE or by caprylic acid precipitation. MoAbs are biotinylated according to published procedures(13).

DDIA to detect sICAM-1

DDIA established in our laboratory was used to measure sICAM-1 levels in serum(8). Briefly, the wells of a plastic microplate (Nunc Immunoplate II, Roskilde, Denmark) were coated with MoAb CL207 by incubating 150 ml of purified MoAb CL207 at 20 μg/ml in phosphate-buffered saline (PBS) overnight. The wells were blocked with PBS, pH 7.4, containing 3% bovine serum albumin for 120 min. The aliquots (150 ml) of serum samples diluted 1/200 in PBS were then added to the plate and incubated for 120 min. After being washed with PBS containing 0.05% Tween 20, the plates were incubated with 150 ml of biotinylated MoAb HA58 (10 μg/ml) for 120 min. Avidin-conjugated peroxidase (Vector, Burlingame, CA) was diluted 1/1000 in 0.05 M PBS with 0.5 M NaCl, pH 8.0, and incubated with the wells for 60 min at room temperature. The degree of substrate reaction was determined with OPD at 492 nm in a Micro-ELISA Autoreader MR580 (Dynatech, Cambridge, MA). Results were expressed as ng/ml

Table 1 *Clinical data of 150 patients studied*

disease	n	age (yr)	AST (IU/L)	ALT (IU/L)	Alkaline phosphatase (IU/L)	Bilirubin (mg/dl)	Albumin (g/dl)
Acute hepatitis	7	27±1	138±39	340±91	254±36	5.8±3.2	3.5±0.3
Chronic persistent hepatitis	18	40±4	46±10	59±13	124±7	0.7±0.1	4.0±0.1
Chronic active hepatitis	41	43±2	53±8	74±14	156±10	0.7±0.1	3.8±0.1
Liver cirrhosis	28	59±2	64±8	60±9	192±15	1.0±0.1	3.5±0.1
Hepatocellular carcinoma	49	60±1	90±12	58±5	325±80	1.8±0.3	3.3±0.1
Primary biliary cirrhosis	7	55±3	63±22	47±20	596±372	2.1±1.5	3.2±0.2
Normal range			10−40	4−37	70−200	0.2−0.8	3.7−5.2

The values are expressed as mean±S. E. M.

calculated from titration curve of purified ICAM-1 antigen. All assays were performed in duplicate.

Statistical analysis

The results are expressed as mean±SEM. The unpaired two-tailed Student's t test was used to detect the significance of the differences between two means. The correlation between measured parameters was determined using Spearman rank correlation coefficient analysis. A level of $p<0.05$ was considered to be significant.

RESULTS

Standard curve for sICAM-1 in DDIA

The purified ICAM-1 antigen (Bender Med Systems, Vienna, Austria) was used to construct a standard curve in DDIA (Fig. 1). The detection limit was 17 ng/ml. The mean intraassay coefficient of variation was 3.2 percent after ten tests of the standard antigen on the same plate. The normal range for sICAM-1 levels in 25 healthy controls was 35 to 229 ng/ml with a mean of 95 ng/ml (Fig.

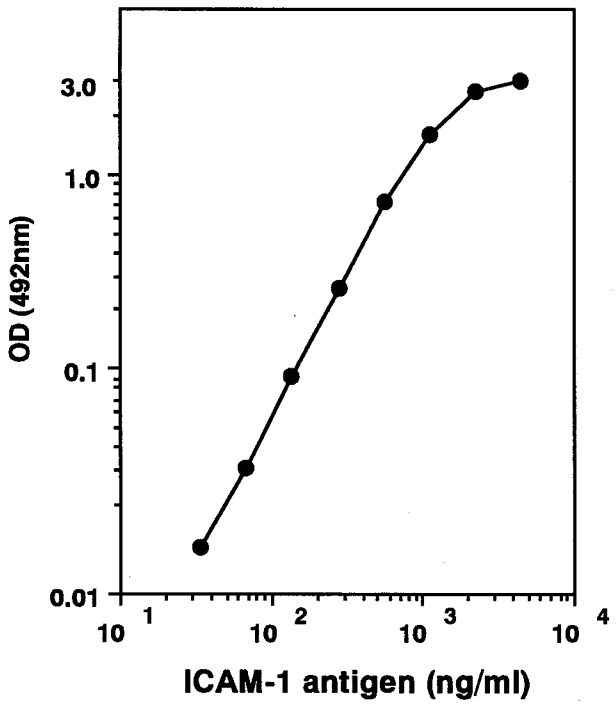

Fig. 1 Standard curve of soluble ICAM-1 antigen assay.

2). The cut off value (mean value+2 standard deviations) was set as 213 ng/m*l*, based on the data of control sera.

sICAM-1 levels in patients with AH

As shown in Fig. 2, the mean of sICAM-1 levels in 7 patients with AH at the time of admission was 985±148 ng/m*l*, which was significantly higher than that of healthy controls ($p<0.001$). The levels of the same individuals in the recovery stage significantly lowered to 228±32 ng/m*l* ($p<0.05$). No significant correlation was seen between sICAM-1 levels and standard liver function tests (Table 2).

sICAM-1 levels in patients with chronic liver diseases

As shown in Fig. 2, 44.4% of 18 patients with CPH, 65.9% of 41 patients with CAH and 89.3% of 28 patients with LC showed increased sICAM-1 levels more than the cut off value. The levels reached as high as 570 ng/m*l*, the mean value being significantly higher compared with healthy controls ($p<0.001$). The mean level of sICAM-1 in patients with LC was significantly higher than those in CPH

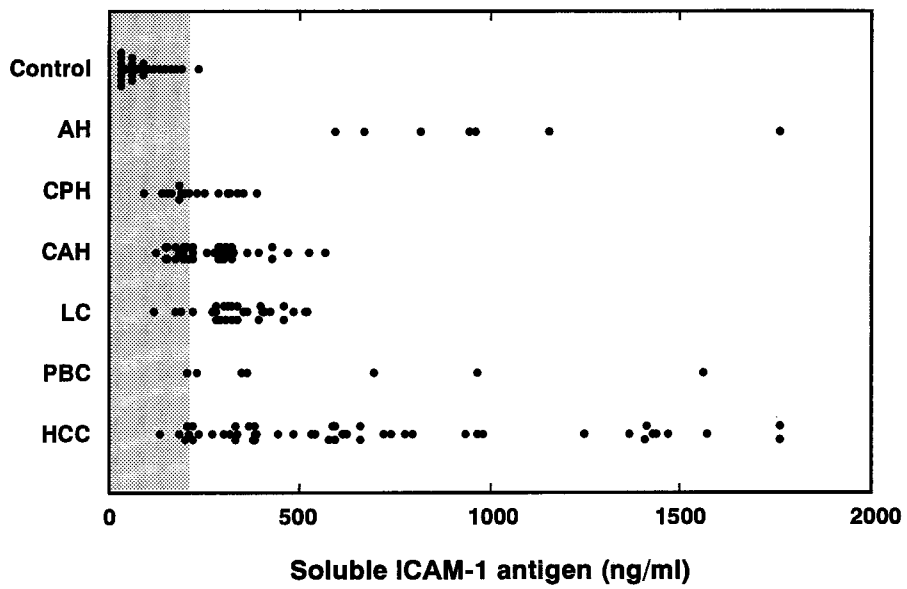

Fig. 2 Soluble ICAM-1 antigen levels in sera from patients with various liver diseases and healthy controls. The cut off value was set at 213 ng/m*l*. AH: acute hepatitis; CPH: chronic persistent hepatitis; CAH: chronic active hepatitis; LC: liver cirrhosis; PBC: primary biliary cirrhosis; HCC: hepatocellular carcinoma.

Table 2 *Spearman rank correlation coeficient for soluble ICAM-1 antigen and standard liver test*

disease	n	AST	ALT	Alkaline phosphatase	Bilirubin	Albumin	AFP
Acute hepatitis	7	−0.464 NS	−0.607 NS	0.214 NS	0.750 NS	−0.786 NS	
Chronic hepatitis	59	0.529 p<0.001	0.453 p<0.01	0.303 p<0.05	−0.213 NS	−0.046 NS	
Liuer cirrhosis	28	0.459 p<0.05	0.284 NS	0.415 p<0.05	0.192 NS	−0.420 p<0.05	0.299 NS
Primary biliary cirrhosis	7	0.857 p<0.05	0.571 NS	0.643 NS	0.847 p<0.05	−0.855 p<0.05	
Hepatocellular carcinoma	49	0.201 NS	−0.141 NS	0.592 p<0.001	0.406 p<0.01	−0.260 NS	0.194 NS

Chronic persistent hepatitis and chronic active hepatitis were analyzed together.
NS ; not significant.

($p<0.01$) and CAH ($p<0.05$), while no difference was found between CPH and CAH. There was a significant correlation in sICAM-1 levels with serum AST and alkaline phosphatase levels in patients with chronic hepatitis or LC (Table 2). Moreover, sICAM-1 levels were significantly higher ($p<0.001$) in patients who showed ALT levels over 100 IU (n=16, 177±25 ng/m*l*) than in those with ALT under 100 IU (n=71, 41±3 ng/m*l*).

Increased sICAM-1 levels over the cut off value were seen six of 7 patients (86%) with PBC. The mean was significantly higher than in healthy controls ($p<0.001$), and the highest was 1,560 ng/m*l*. These were also higher than in patients with CPH ($p<0.005$), CAH ($p<0.001$), and LC ($p<0.005$). sICAM-1 levels showed significantly higher ($p<0.05$) in symptomatic patients who had itching or jaundice (n=3, 1,075±254 ng/m*l*) than in symptom-free patients (n=4, 286±40 ng/m*l*). There was significant correlation between sICAM-1 levels and serum AST, bilirubin and albumin levels (Table 2).

sICAM-1 levels in patients with HCC

Forty-four of 49 patients (90%) with HCC showed increased sICAM-1 levels over the cut off value and the highest level was no less than 1,750 ng/m*l*. The mean level was significantly higher than in healthy controls ($p<0.001$) as well as in CPH ($p<0.005$), CAH ($p<0.001$), and LC ($p<0.001$). The average sICAM-1 level in patients with AFP over 20 ng/m*l* was 737±100 ng/m*l* (n=36), whereas in those under 20 ng/m*l* it was 536±82 ng/m*l* (n=13). There was no difference between the two groups. Though sICAM-1 had no apparent relation with serum

ALT or AST levels, it was shown to be correlated with alkaline phosphatase and bilirubin levels (Table 2).

To evaluate the factors affecting sICAM-1 levels, the relation of sICAM-1 levels to the number and the size of tumors was examined. Patients with multi-nodule HCC (n=24) had higher levels ($p<0.01$) than those with single-nodule HCC (n=25), as shown in Fig. 3. No significant difference of alkaline phosphatase or bilirubin levels, however, was observed for the number of tumors. As shown in Fig. 4, the sICAM-1 levels tended to increase with main tumor size, though this was not statistically significant.

DISCUSSION

The results of this study demonstrate that sICAM-1 is increased in sera from various liver diseases, especially AH, PBC, and HCC.

Cell surface ICAM-1 expression, membrane bound ICAM-1, has been reported in various immune and neoplastic diseases. A strong expression of ICAM-1 was seen on sinusoidal lining cells and on hepatocytes in the areas of inflammation in acute and chronic liver diseases(14). Double-staining for HBcAg and ICAM-1 in liver biopsy sections disclosed expression of ICAM-1 on

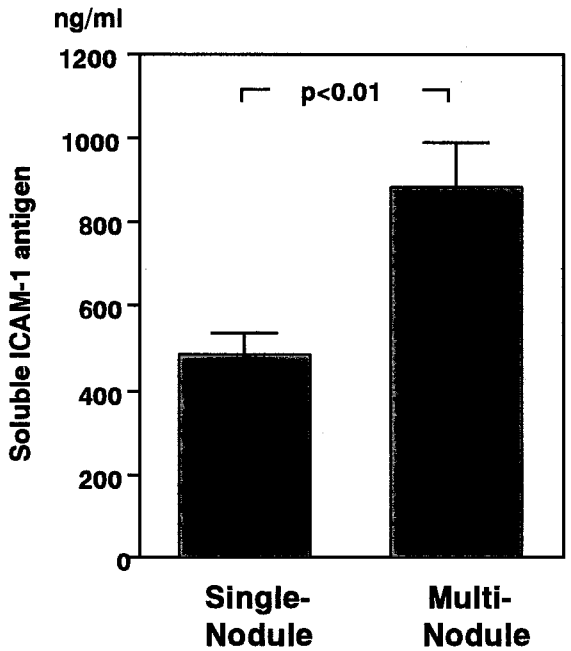

Fig. 3 Soluble ICAM-1 antigen levels and number of tumors.

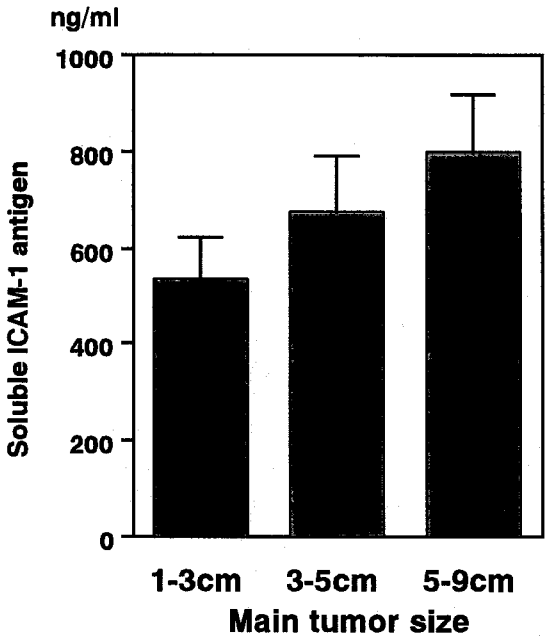

Fig. 4 Soluble ICAM-1 antigen levels and main tumor size. No statistical significance was seen.

hepatocytes, which seemed closely related with inflammatory activity in patients with chronic HBV infection(15). A strong expression of ICAM-1 was also seen on the interlobular bile ducts and proliferating bile ductules in primary sclerosing cholangitis (PSC) and PBC(16).

Seth *et al.* detected a soluble form of ICAM-1 in human sera from healthy controls using an immunological method(9). Rothlein *et al.* indicated that ICAM-1 might be released from the tissues as a result of the tissue damage and inflammation(10). In this study, we demonstrated that sICAM-1 levels in the acute phase of AH were higher than those during recovery. Patients with CPH, CAH and LC also had significantly higher levels than healthy controls; their sICAM-1 levels were correlated with the serum level of AST. These results suggest that the elevation of this protein in acute and chronic viral hepatitis could reflect hepatocellular necrosis and dysfunction due to inflammation of liver tissues.

Recently, Adams *et al.* demonstrated that levels of sICAM-1 were markedly elevated in sera of patients with PBC and PSC over those in other chronic liver diseases(17). We also found high levels in PBC, but noted comparable levels in sera from the acute phase of AH. Notably, there was a positive correlation between this protein and AST levels in serum, and its levels in symptomatic PBC

patients were significantly higher than those in asymptomatic patients. These results confirmed the putative relation between high sICAM-1 and degree of inflammation as suggested above.

The role played by sICAM-1 in patients with viral hepatitis and PBC is unclear. Adams *et al.* speculated sICAM-1 may play an immunoregulatory role after observing that sICAM-1 was derived from activated lymphocytes rather than from bile ducts cells in PBC(17). Their findings and ours suggested that sICAM-1 may act to regulate hepatocellular inflammation in viral hepatitis and PBC by promoting lymphocyte adhesion with LFA-1.

We have already demonstrated that the incidence of positivity for sICAM-1 in malignant diseases is higher than that in benign diseases or in healthy controls (8). In our study of immunostaining of cancerous tissue with MoAb HA58, ICAM-1 was expressed not only on malignant cells but also on stromal cells (mainly fibroblasts) near cancer nests (data not shown). In patients with HCC, sICAM-1 was significantly higher than in patients with CPH, CAH, and LC. Moreover, the levels in patients with multi-nodule tumors were significantly higher than those with single-nodule tumors. Unlike in chronic hepatitis and LC, sICAM-1 levels were not correlated with serum AST levels in patients with HCC.

Release of membrane surface antigen appears to be a common feature of malignant cells *in vitro*(18). The origin of serum sICAM-1 in patients with malignant diseases is thought to be the same mechanism that causes shedding (19). Rothlein *et al.* reported that sICAM-1 in serum could be the result of proteolytic cleavage of cell-bound ICAM-1 close to the cell membrane(10). Moreover, the amount of antigens shed from cell surfaces appears to be correlated with cell growth but not with cell death(20). The result in this study that sICAM-1 levels rise with the size of a growing main tumor in HCC patients is consistent with the above observation.

The role played by sICAM-1 in patients with malignant diseases also remains unclear. It may be shed as a result of the host's immune response to malignant cells and/or surrounding cells. Tomita *et al.* reported that ICAM-1 was frequently expressed in patients with renal-cell cancer (RCC) and that the number of ICAM-1-positive cells in RCC correlated with the degree of T lymphocyte and macrophage infiltration(21). Furthermore, Vogetseder *et al.* demonstrated a marked expression of ICAM-1 in fibrous tissue adjacent to carcinoma, and that its intensity correlated with lymphocyte infiltration in the tissue(22). These observations suggested that the expression of ICAM-1 on malignant cells might augment the host immune reaction. It may, on the other hand, aid somehow in the escape of tumor cells from the host's immune response. The circulating antigen in sera from mice bearing Ehrlich tumor actually absorbed most tumor-

specific rejection antibodies(23). Similarly, there is a possibility that the shed ICAM-1 may block the attachment of cytotoxic T cells or NK cells to HCC cells, since this molecule can bind specifically to LFA-1(10).

We have already reported that sICAM-1 levels showed higher levels in malignant diseases with liver metastasis(8). ICAM-1 is detectable on advanced human melanomas in situ but not on benign melanocytes or early melanomas. From the association between the frequency of ICAM-1 expression on malignant melanomas in situ and tumor growth, Johnson *et al.* speculated that the appearance of ICAM-1 on cell surface could reflect the stage of metastasis, and that it could enable the tumor to escape immune destruction as described above(24).

In conclusion, high levels of sICAM-1 were detected in acute viral hepatitis, chronic hepatitis, liver cirrhosis and primary biliary cirrhosis. Patients with hepatocellular carcinoma also revealed high serum levels of sICAM-1. These results suggest that serum levels of sICAM-1 correlates with the degree of inflammation and the host immune response in liver and carcinoma tissues. The measurement of sICAM-1 may be useful for evaluating the disease states of patients with hepatocellular carcinoma.

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