Journal of Hepatology 1994; **21**: 527–535
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Munksgaard . Copenhagen

Journal of Hepatology
ISSN 0168-8278

Serum levels of macrophage colony stimulating factor (M-CSF) in liver disease

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(Received 29 April 1993)

We investigated the serum level of macrophage colony stimulating factor in acute and chronic liver disease. Levels of macrophage colony stimulating factor (mean±SD, ng/ml) were significantly higher in acute hepatitis (5.67±1.01, $p<0.01$) and chronic active hepatitis (3.34±1.19, $p<0.01$) than in healthy volunteers (1.90±0.25), asymptomatic hepatitis B virus carriers (1.98±0.40), and chronic persistent hepatitis (2.34±0.43). Levels of macrophage colony stimulating factor showed a highly significant correlation with the serum alanine aminotransferase levels in acute hepatitis ($p<0.01$, $r_s=0.903$) and in chronic active hepatitis ($p<0.01$, $r_s=0.672$). Levels of macrophage colony stimulating factor in patients with cirrhosis (cirrhosis; 3.11±0.93 and hepatocellular carcinoma; 3.30±0.74) were significantly higher than in patients with chronic persistent hepatitis although the alanine aminotransferase levels were not significantly different. In cirrhosis, levels of macrophage colony stimulating factor correlated positively with the serum alanine aminotransferase levels ($p<0.05$), total bilirubin levels ($p<0.05$), and indocyanine green clearance ($p<0.05$). An immunohistochemical study showed an increased number of macrophage colony stimulating factor positive mononuclear cells in portal areas in acute hepatitis. Our findings suggest that; (a) the serum levels of macrophage colony stimulating factor represent ongoing hepatocellular necrosis in acute and chronic liver disease, (b) the source of the increase in the serum macrophage colony stimulating factor levels in hepatic inflammation may be, in part, its production by infiltrating mononuclear cells in the liver, and (c) cirrhosis also causes elevated serum levels of macrophage colony stimulating factor. © Journal of Hepatology.

Key words: Cirrhosis; Hepatitis; Infiltrating mononuclear cells; Macrophage colony stimulating factor

Acute or chronic hepatitis is histologically characterized by infiltration of mononuclear cells, including monocytes/macrophages in the liver (1), which suggests that cells of macrophage monocyte lineage are involved in the pathogenesis of viral hepatitis. Tumor necrosis factor α (TNFα) and interleukin-6 (IL–6) are monokines that are mainly produced by monocytes/macrophages. Recently, the association of intrahepatic expression of TNFα and IL-6 with the inflammatory activity of acute or chronic viral hepatitis has been reported (2,3), and a significant correlation has been found between the production of TNFα by peripheral blood mononuclear cells and the inflammatory activity of chronic viral hepatitis (4).

Macrophage colony stimulating factor (M-CSF) is a cytokine secreted by many types of cells, such as monocytes/macrophages, fibroblasts, bone marrow stroma cells, lymphocytes, and endothelial cells (5–7). In addition to TNFα and other monokines, monocytes are one of the main sources of M-CSF (8). M-CSF selectively stimulates the differentiation of macrophage-monocyte progenitors (5). In osteoporotic mice, which are defective in the production of M-CSF, a decreased number of hepatic macrophages has been reported, showing the importance of M-CSF in the differentiation and increase of hepatic macrophages (9). In addition to stimulating the proliferation and differentiation of macrophage-monocyte progenitors,

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human M-CSF has been found to influence immunological activities of mature macrophages, such as tumor cytotoxicity (10), production of TNF (11), and chemotactic activity for monocytes (12). Apart from immunological activity, M-CSF plays important roles in the regulation of plasma cholesterol levels (13) and in the development of the placenta (14).

M-CSF was identified as an 84-kDa homodimeric glycoprotein found in healthy human urine, and is present in the peripheral blood and urine (15). The pleiotropic response to M-CSF was mediated by a receptor, which is identical to the c-fms protooncogene product. The 150-kDa human c-fms-coded glycoprotein was expressed at the cell surface of monocytes/macrophages, which was active as a tyrosine-specific protein kinase *in vitro* (16,17).

Recently, M-CSF was isolated from tissue culture sources (18,19) and several cDNA clones of M-CSF were isolated (20–23). Moreover, it is now possible to measure accurately the M-CSF levels in human sera by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) (24–27). Previous publications have revealed elevated serum and tissue levels of M-CSF in several pathological conditions (28,29); however, the serum M-CSF levels in human liver disease have not yet been investigated in detail. In this study, we measured the serum levels of M-CSF by RIA in acute and chronic liver disease to determine the relationship between the M-CSF concentration and the activity and etiology of these illnesses. We also performed an immunohistochemical study of M-CSF in liver tissue.

Materials and Methods

Sera

Sera were collected from several groups of patients with acute and chronic liver disease, and from healthy volunteers (Table 1). Diagnosis was reached using appropriate serologic, virologic, biochemical, and histologic criteria (30). HBsAg, HBeAg, anti-HBe and IgM anti-HAV antibodies were examined in sera by commercially available RIA kits (Abbott Laboratories, N Chicago, IL, USA), and anti-HCV antibody was determined by second-generation ELISA (Abbott Laboratories). Each healthy volunteer was shown to have normal levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl-transpeptidase (γ-GTP). Patients with a history of liver disease and a significant past medical history other than liver disease were excluded. Among ten patients with acute hepatitis (five type A, one type B, four non-A, non-B), sera were collected serially 2 to 6 times in seven patients. Four of five patients with non-A, non-B acute hepatitis developed an anti-HCV antibody in their clinical course. In all the patients with hepatocellular carcinoma, diagnosis was obtained during the follow up of cirrhosis at our clinic. None of the patients were receiving significant treatment, except for water, electrolytes, and vitamins.

Whole blood was drawn with a sterile syringe, transferred to a centrifuge tube, allowed to clot for 30 min at room temperature and centrifuged at 1500 $\times$g for 15 min at 4°C. Sera were divided into two parts. One part was used for the serologic, virologic, and biochemical examinations, and volumes of 1 ml were stored in microtubes at -20°C to be used in the experiments. Informed consent was obtained from each participant before phlebotomy, and the study was approved by the human research ethics committee of the hospital.

RIA of M-CSF

M-CSF produced by the human T lymphoblastoid cell line CEM-ON was purified and full-length cDNA was isolated as described previously (19,23). DNA encoding M-CSF was introduced into a Chinese hamster ovary expression system and the secreted rhM-CSF was purified to homogeneity (specific activity, 2×10^7 U/mg protein) as previously reported (19). New Zealand white rabbits (Shi-

TABLE 1

Clinical characteristics of the patients with liver disease

Diagnosis (No. studied)	Sex (F/M)	Age (years)	HBeAg/eAb	ALT (IU/l)	γ-GTP (IU/l)	G-gl (g/dl)	T-Bil (mg/dl)	ICG clearance R15 (%)
Healthy volunteer (20)	(8/12)	38±16	0/0	14±8	10±3	1.1±0.2	0.5±0.3	N.D.
HBV asymptomatic carrier (20)	(7/13)	34±7	5/15	21±5	14±8	1.2±0.2	0.4±0.3	N.D.
Chronic persistent hepatitis (12)	(5/7)	45±15	2/1	68±18	34±12	1.2±0.3	0.4±0.3	8±4
Chronic active hepatitis (30)	(12/18)	42±13	7/2	308±201	148±87	1.5±0.4	0.8±0.3	9±4
Acute hepatitis (10)	(3/7)	41±17	1/0	1205±531	197±93	1.7±0.4	8.2±2.6	N.D.
Cirrhosis (20)	(6/14)	58±14	3/2	76±25	61±25	1.7±0.3	1.4±0.5	25±6
Hepatocellular carcinoma (12)	(2/10)	56±12	0/1	65±24	76±28	1.8±0.4	1.8±0.6	33±8

ALT=alanine aminotransferase, γ-GTP=gamma glutamyl transpeptidase, G-gl=gamma globulin, T-Bil=total bilirubin, ICG=indocyanine green, R15=retention ratio in 15 min. Laboratory data of patients with liver disease.
Each laboratory parameter is expressed as mean±SD.

zuoka Laboratory Animal Center, Shizuoka, Japan) were immunized and boosted monthly with 20 μg rhM-CSF in complete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) to obtain rabbit antiserum to rhM-CSF. Of three rabbit antisera developed, one (OCT 511) was selected because of its high titer and high sensitivity. Iodination of rhM-CSF and purification of ^{125}I-rhM-CSF was performed as described by Fraker & Speck (31). Briefly, a 20-μl aliquot (4 μg) of iodogen solution was lyophilized and 10 μl of 0.25 M phosphate buffer was added to each lyophilized sample. Five microliters (0.5 mCi, 37 MBq) of Na^{125}I was added to the tube, followed by 10 μl (1 μg) of the rhM-CSF solution. The mixture was then diluted with 250 μl of 0.05 M phosphate buffer, aspirated from the tube, applied to a 1.0×50 cm column of Ultrogen AcA54 (LKB, Broma, Sweden) and eluted. An aliquot of each 1.0 ml fraction was counted in a γ-spectrometer. The peak radioactivity fractions were analyzed for binding with anti-rhM-CSF antibody (less than 5% was bound non-specifically by normal rabbit serum). The fractions with the most marked binding and the highest specificity were stored at −20°C for later use in the RIA.

The RIA for M-CSF was performed as follows. Duplicate samples of the patients' sera (100 μl) were mixed with ^{125}I-labelled rhM-CSF (10 000 cpm/100 μl) and rabbit antiserum against rhM-CSF (200 μl) diluted 40 000 times. Serial concentrations of rhM-CSF were assayed in parallel for the purpose of standardization. Antigen-antibody binding attained equilibrium within 20 h at room temperature or within 48 h at 37°C. After incubation, the bound product was separated from the free ^{125}I-labelled rhM-CSF by addition of 100 μl of goat anti-rabbit IgG diluted 400 times with phosphate-buffered saline, and 1.0 ml of 6% polyethylene glycol (MW 8000; Wako Pure Chemical Co., Tokyo, Japan) in phosphate-buffered saline. The tubes were shaken and centrifuged at 1000 ×g for 15 min at 4°C. The supernatants were aspirated and the precipitates were counted for 1 min in an automated γ-spectrometer. The detection limit of M-CSF was 0.1 ng/ml in this study. Intra- and inter-assay variations were below 10%.

Immunohistochemical detection of M-CSF positive cells in the liver

Liver biopsy specimens were obtained from five acute hepatitis patients (two type A, one type B, and two type C) and three chronic persistent hepatitis patients (one type B and two type C), the specimens being divided into two parts. One part was fixed with 10% paraformaldehyde and used for routine histology; the other was fixed with 4% paraformaldehyde for 2 h, embedded in OCT compound (Miles Inc., Elkhart, IN, USA), frozen and stored in liquid nitrogen-cooled isopentane, and used for M-CSF staining with an avidin-biotin complex method. In brief, cryostat sections (4 μm thick) were air-dried, rehydrated with phosphate-buffered saline, then soaked in phosphate-buffered saline containing 0.3% H_2O_2 and methanol at room temperature to inactivate endogenous peroxidase. Each section was treated with normal goat serum and incubated with rabbit anti-rhM-CSF antibody (diluted 200 times with phosphate-buffered saline) at 4°C overnight. After washing, each section was applied with avidin-biotin-peroxidase complex (Vectastain ABC kit: Vector Laboratories Inc., Burlingame, CA, USA), then soaked in 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M tris-HCl buffer solution (pH 7.6). All incubations were carried out at room temperature followed by three washes of phosphate-buffered saline. Finally, each section was counterstained with hematoxylin, dehydrated and mounted on a slide.

Statistical analysis

Statistical analysis was performed by means of the Mann-Whitney U test, and the Spearman's rank correlation test. A p-value <0.05 was considered to be significant.

Results

Serum levels of M-CSF were assayed in patients with acute and chronic liver disease (Table 1). The normal M-CSF range in our assay was 1.95±0.44 ng/ml (mean±SD) (27). The levels of M-CSF in the sera of 20 healthy volunteers in this study (10 men and 10 women) were consistently within the normal range (Fig. 1). The asymptomatic HBV carriers were younger than the cirrhosis and hepatocellular carcinoma patients. However, age and sex were independent of the serum M-CSF level (27).

As shown in Fig. 1, the M-CSF level in asymptomatic hepatitis B virus (HBV) carriers was 1.98±0.04 ng/ml, which was not significantly different from that of healthy volunteers (1.90±0.25 ng/ml). Levels of M-CSF were significantly higher in chronic active hepatitis patients than in healthy volunteers ($p<0.01$), asymptomatic HBV carriers ($p<0.01$), and chronic persistent hepatitis patients ($p<0.01$). Levels of M-CSF in cirrhosis and hepatocellular carcinoma patients were significantly higher than that of chronic persistent hepatitis patients, although the serum ALT levels were not significantly different (Table 1). The M-CSF level in acute hepatitis patients was 5.67±1.01 ng/ml, which was significantly ($p<0.01$) higher than that in healthy volunteers, asymptomatic HBV carriers, chronic persistent hepatitis, chronic active hepatitis, cirrhosis and hepatocellular carcinoma patients.

As shown in Fig. 2, we performed serial examination of serum M-CSF levels in acute hepatitis patients (three

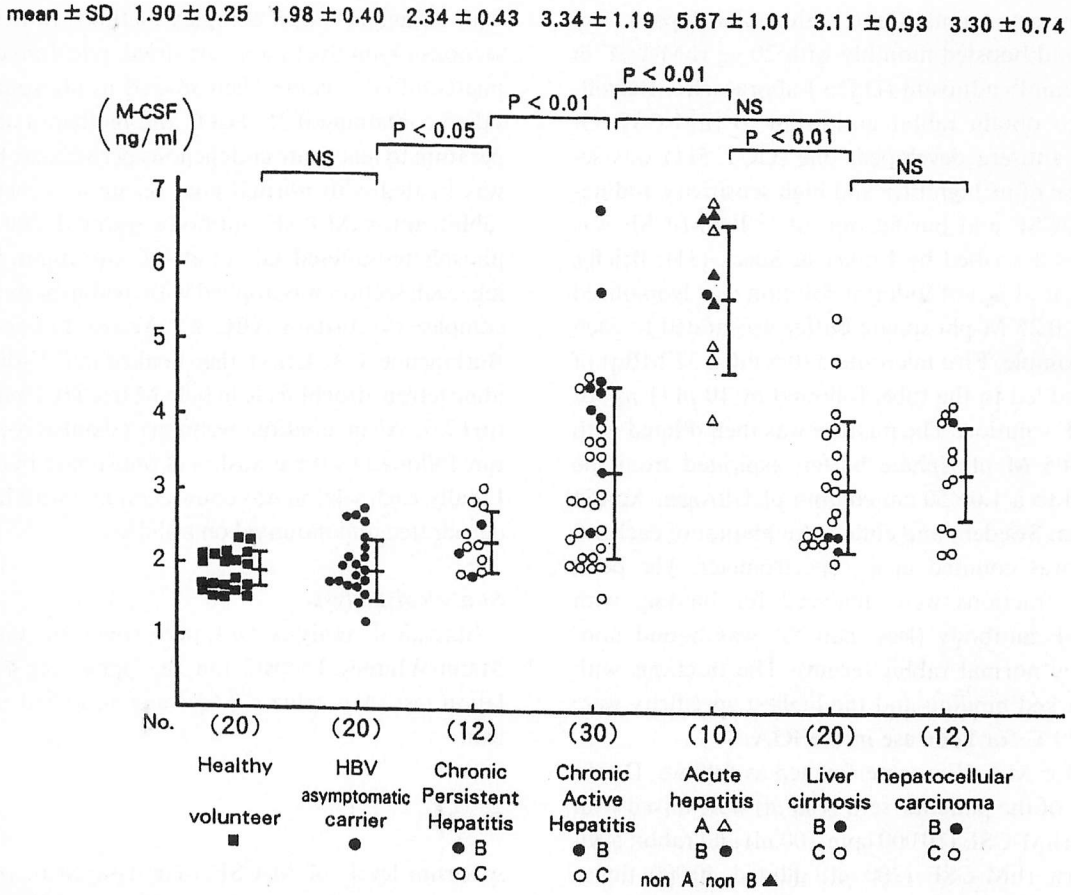

Fig. 1. Serum levels of M-CSF in liver disease. M-CSF was measured using a specific RIA. The mean value (ng/ml; mean±SD) for each group of patients was calculated and statistically analyzed using the Mann-Whitney U test. (■) healthy volunteers, (△) hepatitis A, (•) hepatitis B, (▲) hepatitis non-A non-B.

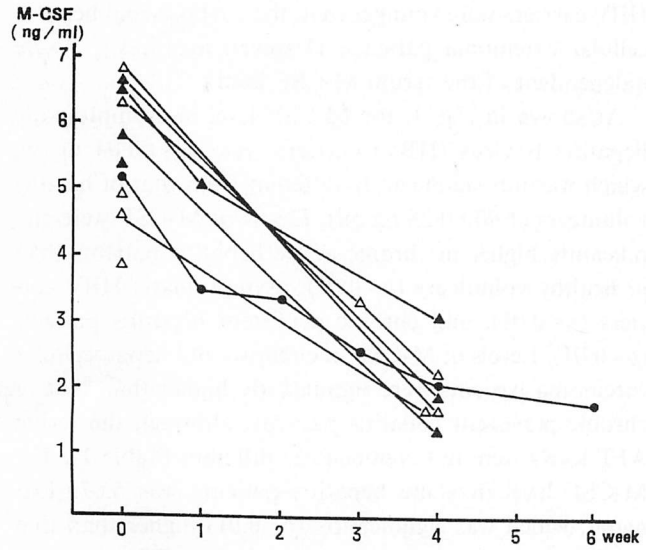

Fig. 2. Serial study of serum M-CSF levels in patients with acute hepatitis. The serum M-CSF levels were measured in patients with acute hepatitis (three hepatitis A, one hepatitis B, and three hepatitis non-A non-B) and levels of M-CSF in three patients (two hepatitis A, and one hepatitis non-A non-B) at the time of consultation. (△) hepatitis A, (•) hepatitis B, (▲) hepatitis non-A, non-B.

type A, one type B, and three type C). The mean M-CSF level in acute hepatitis patients in the acute phase was 5.67±1.01 ng/ml, which gradually declined in the convalescent phase. As shown in Fig. 3A, there was a highly significant positive correlation between the serum ALT and M-CSF levels in acute hepatitis patients (r_s=0.903, p<0.01, n=23). Similarly, in chronic active hepatitis patients (nine type B and 21 type C), a highly significant positive correlation was seen between the serum ALT and M-CSF levels (r_s=0.672, p<0.01) (Fig. 3B). In seven chronic active hepatitis patients who had M-CSF levels higher than 4 ng/ml, the serum ALT levels were above 400 IU/l. In these patients, the M-CSF levels were below 3 ng/ml in the stable phase of hepatitis, when the ALT levels were below 200 IU/l (data not shown).

Correlation between the serum M-CSF levels and laboratory tests was shown in Table 2. In patients without cirrhosis (acute hepatitis and chronic active hepatitis), there was a significant positive correlation between the serum M-CSF and ALT (p<0.01) or AST (p<0.01) levels. Because levels of M-CSF tended to cluster tightly in chronic persistent hepatitis patients, we could not find a

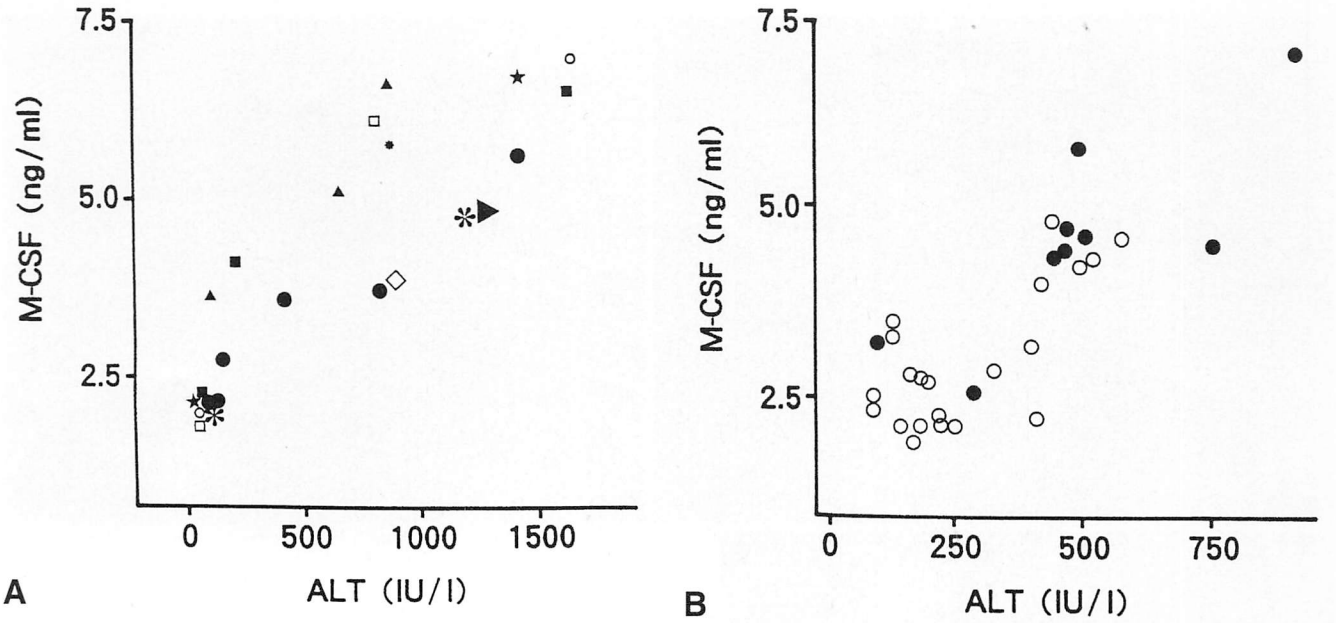

Fig. 3. Correlation between the serum M-CSF and ALT levels in patients with acute hepatitis and chronic active hepatitis. The relationship between the serum M-CSF and ALT levels was studied in 23 samples from 10 patients with acute hepatitis (**A**) and 30 samples from 30 patients with chronic active hepatitis (**B**). **A**. symbols show individual patient. (○, ■, ✻, ▶, ◇) hepatitis A, (•) hepatitis B, (★, ▲, □, ✹) hepatitis non-A non-B. **B**. (•) hepatitis B and (○) hepatitis C. The Spearman's correlation coefficient showed a highly significant correlation in acute hepatitis ($p<0.01$, $r_s=0.903$) and in chronic active hepatitis ($p<0.01$, $r_s=0.672$).

correlation between the serum M-CSF and ALT levels. In acute hepatitis, a positive correlation was seen between the serum M-CSF and total bilirubin levels ($p<0.05$). However, the serum M-CSF level correlated with neither the total bilirubin levels nor the indocyanine green clearance in chronic persistent hepatitis and chronic active hepatitis patients. In patients with cirrhosis, a significant positive correlation was seen between the M-CSF and ALT levels ($p<0.05$). Moreover, the M-CSF level correlated with both the total bilirubin levels ($p<0.05$) and the indocyanine green clearance ($p<0.05$) in cirrhosis and hepatocellular carcinoma patients.

To clarify the mechanism of the increase in serum levels of M-CSF in liver disease, we performed an immunohistochemical study of M-CSF positive cells in liver sections (Fig. 4). Because the mean level of serum M-CSF was prominently different between acute hepatitis patients (5.67 ± 1.01) and chronic persistent hepatitis patients (2.34 ± 0.43), we selected these two groups of patients. In all the specimens from acute hepatitis patients, M-CSF positive mononuclear cells were scattered mainly in the portal areas (Fig. 4A), whereas the number of M-CSF positive cells was small (Fig. 4B) or none (Fig. 4C) in the specimens from chronic persistent hepatitis patients. Kupffer cells, endothelial cells, neutrophils, fibroblasts, and liver parenchymal cells were negative in all the specimens.

TABLE 2

Correlations between laboratory parameters and M-CSF levels in patients with acute and chronic liver disease

Patients (no.)	ALT	AST	T-Bil	ICG (R15)
Non-cirrhosis				
Acute hepatitis (12)	0.903[a]	0.741[a]	0.602[b]	N.D.
Chronic active hepatitis (30)	0.672[a]	0.544[a]	0.328	0.340
Chronic persistent hepatitis (12)	0.571	0.521	0.363	0.298
Cirrhosis				
Liver cirrhosis (20)	0.472[b]	0.391	0.513[b]	0.542[b]
Hepatocellular carcinoma (12)	0.542	0.521	0.661[b]	0.598[b]

Spearman's correlation coefficients are shown. ALT=alanine aminotransferase; AST=aspartate aminotransferase; T-Bil=total bilirubin; ICG=indocyanine green, R15=retention ratio in 15 min.
N.D.=not determined.
[a]=$p<0.01$. [b]=$p<0.05$.

Discussion

The present study has shown that serum levels of M-CSF were significantly higher in patients with acute and chronic liver disease than in healthy volunteers or asymptomatic HBV carriers. The serum M-CSF levels, which were increased in acute hepatitis patients in the acute phase were decreased in the convalescent phase. In acute hepatitis patients and in chronic active hepatitis patients, M-CSF levels increased markedly with serum ALT levels. These findings suggest that serum levels of M-CSF reflect ongoing hepatic inflammation, irrespective of the etiology.

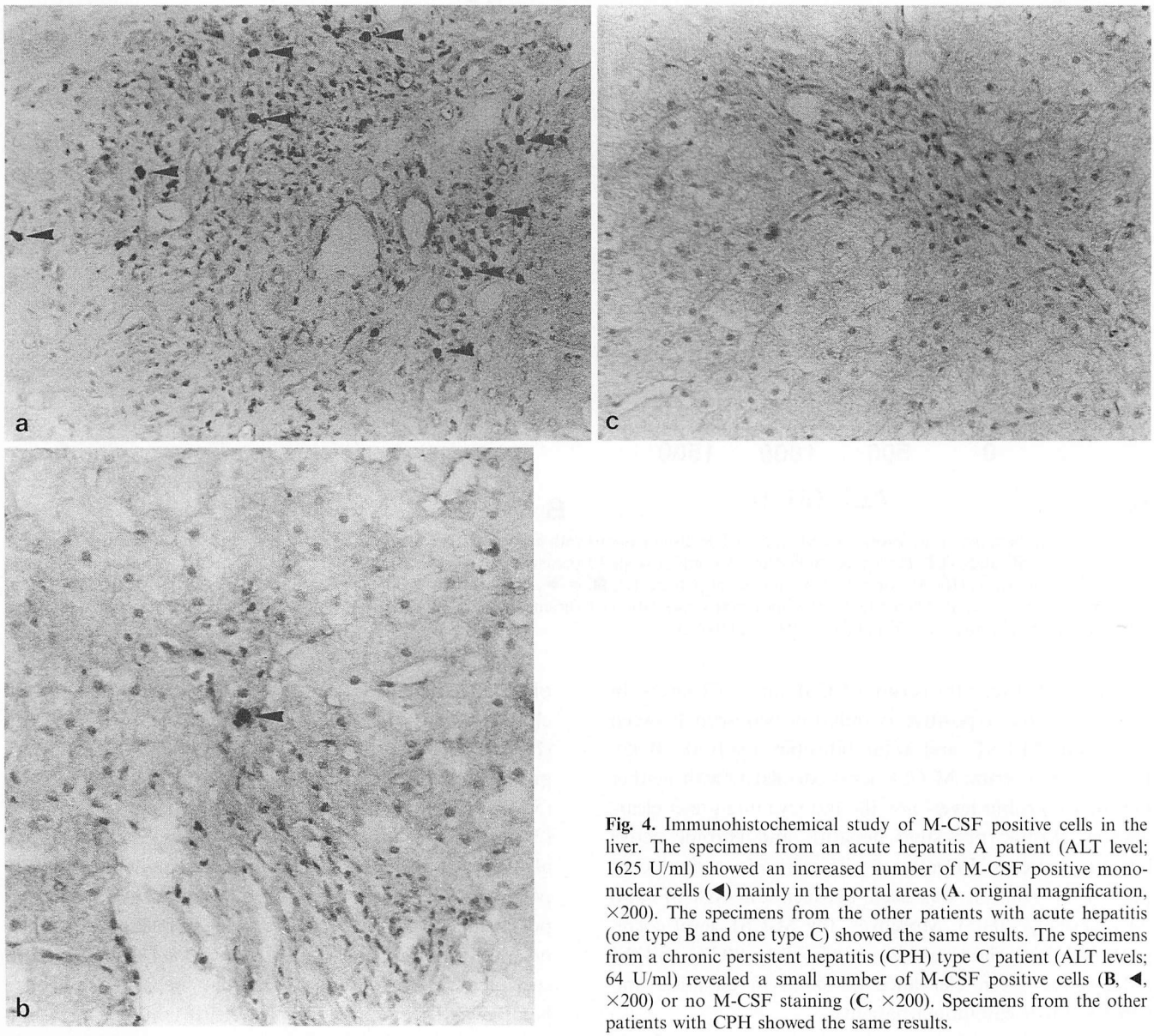

Fig. 4. Immunohistochemical study of M-CSF positive cells in the liver. The specimens from an acute hepatitis A patient (ALT level; 1625 U/ml) showed an increased number of M-CSF positive mononuclear cells (◀) mainly in the portal areas (**A**. original magnification, ×200). The specimens from the other patients with acute hepatitis (one type B and one type C) showed the same results. The specimens from a chronic persistent hepatitis (CPH) type C patient (ALT levels; 64 U/ml) revealed a small number of M-CSF positive cells (**B**, ◀, ×200) or no M-CSF staining (**C**, ×200). Specimens from the other patients with CPH showed the same results.

No significant difference was noted in the serum M-CSF levels in patients infected with different types of viruses when the patients were classified according to serum ALT levels (data not shown).

The present findings on serum M-CSF levels are similar to those obtained by Zöhrens et al. (32) concerning serum soluble intercellular adhesion molecule-1 (ICAM-1) levels, which reflects hepatocellular damage and cirrhosis. Zührens et al. (32) speculated that the elevated ICAM-1 in hepatic inflammation was derived from hepatocytes by shedding or by necrosis. They also proposed that impaired clearance from the circulation in patients with cirrhosis elevated the serum ICAM-1 levels.

Tomiya et al. (33) suggested that serum levels of hepatocyte growth factor also reflect hepatocellular necrosis. They speculated that increased serum levels of hepatocyte growth factor are related to the activation of hepatic macrophages because activated Kupffer cells are the main source of hepatocyte growth factor in the damaged liver (34). Our immunohistochemical study of M-CSF positive cells in liver specimens revealed an increased number of M-CSF-positive mononuclear cells in acute hepatitis. However, in chronic persistent hepatitis patients, there were no M-CSF positive cells or only a small number. Considering the finding that the serum level of M-CSF increases with the degree of hepatic inflammation, the source of increased serum levels of M-CSF in hepatic inflammation may, in part, be due to its production by infiltrating mononuclear cells in the liver.

Pinzani et al. (35) reported that fat-storing cells from

mouse liver are activated to secrete M-CSF in response to inflammatory products of macrophages, such as platelet-derived growth factor (36), suggesting a paracrine cytokine network involved in the monocyte- or macrophage-mediated immune reaction in the hepatic sinusoid. However, in our immunohistochemical study, only the infiltrating mononuclear cells were positive for M-CSF, and fat-storing cells were negative. The fat-storing cells may have produced very little or no M-CSF in human liver disease. Another possibility is that fat-storing cells produce M-CSF which is antigenetically different from the M-CSF produced by mononuclear cells.

Recently, immunohistochemical studies have shown an increase in the number of intrahepatic interferon-γ (IFNγ)-positive mononuclear cells (37) and TNFα-positive mononuclear cells (2) in chronic active hepatitis patients in comparison with chronic persistent hepatitis patients. These studies demonstrate that the IFNγ or TNFα positive cells in the liver were activated lymphocytes or monocytes. Because the production of M-CSF by monocytes is enhanced by IFNγ or TNFα alone (38,39) or in combination with GM-CSF (40), which are secreted by activated T cells (5), the increased production of IFNγ and TNFα in the infiltrating mononuclear cells in the liver may upregulate M-CSF production by infiltrating mononuclear cells in a paracrine or an autocrine manner. Although very little is known about the organs and cell types responsible for *in vivo* M-CSF synthesis (41), infiltrating mononuclear cells in the liver may be one of the most likely candidates for the source of increased M-CSF production in patients with acute or chronic liver disease *in vivo*.

We also examined the effects of sera from the patients on M-CSF secretion by peripheral blood monocytes from a healthy control. The sera from acute hepatitis patients slightly increased the production of M-CSF by peripheral blood monocytes in comparison with the sera from other groups of patients and controls although no statistical significance was noted (date not shown). Because the production of M-CSF is regulated by the interaction of several cytokines, such as M-CSF itself, GM-CSF, TNFα, and IFNγ in a complex way (40), the secretion of M-CSF by peripheral blood monocytes *in vitro* might be influenced by the interaction of components in sera, including M-CSF.

In patients with cirrhosis and hepatocellular carcinoma, the M-CSF levels were significantly higher than that in chronic persistent hepatitis patients, although the ALT levels in patients with cirrhosis were not. In patients with cirrhosis, the serum M-CSF levels correlated positively not only with the ALT levels, but also with the total bilirubin levels and the indocyanine green clearance, which represent the severity of cirrhosis (42). In hepatocellular carcinoma patients, there was also a significant correlation between the serum M-CSF levels and the total bilirubin levels or the indocyanine green clearance. Considering previous studies which revealed that macrophages, especially the Kupffer cells, metabolize most of the M-CSF in the circulation and regulate the serum concentration of M-CSF *in vivo* (43), and that receptor-mediated endocytosis of mannose or immunoglobulin-G by Kupffer cells is impaired in cirrhosis (44), the ability of Kupffer cells to metabolize M-CSF might be also impaired in cirrhosis. Another possibility is that the clearance of M-CSF from the circulation might be impaired due to the internal and external shunts in cirrhosis. No significant correlation was noted between the serum M-CSF and ALT levels in hepatocellular carcinoma patients in this study. Because the severity of cirrhosis as assessed by serum bilirubin levels and indocyanine green clearance in hepatocellular carcinoma patients was more severe than in patients with cirrhosis (Table 1), correlation between these two parameters may be impaired. These explanations may account for the discrepancy between the observed rise in M-CSF levels and lower ALT levels in cirrhosis and hepatocellular carcinoma patients.

Although M-CSF is a growth factor for monocyte-macrophages, no significant increase in monocyte count was observed in patients with acute hepatitis or chronic active hepatitis who had high serum M-CSF levels (data not shown). Similarly, treatment with M-CSF *in vivo* (45) or augmentation of the serum M-CSF level by administering IL-2 *in vivo* (46) did not significantly increase the monocyte count in peripheral blood. The pathophysiology of elevated serum M-CSF levels in acute and chronic liver disease remains to be clarified. The fact that a substantial amount of M-CSF is constantly present in the peripheral blood (24–27) raises the question of its pathophysiological significance. However, our finding that serum M-CSF level reflects the activity of hepatitis suggests that the macrophage-monocyte lineage plays a crucial role in the pathogenesis of acute and chronic liver disease.

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