Reprinted from

International Hepatology Communications

An International Journal for the Rapid Dissemination of Original Short Communications in Hepatology

Volume 1
(1993)

ELSEVIER
AMSTERDAM – LONDON – NEW YORK – TOKYO

International Hepatology Communications, 1993; 1: 1–4

HEPCOM 00011

Macrophage colony stimulating factor and hepatitis

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(Received 25 September 1992; accepted 30 November 1992)

We investigated the serum level of macrophage colony stimulating factor (M-CSF) in patients with hepatitis using a newly developed radioimmunoassay (RIA). The mean level of M-CSF in patients with chronic active hepatitis was significantly higher than that of the healthy controls ($P < 0.01$). The mean level of M-CSF in patients with acute hepatitis in the acute phase was prominently higher than that of the patients with chronic active hepatitis ($P < 0.001$). However, in the convalescence phase of acute hepatitis, the mean level of M-CSF decreased to the lower level comparable to the mean level of the patients with chronic active hepatitis. These data suggest that the serum level of M-CSF is one of the factors which represents the inflammatory activity of liver diseases.

Key words: M-CSF; RIA; Hepatitis

M-CSF is a cytokine which promotes the growth or differentiation of cells of macrophage monocyte lineage [1]. In addition to its hematopoietic effects, M-CSF is reported to enhance several functions of murine monocytes such as production of interferon and induction of chemotaxis [2,3]. M-CSF was isolated from urine [4] or culture media of cell lines [5] and the cDNA clones [6,7] have been isolated. Recently, it has become possible to measure the serum level of M-CSF by the enzyme-linked immunoassay or RIA [8,9]. In this paper, we have measured the serum level of M-CSF in patients with acute hepatitis and chronic active hepatitis by RIA and investigated its correlation with the clinical course.

Patients and Methods

We investigated the serum level of M-CSF in 133 healthy volunteers (117 men and 16 women), five patients with acute hepatitis, and 20 patients with chronic active hepatitis (Table 1). Clinical diagnosis of all the patients was determined by biochemical, serological,

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TABLE 1
Clinical features and serum M-CSF levels of the patients

	No.	Virus marker (A/B/C)	ALT (IU/l)	M-CSF (ng/ml)
Control	133	0/0/0	14 ± 8	1.95 ± 0.44
Acute hepatitis				
in the acute phase	5	3/2/0	1128 ± 458	5.16 ± 1.24
in the convalescence phase	5	3/2/0	62 ± 16	2.23 ± 0.25*,#
Chronic active hepatitis	20	0/6/14	153 ± 38	2.38 ± 0.46*,#

Mean values ± SD are presented for each group of patients.
Data were analyzed by the two sample *t*-test.
*$P < 0.01$ vs. control.
#$P < 0.001$ vs. acute hepatitis in the acute phase.

and histological data. HBsAg and anti-hepatitis A virus antibody (IgM) were examined by the commercially available radioimmunoassay kits (Abbott Lab., USA). Each healthy control was verified within the normal level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltranspeptidase. All the patients with hepatitis had no other significant diseases. The patients with acute hepatitis in the acute phase were defined as those who had ALT levels higher than 500 IU/l to distinguish them from the histologically determined chronic active hepatitis patients showing less than 200 IU/l of ALT and the acute hepatitis patients in the convalescence phase showing less than 100 IU/l of ALT. Blood was collected from each patient, centrifuged, and the sera were stored at −20°C until the RIA was performed. Informed consent was obtained from each patient and healthy control, and this study was approved by the ethical committee of the hospital.

The human recombinant M-CSF (rhM-CSF) was manufactured as follows. DNA encoding M-CSF was introduced into a Chinese hamster ovary expression system and the secreted recombinant human M-CSF (rhM-CSF) was purified to homogeneity [6]. The New Zealand white rabbit was used to obtain rabbit anti-rhM-CSF. Iodination of rhM-CSF and purification of [^{125}I]rhM-CSF were performed using the method described by Fraker and Speck [10]. The RIA of M-CSF was performed according to the previously reported method [11]. Briefly, duplicate samples (100 μl) and rhM-CSF (standard) were mixed with a ^{125}I-labeled rhM-CSF (10 000 cpm/100 μl) and rabbit anti-serum against rhM-CSF (200 μl) suspension. After incubation for 48 h at 37°C, the binding product was separated from the free ^{125}I-labeled rhM-CSF by the addition of anti-rabbit IgG and 6% poly(ethylene glycol) (molecular weight 8000). The tubes were centrifuged and the supernatants were aspirated. The precipitates were counted for 1 min in a gamma spectrometer. The sensitivity limit was 0.1 ng/ml in this assay. The data were analyzed by a two sample *t*-test.

Results

The mean serum M-CSF level in the controls was 1.91 ± 0.48 ng/ml (mean ± SD). No correlation between the sex or age and the serum M-CSF level was noted (data not shown).

The mean M-CSF level in sera of patients with acute hepatitis in the acute phase was 5.16 ± 1.24 ng/ml, which was significantly higher than the data in the convalescence phase (2.23 ± 0.25 ng/ml, $P < 0.001$) and the data of patients with chronic active hepatitis (2.38 ± 0.46 ng/ml, $P < 0.001$). The mean M-CSF level in sera of patients with chronic active hepatitis was 2.38 ± 0.46 ng/ml, which was significantly ($P < 0.01$) higher than the data of the control patients.

Discussion

Previously, the serum level of M-CSF was investigated during the infection of Listeria monocytogenes in mice [12]. However, little has been reported about the activity of any diseases in humans. In this paper, we demonstrated, for the first time, that the serum M-CSF level was elevated in patients with viral hepatitis. The mean M-CSF level in patients with chronic active hepatitis (including type B and type C) was significantly higher than the mean level for the healthy controls. The mean M-CSF level in patients with acute hepatitis (including type A and type B) in the acute phase was prominently higher than the mean level of the other groups.

These data suggest that the system to activate the proliferation of cells of macrophage monocyte lineage is functioning in vivo in acute or chronic hepatitis. Further study of the serum M-CSF level is needed in patients with various kinds of liver diseases. Also, we have to investigate the mechanism of the increased level of M-CSF in hepatitis. We are now investigating to determine whether the secretion of M-CSF by the peripheral blood mononuclear cells is enhanced in vitro or whether or not the production within the liver is increased in patients with acute hepatitis.

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