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〔Original Paper〕

Serum levels of macrophage colony stimulating factor in patients with unstable angina pectoris

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SUMMARY

The purpose of this study was to examine serum levels of macrophage colony stimulating factor (M-CSF) in patients with unstable angina pectoris and in those with stable effort angina pectoris of different pathophysiologies.

Methods: Serum samples were obtained from 20 patients with unstable angina pectoris (UAP), 20 patients with stable effort angina pectoris (EAP), and 15 healthy volunteers (NOR). Serum levels of M-CSF were measured by enzyme-linked immunosorbent assays.

Results: Serum levels of M-CSF in the UAP group, the EAP group and the NOR group were 695±240 pg/ml, 322±69 pg/ml and 229±44 pg/ml, respectively. Levels of M-CSF were significantly higher in the UAP group than in the EAP group ($P<0.0001$). In the UAP group, levels of M-CSF were substantially higher in IIIB group (801±268 pg/ml) than IIB or IB group (566±114 pg/ml) ($P<0.05$), according to Braunwald classification. M-CSF levels showed a negative relationship with clinical improvement in the UAP group.

Conclusion: Increased level of M-CSF is suggested to a pathophysiological role of unstable angina pectoris.

Key words: macrophage colony stimulating factor, unstable angina pectoris, acute coronary syndrome, enzyme-linked immunosorbent assay,

I. Introduction

Macrophage colony stimulating factor (M-CSF) was first characterized as a glycoprotein that induces the formation of monocyte and macrophage colonies from precursors in murine bone marrow culture[1]. M-CSF may potentiate a number of inducible monocyte functions including phagocytosis, microbial killing, cytotoxicity for tumor cells, as well as enhance

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the synthesis of inflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor and interferon[2]. Unlike the other primary myeloid colony stimulation factors (IL-3, granulocyte-macrophage colony stimulating factor and granulocyte colony stimulating factor), M-CSF is continuously present at biologically active concentrations in the serum of normal animals and is required for the survival of circulating monocytes and tissue macrophages[3].

In coronary arteries, vascular endothelial cells and smooth muscle cells reportedly produce M-CSF, which induces the proliferation and differentiation of macrophages[3]. Immune cells infiltrating coronary lesions, including monocytes, macrophages, T lymphocytes, and mast cells have been suspected to contribute to plaque instability[4-9]. Coronary plaque disruption with consequent thrombus formation and acute inflammatory reaction are considered to be major pathogenic mechanisms responsible for acute coronary syndrome[10, 11]. Therefore, patients with acute coronary syndrome may have increased serum levels of M-CSF according to the severity of disease. In this study, we measured serum M-CSF levels in patients with unstable angina pectoris and in those with stable effort angina pectoris of different pathophysiologies.

II. Materials and Methods

Patient Selection

We studied 20 patients with primary unstable angina pectoris (UAP) (type IB, type IIB or type IIIB according to the Braunwald classification[12]), 20 patients with stable effort angina pectoris (EAP), and 15 apparently healthy volunteers (NOR) of equivalent age and life style and without any limitation in physical activity. In unstable angina group, 11 patients had one episode or more at rest within the 48 hours preceding admission (group A, type IIIB), and 9 patients had no episodes at rest within the 48 hours preceding admission (group B, type IB or type IIB). Unstable angina pectoris was defined by typical chest pain occurring at rest or with minimal effort without any increase in the serum creatine kinase MB fraction and with angiographic evidence of coronary artery disease. In the UAP group, serum samples were obtained at the time of admission and on the 7th day after admission. In the EAP group, serum samples were obtained at the time of admission.

Coronary Angiography

Coronary angiography was performed by the Judkins technique. The occurrence and severity of coronary angiographic lesions were evaluated from at least three projections by the score of the American Heart Association.

Measurement of M-CSF Levels

Serum levels of M-CSF were measured using a human M-CSF kit, Parameter, from R&D Systems (Abingdon, United Kingdom). All samples were diluted 1 : 5 with calibrator diluent. Then 100 μl of assay diluent was added to 96 well polystyrene microtiter plates coated with a murine monoclonal antibody against M-CSF, and 100 μl of M-CSF standard or diluted samples were added to each well. After incubation for 90 minutes at room temperature, aspiration and washing, 200 μl of M-CSF conjugate (polyclonal antibody against M-CSF conjugated to horseradish peroxidase) were added to each well. Then the wells were incubated for 90 minutes at room temperature. After another aspiration and washing, 200 μl of substrate solution were added to each well. The wells were incubated for 30 minutes at room temperature, and 50 μl of stop solution were added to each well. The amount of conjugate bound

to the well was detected by reaction with the substrate tetramethylbenzidine. The mean absorbance values in each set of duplicate standard or diluted sample was calculated. Each assay was calibrated by a standard curve of M-CSF standard concentration. The minimum detectable dose of M-CSF was found to be 20pg/ml.

Statistical Analysis

Data are expressed as mean ± standard deviation. One-way analysis of variance and the multiple comparison method of Scheffe were used to test for statistically significant differences among the three groups. Paired or unpaired *t* tests were used to compare the two groups. A p-value<0.05 was considered statistically significant.

Ⅲ. Results

Clinical Characteristics

All patients with unstable angina and stable effort angina had anginal attacks before admission and underwent a treatment with nitrates, calcium antagonists, aspirin and heparin. All patients showed angiographic evidence of coronary artery disease without significant difference in severity or in extent of angiographic coronary lesions between the groups of patients. During the observation, no patient died or suffered from myocardial infarction. In the UAP group, 12 patients underwent percutaneous transluminal coronary angioplasty (PTCA) and 2 patients underwent coronary artery bypass grafting (CABG). No patient had these revascularization procedures within 7 days after admission. In the EAP group, 13 patients underwent PTCA and 2 patients underwent CABG. The characteristics of patients and control subjects are shown in Table 1. There were no significant differences in age and sex among the three groups.

Table 1. The characteristics of patients with unstable angina pectoris (UAP), stable effort angina pectoris (EAP), and healthy volunteers (NOR).

	UAP	EAP	NOR
Numbers	20	20	15
Age (mean±SD)	66.1±10.5	65.4±9.8	60.5±8.4
Male : Female	12 : 8	13 : 7	10 : 5

Circulating M-CSF Levels

Serum levels of M-CSF in the UAP group, the EAP group and the NOR group were 695 ±240 pg/ml, 322±69 pg/ml and 229±44 pg/ml, respectively. Levels of M-CSF were significantly higher in the UAP group than in the EAP group or the NOR group ($P<0.0001$). No significant difference in the level of M-CSF was found between the EAP group and the NOR group (Fig.1).

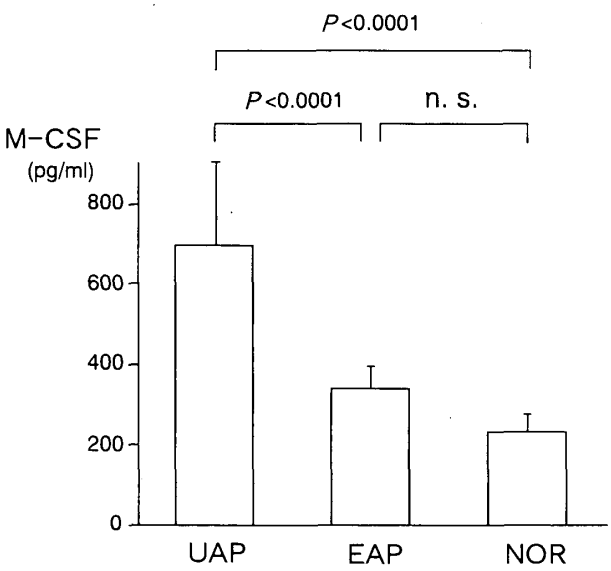

Fig 1. Levels of macrophage colony stimulating factor (M-CSF) in patients with unstable angina pectoris (UAP) or stable effort angina pectoris (EAP), and healthy volunteers (NOR). Levels of M-CSF were higher in the UAP group than in the EAP group or the NOR group ($P<0.0001$). No significant differences in levels of M-CSF were found between the EAP group and the NOR group.

M-CSF Levels and the Braunwald Classification

In the UAP group, serum levels of M-CSF at 7th day after admission were 469 ± 126

pg/ml. Levels of M-CSF were significantly higher at admission (695±240 pg/ml) than at the 7th day ($P<0.0005$) (Fig.2). In group A, serum levels of M-CSF at admission and the 7th day were 801±268 pg/ml and 506±141 pg/ml, respectively. Levels of M-CSF in the group A were significantly higher at admission than the 7th day ($P<0.005$). In group B, serum levels of M-CSF at admission and the 7th day were 566±114 pg/ml and 424±93 pg/ml, respectively. Levels of M-CSF in the group B were significantly higher at admission than the 7th day ($P<0.05$) (Fig.3). Levels of M-CSF at admission in the two UAP groups were significantly higher in the IIIB group than the IB or the IIB group according to Braunwald classification. There was no significant difference in levels of M-CSF between the two groups on the 7th day after admission (Fig.4). M-CSF levels decreased with clinical improvement in UAP group.

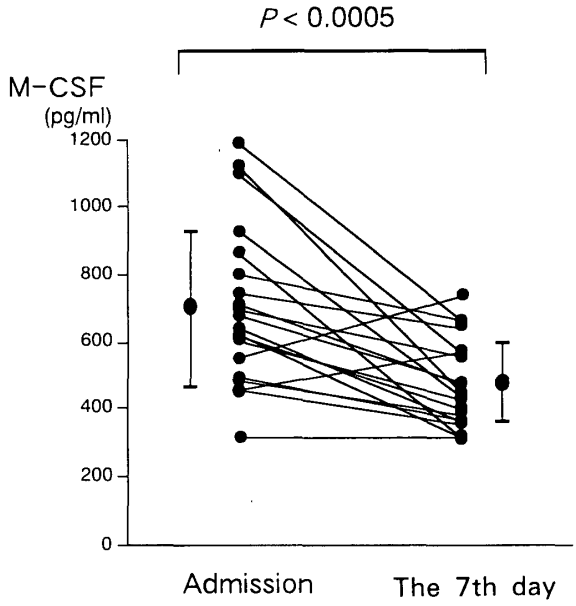

Fig 2. Levels of macrophage colony stimulating factor (M-CSF) in the unstable angina group. Levels of M-CSF were significantly higher at admission than on the 7th day ($P<0.0005$).

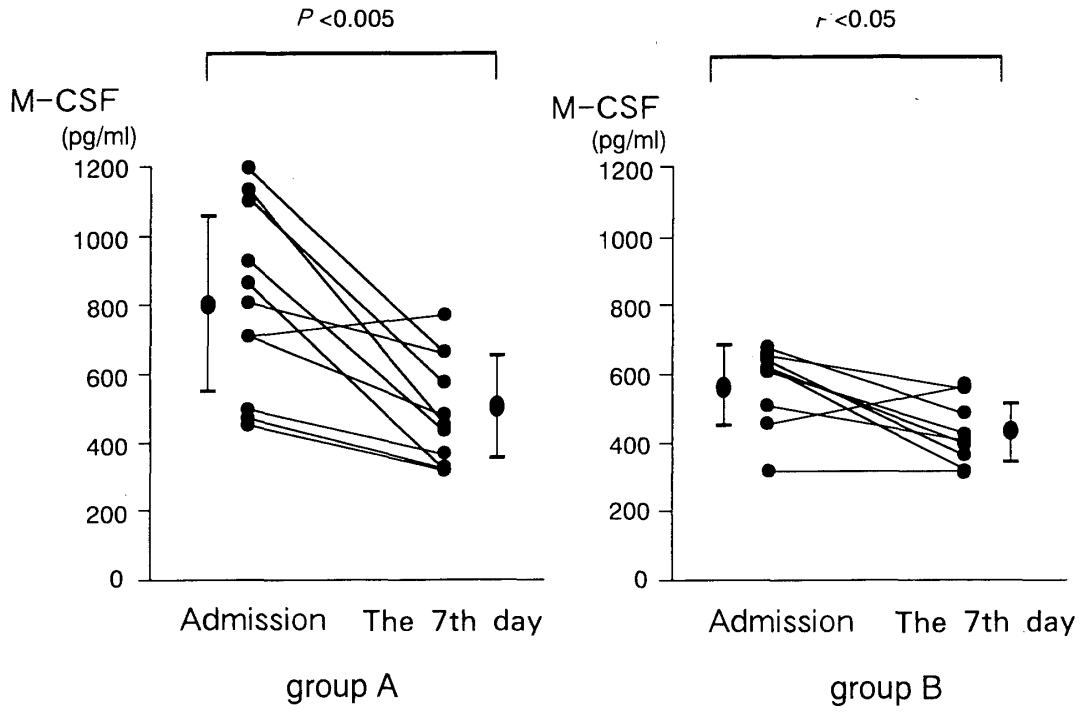

Fig 3. Levels of macrophage colony stimulating factor (M-CSF) in group A (type IIIB) and group B (type IB and type IIB). Levels of M-CSF in group A and B were significantly higher at admission than the 7th day.

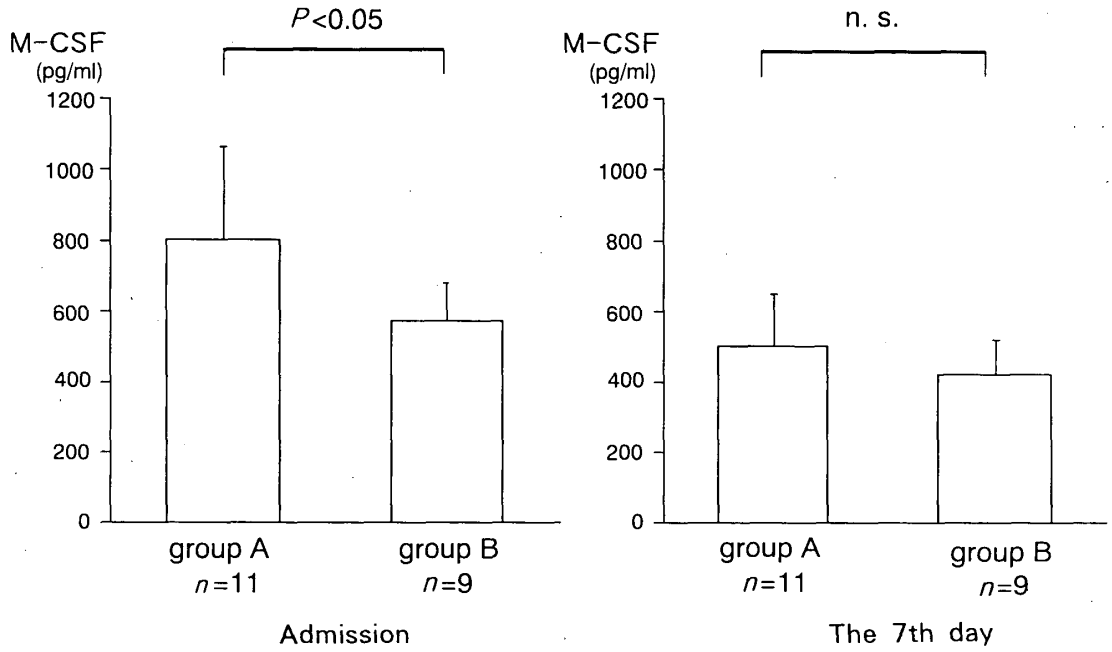

Fig 4. Different levels of macrophage colony stimulating factor (M-CSF) between group A (type IIIB) and group B (type IB and type IIB). At admission, levels of M-CSF in the unstable angina group were significantly higher in the IIIB group than the IB or the IIB group according to Braunwald classification. There was no significant difference in levels of M-CSF between the two groups at 7 days after admission.

Ⅳ. Discussion

Coronary plaque disruption with consequent platelet aggregation and thrombus formation, often with the contribution of dynamic change of vascular tone (as suggested by experimental observations on cyclic flow variation[13]), is considered to be one of the most important mechanisms responsible for occurrence of acute coronary syndromes, including unstable angina and myocardial infarction[5,14]). Recent studies provided evidence of a role of inflammation in plaque disruption in addition to biomechanical factors, such as shear stress and vasospasm[5,15-17]. CD4 + and CD8 + circulating lymphocytes are activated in patients with UAP[6], and the immediate site of plaque rupture or erosion is always marked by inflammatory processes (accumulation of macrophages and T cells)[16]. This is consistent with the hypothesis that inflammation plays an important role in the pathophysiology of unstable angina.

M-CSF enhances proliferation and differentiation of monocyte progenitors and is required for survival and activation of mature monocytes and macrophages. Expression of the M-CSF gene was recognized in cultured human vascular endothelial cells and smooth muscle cells as well as in atheromatous lesions from rabbit and human[3]. In this study, there were increased levels of M-CSF in patients with unstable angina pectoris compared to patients with stable effort angina and healthy volunteers. Therefore, M-CSF production may be increased in patients with unstable angina, leading to macrophage infiltration at the site of plaque rupture.

Levels of M-CSF in unstable angina pectoris were significantly higher in the IIIB group than the IB or the IIB group according to Braunwald classification. Among the three

classes of unstable angina pectoris, the IIIB group is considered to be the most severe. After antianginal treatment, M-CSF levels decreased with clinical improvement in patients with type IB, IIB and IIIB unstable angina. It is proposed that levels of M-CSF in unstable angina pectoris are going down according to stabilization of the unstable plaque. Taken together, these data suggest that the levels of M-CSF are associated with the disease severity of unstable angina.

In conclusion, M-CSF may play a role in the pathophysiology of unstable angina. Detection of increased levels of serum M-CSF may have diagnostic utility in patients with unstable angina pectoris.

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要　旨

不安定狭心症の病態発現には粥腫の破綻および血栓形成が関与する。粥腫の破綻に続き血管壁に進入した血液はマクロファージに貪食される。一方マクロファージの遊走，活性化に関与するM-CSFはリンパ球，単球だけでなく，血管内皮細胞や平滑筋細胞からも産生される。本研究では不安定狭心症患者のM-CSF値を検討した。

対象はUAP群：不安定狭心症患者20例，EAP群：安定労作性狭心症患者20例，NOR群：健康成人15例で末梢静脈より採血を行ないELISA法を用いてM-CSF値を検討した。なおUAP群では入院時と7日後も測定し，さらにBraunwald分類によりIIIB群とIIBおよびIB群に分けて検討した。

M-CSF値はUAP群695±240pg/ml，EAP群322±69pg/ml，NOR群229±44pg/mlであり，UAP群のM-CSF値はEAP群およびNOR群に比し有意に高かった（$P<0.0001$）。UAP群の入院時M-CSF値は7日後（469±126 pg/ml）に比し有意に高かった（$P<0.0005$）。さらに入院時UAP群のうち，Braunwald分類IIIB群（801±268pg/ml）のM-CSF値はIIBおよびIB群（566±114pg/ml）に比し有意に高かった（$P<0.05$）。

狭心症の不安定期にはM-CSF値は高値を示すと考えられる。このことと，狭心症の不安定期に起こる血管壁へのマクロファージの遊走との間に関連があると推測された。なお，血清M-CSF値は狭心症の病勢を示す指標となりえる可能性が示唆された。

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