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Clinical Significance of Increased Plasma Concentration of Macrophage Colony–Stimulating Factor in Patients With Angina Pectoris

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OBJECTIVES To determine the effect of macrophage colony–stimulating factor (MCSF) on atherogenesis in patients with coronary artery disease (CAD), we assessed the relation between the plasma concentration of MCSF and the incidence of acute coronary events in patients with CAD.

BACKGROUND Cytokines such as MCSF play a central role in inflammatory and proliferative responses in patients with acute coronary syndromes. However, the effect of MCSF on the clinical course in patients with CAD is still not known.

METHODS We measured the plasma MCSF concentration in 142 patients with documented CAD (62 ± 9 years) and followed up for a mean period of 14 ± 6 months. The study included 97 patients with stable angina (SA), 45 patients with unstable angina (UA) and 22 age-matched control subjects. The predictors of coronary events were analyzed by using a Cox proportional hazards model.

RESULTS The mean plasma MCSF concentration in patients with UA was significantly higher than that in patients with SA and in control subjects (981 ± 277 vs. 693 ± 223 vs. 680 ± 158 pg/ml, $p < 0.001$). The mean plasma MCSF concentration in the 20 patients with coronary events was significantly higher than that in patients without coronary events (1,192 ± 232 vs. 690 ± 213 pg/ml, $p < 0.001$). The predictors of unfavorable outcome were an increased MCSF concentration, the presence of CAD and a low ejection fraction.

CONCLUSIONS These findings suggest that an increased circulating MCSF concentration reflects atherosclerotic progression in patients with CAD and predicts future cardiac events. (J Am Coll Cardiol 2000;35:655–65) © 2000 by the American College of Cardiology

The primary function of macrophage colony–stimulating factor (MCSF) is the regulation of the growth, differentiation and maturation of monocytes and macrophages (1–3). The expression of MCSF messenger ribonucleic acid and the presence of MCSF protein have been demonstrated in atherosclerotic lesions in humans (4). Further, the foam cells from atheroma are derived primarily from macrophages (5). The administration of MCSF in vivo has been shown to lower plasma cholesterol concentrations rapidly in various animal models (1,6). Based on these findings, increases in circulating MCSF concentration may be due to increases in the cholesterol concentration, resulting in the activation of monocytes in the arterial wall (2,6,7). Therefore, persistently elevated circulating concentrations of MCSF may reflect active systemic atherogenesis. Furthermore, several investigators have demonstrated that a high macrophage content is present in samples of coronary atherosclerotic plaque tissue from patients with acute coronary syndromes or from patients undergoing percutaneous transluminal coronary angioplasty (PTCA) (8–11).

Macrophages phagocytose lipids and are transformed into foam cells. Then the macrophages release the lipids into the necrotic center of the atherosclerotic plaque. Finally, after the phagocytosis of the lipids, the activated macrophages move from the lipid core to the fibrous cap, weakening the barrier of the fibrous cap (12). The mechanism responsible for macrophage involvement in the genesis of cardiac events may include thrombus organization, smooth muscle cell migration and proliferation and constrictive scarring of the adventitia. However, the mechanism has not been clearly established. In addition, it is unclear whether the circulating

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Abbreviations and Acronyms

CABG = coronary artery bypass graft surgery
CAD = coronary artery disease
HDL = high density lipoprotein
LDL = low density lipoprotein
LVEF = left ventricular ejection fraction
MCSF = macrophage colony–stimulating factor
PTCA = percutaneous transluminal coronary angioplasty
SA = stable angina
UA = unstable angina
VLDL = very low density lipoprotein

MCSF concentration can predict the clinical course or prognosis of patients with coronary artery disease (CAD). In the present study, we investigated the relation between the incidence of acute coronary events and the plasma concentration of circulating MCSF in patients with angina pectoris.

METHODS

Study group. The study was approved by the Nippon Medical School Human Ethics Committee, and written, informed consent was obtained from all of the patients. One hundred seventy-eight consecutive patients (137 men and 41 women, mean age 61 ± 10 years) with documented CAD by coronary arteriography were enrolled. Thirty-one patients were excluded from the study because of the presence of the following diseases influencing the MCSF concentration: 1) acute myocardial infarction (13); 2) arrhythmia (supraventricular or ventricular) or atrial fibrillation/flutter; 3) renal dysfunction with a creatinine concentration ≥133 μmol/liter or evidence of gout; 4) neoplastic disease within the previous five years; 5) infectious diseases or autoimmune diseases; 6) pregnancy; or 7) extracardiac atherosclerotic macroangiopathy. Furthermore, patients who had undergone coronary artery bypass graft surgery (CABG) were excluded from the study because the operation could trigger an increase in the plasma MCSF concentration.

All patients were characterized according to age, gender, history of myocardial infarction, previous revascularization with CABG or PTCA, standard risk factor assessment and history of angina. Patients with angina were treated with medical therapy. Unless the angina resolved with medical treatment, revascularization with PTCA or CABG was performed. Patients with effort-induced angina were defined by the following criteria: 1) significant coronary stenoses by coronary arteriography (>50% diameter stenosis); and 2) significant ST segment depression during 24-h Holter monitoring or treadmill exercise testing with associated anginal chest pain. Patients with vasospastic angina were characterized by spontaneous episodes of chest pain at rest with accompanying ischemic ST segment elevations or angiographic evidence of >50% vasoconstriction induced by acetylcholine infusion in one or more segments of the epicardial arteries with associated ischemic ST segment elevations.

The 22 control subjects included 10 healthy volunteers and 12 patients with atypical chest pain but without abnormal global cardiac function, evidence of significant coronary stenosis, diabetes mellitus, hypertension or gout. None of the control subjects received any drugs.

Study design and assays. After an overnight fast, blood and urine were collected for the analysis of serum lipids, renal function and plasma glucose. The MCSF concentration and blood pressure were measured early in the morning on the day after admission for patients with stable angina (SA). In contrast, blood sampling in patients with unstable angina (UA) and patients undergoing PTCA was performed 7 to 10 days after hospital admission or PTCA. Because intravenous heparin and the other intravenous anticoagulant drugs used during the early treatment stage for acute coronary syndromes can influence the MCSF concentration (14), blood sampling in in-patients with UA was performed after the stabilization of anginal attacks. Low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, high density lipoprotein (HDL) cholesterol, lipoprotein (a) and plasma MCSF concentration were measured in patients with angina pectoris and in control subjects. Blood sampling in out-patients was performed after an overnight fast. The plasma MCSF concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R & D System Inc., Minneapolis, Minnesota). The sensitivity of the MCSF assay was 40 pg/ml. The interassay and intra-assay variations of the ELISA measurements were less than 5% to 10%. We assessed the following risk factors within two weeks of the MCSF measurement: serum uric acid concentration, plasma lipid concentration, body weight and smoking habit.

Cardiac catheterization. A standard protocol for right- and left-sided cardiac catheterization and coronary angiography was performed. Significant coronary artery stenosis was defined as ≥50% reduction in the luminal diameter in the main truncus and ≥75% at all other sites. The left ventricular ejection fraction (LVEF) was calculated during left ventriculography using the modified area–length method. Calcium antagonists and long-acting nitrates were discontinued in patients with vasospastic angina at least 48 h before cardiac catheterization. The vasomotor response to intracoronary acetylcholine chloride infusion (Daiichi Pharmaceutical, Tokyo, Japan) was determined. Coronary arteriograms were performed when acetylcholine-induced vasospasm (>50% reduction in luminal diameter in one or more segments of the epicardial coronary arteries) with ischemic electrocardiographic (ECG) changes (≥1 mm ST segment depression or elevation) occurred.

Follow-up. One hundred forty-seven patients with angina pectoris met the inclusion criteria and constitute the study group. However, five patients could not be contacted during follow-up. Therefore, 142 patients completed the follow-up (112 men and 30 women, mean age 62 ± 9 years [range 41 to 80]). The mean follow-up period was 14 ± 6 months.

Most patients returned for out-patient evaluation at one-month intervals. Clinical assessment, including evaluation of cardiac status, electrocardiography and assessment of drug compliance, was performed at each visit. The start of the follow-up period was the day of MCSF sampling. The clinical end points included the occurrence of acute coronary events: 1) cardiac death; 2) acute myocardial infarction; 3) hospital admission for UA; 4) emergent revascularization with PTCA or CABG because of new or worsening symptoms; and 5) the exclusion criteria described earlier. In the present study, the clinical end points only represented acute coronary events, not progression of cardiac disease. Therefore, the use of elective PTCA or CABG, which represented therapy for progression of disease, was not included as a clinical end point. Deaths were classified as cardiac or noncardiac. Myocardial infarctions were confirmed by standard ECG criteria and increases in the serum creatine kinase isoenzyme activity. Hospital admissions for UA were diagnosed on the basis of worsening exertional angina or new-onset angina with typical ischemic ECG changes but without increases in the serum creatine kinase activity.

The patients were monitored every six months by treadmill exercise testing. Those patients who underwent PTCA at the start of the follow-up period had a second coronary arteriogram performed at six to 12 month intervals after the initial PTCA. Those who had silent myocardial ischemia or anginal pain during treadmill exercise testing or who developed restenosis or a new stenotic region based on the second coronary arteriogram without worsening angina underwent elective PTCA or CABG. Reevaluation of the plasma MCSF concentration in patients with acute coronary events was performed immediately after re-admission to the hospital.

Statistical analysis. All data are expressed as the mean value ± SD. The unpaired Student *t* test was used to compare the two groups. The Levene test was used for homogeneity of variance testing, and one-way analysis of variance was used to assess any differences in multiple group data. Post hoc analysis was performed with Dunnett's test or the Games-Howell test. The chi-square test with Yates' correction or the Fisher exact probability test was applied for dichotomous and categoric data. Correlations between clinical variables were determined using the Spearman correlation coefficient. Estimation of the cumulative probability of event-free survival was performed with the Kaplan-Meier method, and differences between the curves were assessed with the Wilcoxon test. To determine the predictors of acute coronary events, the Cox proportional hazards model was used with the stepwise selection method. This model was constructed using the following variables: age, gender, body mass index, any smoking habit, history of diabetes mellitus, history of hypertension, history of hyperuricemia, history of previous myocardial infarction, nonuse of calcium antagonists, nonuse of beta-blockers, nonuse of oral nitrates, nonuse of antiplatelet agents, nonuse of aspirin, nonuse of lipid-lowering drugs, nonuse of angiotensin-coverting enzyme inhibitors, plasma LDL cholesterol concentration, VLDL cholesterol concentration, HDL cholesterol concentration, lipoprotein (a) concentration, plasma MCSF concentration, extent of CAD and left LVEF. The appropriateness of the hazard assumption was examined by preparing log (−log) plots of the survival function. Furthermore, multivariate-adjusted risk ratios (RR) and 95% confidence intervals (CI) were reported. This model influence of profile and interaction was examined by regression diagnosis. Two-tailed p values <0.05 were considered statistically significant. Statistical analyses were performed with a computer and the Statistical Package for the Social Sciences (SPSS, 1997) system 7.5.2J software.

RESULTS

Patient characteristics. Baseline characteristics of the study patients are summarized in Table 1. The mean age of the study group was 62 ± 9 years (range 36 to 80). The study included 97 patients with SA and 45 patients with UA; 49 of the patients had a previous myocardial infarction. Furthermore, 103 patients had exertional angina and 39 patients had vasospastic angina. The mean creatinine concentration in the patients with UA and SA was significantly higher than that in the control subjects (88 ± 18 vs. 88 ± 18 vs. 71 ± 9 μmol/liter, $p < 0.001$). Although there were no significant differences in the plasma concentrations of LDL cholesterol and lipoprotein (a) between the patients with UA and SA and the control subjects, the plasma concentration of HDL cholesterol in the patients with UA and SA was significantly lower than that in the control subjects ($p < 0.001$). Of the control subjects, four had a mild degree of hyperlipidemia and four had hyperuricemia. About one-half of the study patients had hypertension or diabetes mellitus.

With respect to therapy, 61 patients with angina pectoris had undergone PTCA at the start of the follow-up period, and the incidence of PTCA between inpatients with UA and patients with SA was similar. Further, there were no significant differences in the frequency of the use or type of anti-ischemic agent, aspirin or antiplatelet agents used by the physicians between inpatients with UA and patients with SA. However, the frequency of treatment with lipid-lowering drugs in patients with UA was significantly lower than that in patients with SA ($p < 0.02$).

With respect to coronary arteriography, 34 (92%) of 37 patients who had major epicardial coronary arteries with

Table 1. Baseline Characteristics of Study Group

Variables	Patients With Angina Pectoris			Control Group (n = 22)	p Value*
	Total (n = 142)	UA (n = 45)	SA (n = 97)		
Age (yrs)	62 ± 9	65 ± 8	61 ± 9	60 ± 7	0.02
Men	112 (79%)	35 (78%)	77 (79%)	15 (68%)	0.26
BMI (kg/m^2)	23.3 ± 2.6	23.2 ± 2.7	23.4 ± 2.6	22.5 ± 2.9	0.39
Creatinine (μmol/liter)	88 ± 18	88 ± 18	88 ± 18	71 ± 9	<0.001
Previous MI	49 (35%)	15 (33%)	34 (35%)	0	0.84
Plasma lipid concentration					
LDL-c (mmol/liter)	3.1 ± 0.9	3.2 ± 1.1	3.0 ± 0.7	2.9 ± 0.5	0.21
HDL-c (mmol/liter)	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	1.5 ± 0.2	<0.001
Lipoprotein (a) (mg/dl)	26 ± 24	32 ± 31	23 ± 20	23 ± 15	0.09
VLDL-c (mg/dl)	207 ± 123	186 ± 115	226 ± 121	187 ± 133	0.03
Coronary risk factors					
Hypertension (%)	80 (56%)	25 (56%)	55 (57%)	—	0.90
Diabetes (%)	48 (34%)	14 (31%)	34 (35%)	—	0.64
Hyperlipidemia (%)	96 (68%)	31 (69%)	65 (67%)	4 (18%)	<0.001
Smoking (%)	74 (52%)	24 (53%)	50 (52%)	8 (36%)	0.69
Therapy					
PTCA (%)	61 (43%)	19 (42%)	42 (43%)	—	0.90
Oral nitrates (%)	89 (63%)	28 (62%)	61 (63%)	—	0.94
Calcium antagonists (%)	98 (69%)	34 (75%)	64 (66%)	—	0.25
Beta-blockers (%)	21 (15%)	3 (7%)	18 (19%)	—	0.06
ACE inhibitors (%)	50 (35%)	17 (38%)	33 (34%)	—	0.66
Lipid-lowering drugs (%)	52 (37%)	10 (22%)	42 (43%)	—	0.02
Aspirin (%)	64 (45%)	22 (49%)	42 (43%)	—	0.53
Antiplatelet agents (%)	66 (46%)	23 (51%)	43 (44%)	—	0.45
No. of diseased coronary arteries					
Zero vessels (%)	37 (26%)	12 (27%)	25 (26%)	—	0.01
One vessel (%)	51 (36%)	9 (20%)	42 (43%)	—	—
Two vessels (%)	27 (19%)	9 (20%)	18 (19%)	—	—
Three vessels (%)	27 (19%)	15 (33%)	12 (12%)	—	—
LVEF (%)	64 ± 14%	62 ± 17%	65 ± 12%	—	0.27

*Indicates statistical differences between patients with UA, those with SA and control subjects in multiple comparisons using analysis of variance. Data are presented as the mean value ± SD or number (%) of patients or subjects.

ACE = angiotensin-converting enzyme; BMI = body mass index; HDL-c = high density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty; SA = stable angina; UA = unstable angina; VLDL-c = very low density lipoprotein cholesterol.

≤50% diameter stenosis were diagnosed as having vasospastic angina.

Circulating MCSF concentration. The mean MCSF concentration at the start of the follow-up period in patients with UA was significantly higher than that in patients with SA and in control subjects (981 ± 277 vs. 693 ± 223 vs. 680 ± 158 pg/ml, $p < 0.001$) (Fig. 1). The mean MCSF concentrations in patients with zero, one, two and three diseased coronary arteries were 689 ± 248, 747 ± 223, 860 ± 289 and 911 ± 331 pg/ml, respectively, which indicated a significant positive correlation between the MCSF concentration and the extent of CAD ($r = 0.328$, $p < 0.001$) (Fig. 2). The clinical features of the patients with different degrees of CAD are summarized in Table 2.

Cardiac events. Twenty acute coronary events occurred within two years of follow-up. The mean time from the beginning of the follow-up period to coronary events was 3.8 ± 2.5 months (Table 3). Of the acute coronary events, there were seven patients with acute myocardial infarctions, eight patients with UA requiring hospital admission and five patients with requiring emergent PTCA or CABG. Fifteen patients were excluded during the follow-up because they underwent elective PTCA or CABG, and four patients experienced cerebral ischemic accidents or arteriosclerotic obliteration. In a multiple comparison among patients with acute coronary events, patients with elective PTCA or CABG, patients with other atherosclerotic events and patients without coronary events (none in Table 3), those with acute coronary events had a significantly higher plasma MCSF concentration than those without coronary events ($p < 0.001$). The mean plasma MCSF concentration at the start of the follow-up period in the 15 patients who underwent elective PTCA or CABG during follow-up was higher than that in patients (n = 59) without any cardiac events and who did not receive PTCA at the start of the

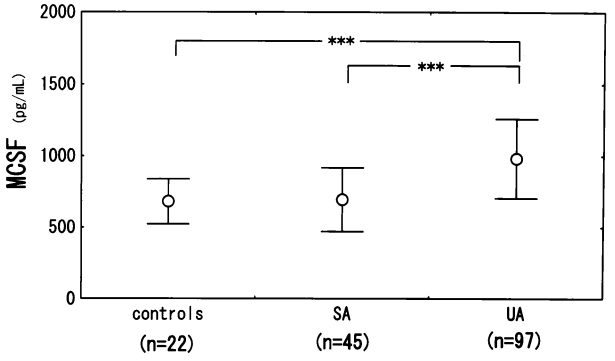

Figure 1. Circulating MCSF concentrations in patients with UA, patients with SA and control subjects. ***$p < 0.001$ (by post hoc analysis).

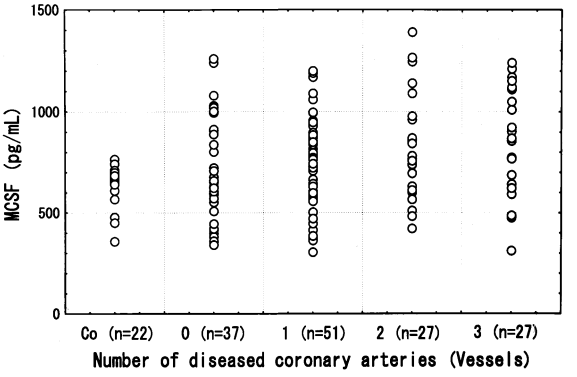

Figure 2. Changes in the circulating MCSF concentration in patients with CAD and in control subjects. The plasma MCSF concentration increased in proportion to the number of diseased coronary arteries, giving a significant positive correlation between the MCSF concentration and the number of diseased coronary arteries ($r = 0.328$, $p < 0.001$). Co = control subjects.

follow-up period or in those ($n = 44$) who underwent PTCA at the start of the follow-up period and did not experience any cardiac events (803 ± 177 vs. 669 ± 225 vs. 725 ± 170 pg/ml, respectively).

With respect to the correlation between the degree of CAD and the incidence of acute coronary events, three coronary events occurred in patients with one-vessel disease, eight in patients with two-vessel disease and nine in patients with three-vessel disease (Table 4). There were no coronary events in patients without CAD. Of the patients with acute coronary events, nine patients (45%) had undergone elective PTCA at the beginning of the study. In 10 of the 20 patients who developed acute coronary events, measurement of MCSF was performed on re-admission to the hospital. The mean plasma concentration of MCSF was 1,234 ± 477 pg/ml, which correlated significantly with the concentration of MCSF at the start of the follow-up period ($r = 0.66$, $p = 0.039$). Of the 15 patients who underwent elective PTCA or CABG, four underwent elective PTCA because of continued angina. The mean plasma MCSF concentration at the time of re-admission in this group of patients was higher than that at the start of the follow-up period, although the difference did not reach statistical significance (1,146 ± 215 vs. 727 ± 370 pg/ml, $p = 0.16$).

Predictors of cardiac events. Based on univariate analysis using the Cox hazards regression model, seven factors were found to be related to the development of acute coronary events (Table 5): 1) plasma MCSF concentration ≥950 pg/ml; 2) presence of multivessel CAD; 3) presence of UA on hospital admission; 4) LVEF <40%; 5) lipoprotein (a) concentration ≥40 mg/dl; 6) HDL cholesterol concentration <1.0 mmol/liter; and 7) no lipid-lowering drug therapy use. Based on multiple regression analysis (Table 5), a plasma MCSF concentration ≥950 pg/ml was an independent risk factor for an unfavorable outcome, with an increased relative risk of acute coronary events (RR 23.7, 95% CI 5.4 to 103.8). Furthermore, multivessel CAD and a low LVEF were also independent predictors of acute coronary events (RR 6.8, 95% CI 1.9 to 23.9 and RR 3.1, 95% CI 1.1 to 8.7, respectively).

Clinical features of patients with MCSF concentrations ≥950 pg/ml. The cumulative two-year cardiac event-free rate in patients with an MCSF concentration ≥950 pg/ml is significantly lower than that in patients with an MCSF concentration <950 pg/ml ($p < 0.001$) (Fig. 3). The clinical characteristics of patients with an MCSF concentration ≥950 pg/ml are summarized in Table 6. Those patients with a high plasma MCSF concentration ≥950 pg/ml were significantly older and had a higher incidence of UA and a lower plasma HDL cholesterol concentration as compared with those patients with a plasma MCSF concentration <950 pg/ml.

DISCUSSION

We quantified the circulating MCSF concentration and assessed its relation with the development of acute coronary events in patients with angina pectoris. The results demonstrate that the plasma MCSF concentration in patients with UA was significantly higher than that in patients with SA. Furthermore, the greater the extent of CAD, the greater the circulating MCSF concentration.

MCSF concentration in patients with UA and SA. In the present study, the plasma MCSF concentration in patients with UA was significantly higher than that in patients with SA. The results are consistent with findings from previous reports (15). Furthermore, Moreno and other investigators (8–10,16,17) demonstrated that there is a significantly greater amount of macrophage-rich plaque in patients with UA or restenosis after PTCA than in patients with SA or no restenosis after PTCA. In these studies, there are greater amounts of thrombi and inflammatory macrophages in primary atherosclerotic plaques in patients with acute coronary syndromes than in patients with SA. Further, the

Table 2. Clinical Features of Patients With Coronary Artery Disease

	Extent of CAD				
	Zero Vessels (n = 37)	One Vessel (n = 51)	Two Vessels (n = 27)	Three Vessels (n = 27)	p Value
Age (yrs)	59 ± 8	62 ± 9	63 ± 9	64 ± 8	0.08
Men	6 (70%)	40 (78%)	24 (89%)	22 (81%)	0.34
Previous MI	2 (5%)	24 (47%)	7 (26%)	16 (59%)	<0.001
LDL-c (mmol/liter)	3.0 ± 0.8	3.2 ± 0.7	3.1 ± 1.3	3.2 ± 0.7	0.70
HDL-c (mmol/liter)	1.3 ± 0.3	1.2 ± 0.3	1.0 ± 0.2	1.2 ± 0.2	0.01
VLDL-c (mg/dl)	193 ± 113	246 ± 128	197 ± 109	196 ± 117	0.11
Lipoprotein (a) (mg/dl)	20 ± 18	23 ± 20	32 ± 31	34 ± 30	0.05
Coronary risk factors					
Hypertension (%)	19 (51%)	28 (55%)	19 (70%)	14 (52%)	0.42
Diabetes (%)	3 (8%)	17 (33%)	9 (33%)	19 (70%)	<0.001
Hyperlipidemia (%)	18 (49%)	42 (82%)	20 (74%)	16 (59%)	0.01
Smoking (%)	15 (41%)	22 (43%)	15 (56%)	16 (59%)	0.35
Therapy					
PTCA (%)	2 (5%)	24 (47%)	18 (67%)	17 (63%)	<0.001
Oral nitrates (%)	15 (41%)	36 (71%)	20 (74%)	18 (67%)	0.01
Calcium antagonists (%)	27 (73%)	35 (69%)	18 (67%)	18 (67%)	0.94
Beta-blockers (%)	1 (3%)	10 (20%)	7 (26%)	3 (11%)	0.04
ACE inhibitors (%)	7 (19%)	18 (35%)	13 (48%)	12 (44%)	0.06
Lipid-lowering drugs (%)	8 (22%)	26 (51%)	10 (37%)	8 (30%)	0.03
Aspirin (%)	4 (11%)	23 (45%)	16 (59%)	21 (78%)	<0.001
Antiplatelet agents (%)	6 (16%)	27 (53%)	17 (63%)	16 (59%)	<0.001
Acute CE (%)	0	3 (6%)	8 (30%)	9 (33%)	<0.001

Data are presented as the mean value ± SD or number (%) of patients.
CAD = coronary artery disease; CE = cardiac events; other abbreviations as in Table 1.

macrophage and thrombus contents are correlated. Finally, a greater amount of tissue factor is present in primary coronary plaque tissue (often macrophage-rich or thrombi-containing plaque). Macrophages may be involved in restenosis because of their capacity to express numerous growth factors, cytokines (11) and metalloproteinases (18–20). In addition, restenosis after PTCA is associated with monocyte activation at the time of PTCA (21). The mechanisms responsible for macrophage involvement in cardiac events may include thrombus organization, smooth muscle cell migration and proliferation and constrictive scarring of the adventitia. Previous reports have demonstrated that smooth muscle cells express granulocyte-macrophage colony–stimulating factor in both normal and atherosclerotic human coronary arteries (22). These results suggest that MCSF is involved in the progression of coronary atherosclerosis and in the destabilization of atheromatous plaque.

In the present study, most of the patients with high

Table 3. Atherosclerotic Events and Macrophage Colony–Stimulating Factor Concentrations in Study Patients

Events	No. of Patients	MCSF-1* (pg/ml)	No. of PTCAs†	MCSF-2* (pg/ml)
Acute coronary events (%)	20 (14%)	1,192 ± 232	9 (45%)	1,158 ± 239
Cardiac death (%)	0		0	
Acute MI (%)	7 (5%)	1,232 ± 203	3 (43%)	1,153 ± 213
Unstable angina (%)	8 (6%)	1,161 ± 229	5 (63%)	1,165 ± 302
Emergent PTCA or CABG (%)	5 (3%)	1,186 ± 311	1 (20%)	1,140
Elective PTCA or CABG (%)	15 (11%)	803 ± 177	8 (53%)	788 ± 324
Other atherosclerotic events (%)	4 (3%)	1,088 ± 111	0	
Cerebrovascular ischemic events (%)	2 (1%)		0	
Arteriosclerotic obliteration (%)	2 (1%)		0	
None (%)	103 (72%)	690 ± 213	44 (43%)	725 ± 170

*$p < 0.001$ in multiple comparisons, using analysis of variance, of patients with acute coronary events, those with elective PTCA or CABG, those with other atherosclerotic events (only in MCSF-1) and those with none of these conditions. †Number of patients undergoing PTCA at the start of the follow-up period.
CABG = coronary artery bypass graft surgery; MCSF-1 = plasma macrophage colony–stimulating factor concentration in patients with or without coronary events; MCSF-2 = plasma macrophage colony–stimulating factor concentration in patients who had PTCA at the start of the follow-up period; other abbreviations as in Table 1.

Table 4. Coronary Angiographic Findings and Macrophage Colony–Stimulating Factor Concentrations in Patients With Acute Coronary Events

Pt no.	Gender	Age (years)	No. of Medications*	First MCSF	Second MCSF	First CAG†	Follow-Up (months)	Coronary Events	Second CAG
1	M	55	4	1,120	982	#2 (75%), #5 (50%)	6	UA	#2 (99%), #3 (90%), #7 (75%)
2	M	54	3	1,050	923	#10 (75%), #12 (90%→PTCA 50%)	1	UA	#10 (90%), #12 (90%)
3	F	64	6	1,640	1,357	#1 (99%→PTCA 50%), #7 (90%)	2	UA	#1 (90%), #7 (99%)
4	M	67	4	1,190	—	#1 (90%), #13 (75%)	4	UA	#1 (90%), #13 (99%)
5	M	66	4	816	974	#6 (90%→PTCA 25%)	2	UA	#6 (90%)
6	F	73	2	1,120	1,911	#7 (90%→PTCA 25%)	2	UA	#7 (90%)
7	F	68	4	1,152	—	#6 (90%), #13 (75%)	5	UA	#6 (99%), #13 (75%)
8	M	43	4	1,200	—	#9 (90%→PTCA 25%)	6	UA	#9 (90%)
9	M	58	2	1,090	—	#7 (100%→PTCA 25%), #11 (90%)	8	AMI	#7 (100%), #11 (90%)
10	M	75	5	978	891	#4 (90%), #6 (99%→PTCA 50%)	4	AMI	#4 (90%), #6 (100%)
11	M	79	4	1,580	1,306	#1 (90%), #6 (75%), #13 (90%)	6	AMI	#1 (100%), #6 (90%), #13 (90%)
12	M	62	3	1,211	—	#1 (90%), #6 (90%), #11 (90%)	2	AMI	#1 (99%), #6 (100%), #11 (90%)
13	F	65	1	1,265	—	#6 (90%), #11 (75%)	1	AMI	#6 (100%), #11 (75%)
14	M	51	4	1,390	—	#2 (100%→PTCA 25%), #13 (50%)	4	AMI	#2 (25%), #11 (90%), #13 (100%)
15	M	71	2	1,110	994	#2 (75%), #7 (75%), #11 (90%)	10	AMI	#2 (75%), #7 (75%), #12 (100%)
16	M	63	5	1,140	917	#2 (90%→PTCA 50%), #12 (75%)	6	EM-PTCA	#2 (99%), #12 (90%)
17	F	72	1	903	—	#1 (75%), #6 (90%), #12 (90%)	2	EM-CABG	#1 (99%), #6 (90%)
18	M	79	2	1,710	2,234	#1 (90%), #5 (50%), #6 (90%)	2	EM-CABG	Same as first CAG
19	M	57	3	1,170	—	#1 (100%), #10 (90%), #13 (100%)	3	EM-CABG	Same as first CAG
20	M	79	5	1,010	—	#2 (90%), #5 (75%), #11 (90%)	2	EM-CABG	#2 (90%), #5 (90%), #11 (90%)

*Antianginal agents, lipid-lowering drugs, antiplatelet agents, aspirin and angiotensin-converting enzyme inhibitors. †The coronary arteriographic (CAG) findings indicate the stenotic site (#) and degree of stenosis (%).
AMI = acute myocardial infarction; EM = emergent; F = female; M = male; MCSF = macrophage colony–stimulating factor plasma concentration (pg/ml); other abbreviations as in Tables 1 and 3.

Table 5. Cox Proportional Hazards Regression Analysis for the Predictors of Acute Coronary Events

Variables	Univariate Analysis		Multivariate Analysis	
	p Value	RR (95% CI)	p Value	RR (95% CI)
Age	0.06	1.1 (1.0–1.1)	NS	NS
Male gender	0.73	0.8 (0.3–2.3)	NS	NS
Previous MI	0.17	1.8 (0.8–3.9)	NS	NS
Unstable angina	<0.001	7.9 (2.9–21.7)	NS	NS
MCSF ≥ 950 pg/ml	<0.001	34.9 (8.1–151.0)	<0.001	23.7 (5.4–103.8)
Plasma lipid concentration				
LDL-c ≥4 mmol/liters	0.97	1.0 (0.2–4.4)	NS	NS
VLDL-c ≥ 250 mg/dl	0.27	0.5 (0.2–1.6)	NS	NS
HDL-c <1.0 mmol/liters	0.004	3.6 (1.5–8.8)	NS	NS
Lipoprotein (a) >40 mg/dl	0.003	3.8 (1.5–9.2)	NS	NS
Coronary risk factor				
Diabetes	0.09	0.4 (0.2–1.2)	NS	NS
Smoking	0.21	0.6 (0.3–1.3)	NS	NS
Hyperuricemia	0.59	0.7 (0.3–2.1)	NS	NS
Hypertension	0.63	0.8 (0.3–2.1)	NS	NS
Therapy				
PTCA	0.07	2.3 (0.9–5.6)	NS	NS
Nonuse of oral nitrates	0.75	1.2 (0.5–2.9)	NS	NS
Nonuse of lipid-lowering drugs	0.05	3.5 (1.0–12.0)	NS	NS
Nonuse of aspirin	0.07	2.5 (0.9–6.6)	NS	NS
Nonuse of ACE inhibitors	0.05	2.4 (1.0–5.4)	NS	NS
Nonuse of beta-blockers	0.24	0.3 (0.1–2.2)	NS	NS
Nonuse of antiplatelet agents	0.18	1.8 (0.8–4.5)	NS	NS
Nonuse of calcium antagonists	0.60	0.8 (0.3–2.0)	NS	NS
CAD (two or three vessels)	<0.001	11.7 (3.4–40.2)	0.003	6.8 (1.9–23.9)
LVEF <40%	0.002	5.1 (1.8–14.4)	0.037	3.1 (1.1–8.8)

CAD = coronary artery disease; CI = confidence interval; MCSF = macrophage colony–stimulating factor; RR = relative risk. Other abbreviations as in Table 1.

plasma MCSF concentrations had acute coronary events. Eight of these patients had progression of the atherosclerotic lesion within 10 months after PTCA, resulting in restenosis or total occlusion. The MCSF concentration in these patients was higher than that in those patients undergoing elective PTCA. However, we could not determine the reason for this finding because of differences in underlying factors. Accordingly, further prospective studies will be needed to determine the responsible mechanism. However, the increase in the plasma MCSF concentration suggests that it may play a part in the progression of atherosclerotic lesions.

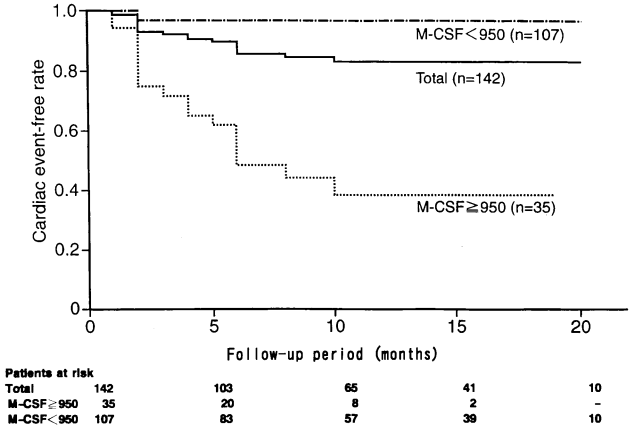

Figure 3. Cumulative two-year cardiac event-free survival curves for patients with angina pectoris. The cardiac event-free rate in patients with an MCSF concentration ≥950 pg/ml is significantly lower than that in patients with an MCSF concentration <950 pg/ml ($p < 0.001$).

Circulating concentrations of MCSF in CAD. The present study demonstrated that the plasma MCSF concentration in our study patients had a significant positive correlation with the extent of CAD. Repeat coronary arteriography in 20 patients with acute coronary events demonstrated restenosis or total occlusion after PTCA in nine patients (45%) during the follow-up period. Moreover, the progression of significant stenotic lesions in 8 of the 11 remaining patients resulted in coronary events, and the development of a new stenotic lesion in one patient was confirmed. In these patients, it is likely that atherosclerotic progression also occurred in major arteries other than the coronary arteries. However, such progression was not con-

Table 6. Clinical Features of Patients With a Macrophage Colony–Stimulating Factor (MCSF) Concentration ≥950 pg/ml or <950 pg/ml

Variables	MCSF Concentration ≥950 (pg/ml) (n = 35)	MCSF Concentration <950 (pg/ml) (n = 107)	p Value
Age (years)	65 ± 9	61 ± 9	0.009
Men	28 (80%)	84 (79%)	0.99
Previous MI	11 (31%)	38 (35%)	0.69
Unstable angina	25 (71%)	20 (19%)	<0.001
Plasma lipid concentration			
LDL-c (mmol/liters)	3.2 ± 1.2	3.1 ± 0.7	0.62
HDL-c (mmol/liters)	1.1 ± 0.2	1.3 ± 0.4	0.003
Lipoprotein (a) (mg/dl)	31 ± 23	24 ± 25	0.17
Coronary risk factors			
Hyperlipidemia (%)	24 (69%)	72 (67%)	0.99
Hypertension (%)	14 (40%)	48 (45%)	0.69
Diabetes (%)	24 (69%)	70 (65%)	0.84
Hyperuricemia (%)	10 (29%)	24 (22%)	0.50
Smoking (%)	21 (60%)	53 (49%)	0.33
Therapy			
PTCA	7 (49%)	44 (41%)	0.56
Oral nitrates (%)	20 (57%)	69 (65%)	0.54
Calcium antagonists (%)	21 (60%)	77 (72%)	0.21
Beta-blockers (%)	2 (6%)	19 (18%)	0.10
ACE inhibitors (%)	18 (51%)	32 (30%)	0.03
Lipid-lowering drugs (%)	9 (26%)	43 (40%)	0.16
Aspirin (%)	20 (57%)	44 (41%)	0.12
Antiplatelet agents (%)	18 (51%)	48 (45%)	0.56
Multivessel disease			
Two or three vessels (%)	21 (60%)	33 (31%)	0.003
LVEF (%)	62 ± 17%	63 ± 14%	0.74

Data are presented as the mean value ± SD or number (%) of patients.
Abbreviations as in Table 1.

firmed by peripheral angiography or radiologic imaging. Therefore, our results are consistent with previous reports (8,10) and suggest that increases in the circulating MCSF concentration reflect the activity or progression of atherogenesis in the coronary arteries of patients with coronary events. In addition, our results suggest that macrophage tissue factor expression may be responsible for prolonged luminal surface thrombogenicity after balloon injury (23).

MCSF and acute coronary events. In the present study, 20 patients with acute coronary events had significantly higher concentrations of circulating MCSF at the start of the follow-up period, as compared with those without coronary events. In 10 of these patients with coronary events, the circulating MCSF concentration was measured on re-admission to the hospital and demonstrated an increase in the MCSF concentration in three patients and a decrease in seven patients. However, there were no significant differences in the mean MCSF concentration between the value at the beginning of the follow-up period and that at the end point. This suggests that the MCSF concentration was maintained at a high level during the clinical course of these 10 patients. In the remaining patients experiencing cardiac events, however, it was impossible to measure or assess the circulating MCSF concentration because of differences in the time of blood sampling.

The plasma MCSF concentration in the 15 patients who underwent elective PTCA or CABG during the follow-up period was higher than that in patients who had not undergone PTCA at the start of the follow-up period or in patients who had undergone PTCA at the start of the follow-up period and had no subsequent cardiac events. The MCSF concentration in the four patients who underwent elective PTCA tended to be greater than that at the start of the follow-up period in all of the patients. This result suggests that inflammation in the coronary arterial wall is progressive and leads to the development of acute coronary syndromes.

Predictive value for cardiac events. Based on the results, the overall sensitivity of a high MCSF concentration (≥950 pg/ml) for predicting cardiac events at six months is 88.9%, and the specificity of a low MCSF concentration (<950 pg/ml) for predicting cardiac events at six months is 84.3%. Therefore, the accuracy of a high MCSF concentration for predicting cardiac events is 85%. However, we

could not evaluate the predictive value of a high MCSF concentration for determining restenosis or occlusion in patients with PTCA, because the patients who underwent PTCA at the start of the follow-up period did not have a second coronary arteriogram.

High concentrations of MCSF in patients without cardiac events. In the present study, there were 13 patients with a high concentration of MCSF (≥950 pg/ml) but who had no acute coronary events. Of these patients, two had diabetes mellitus, one had hypertension, one had hyperlipidemia and four had diabetes mellitus complicated by hyperlipidemia. However, only three patients had poorly controlled diabetes mellitus and subsequently were treated with oral agents. Liu (24) and Saini (25) and colleagues have demonstrated that hyperglycemia reduces macrophage phagocytic function and enhances the growth response of macrophages to MCSF. Thus, the effect of circulating MCSF in patients with diabetes mellitus might be enhanced by continuous hyperglycemia. With respect to hyperlipidemia, there is a tendency for MCSF to decrease after therapy with lipid-lowering agents. However, there were several patients with hyperlipidemia and high MCSF concentrations whose MCSF value did not decrease despite a decrease in lipid concentrations with lipid-lowering agents. The lack of a decrease in the MCSF concentration could not be clarified in this study.

In the other patients, the cause of a high MCSF concentration is unknown. None of the patients had neoplasms, infectious diseases or collagen vascular diseases during the follow-up period. Although a few of these patients had hyperuricemia or obesity, the mean uric acid concentration and body mass index were similar to those values of patients with normal concentrations of MCSF. A few of the patients with a high MCSF concentration had similar values two to four months later (data not presented), without increases in markers of inflammation, such as C-reactive protein. Although it is possible that macroangiopathy was present, further studies are necessary to determine the cause of unexplained increases in MCSF.

Study limitations. Although the present study showed that the circulating concentration of MCSF can predict future coronary events, the measurement of circulating MCSF is influenced by aging, diet, gender and medications. Several investigators have demonstrated the effect of aspirin or antiplatelet agents on the MCSF concentration (14). The MCSF promotes the ability of macrophages to phagocytose oxidized LDL generated by eating. We confirmed circadian variations in the circulating MCSF concentration in several patients enrolled in the present study, with a peak in the concentration at about midnight and a trough in the concentration at noon (data not presented). In addition, there are no reports on the day-to-day variation in circulating MCSF concentrations. Therefore, further evaluation of circadian variations and day-to-day variations in the MCSF concentration are needed. There were only 10 patients with coronary events in the present study, making the comparison of MCSF concentrations difficult. The period before acute coronary events in these patients was relatively short (about four months), resulting in minimal variability. However, we could not determine whether the increase in the circulating MCSF concentration was constant in patients with coronary events.

The study design did not allow us to determine whether the increase of MCSF in patients with angina pectoris is a cause or consequence of plaque instability. The increase in MCSF in the systemic circulation may simply be a reflection of events occurring in the coronary circulation. However, although the clinical manifestations of acute coronary syndromes are determined at the level of the coronary circulation, the demonstration of a systemic inflammatory response supports the hypothesis that systemic factors may contribute to plaque instability.

Conclusions. The results of the present study demonstrate that the concentration of circulating MCSF is an independent predictor of near future coronary events in patients with angina pectoris. However, further studies are required to establish the mechanistic relations between the circulating MCSF concentrations and the development of coronary events and to investigate whether a time-dependent inflammatory process occurs, at least in patients with increased circulating concentrations of MCSF.

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