Plasma profiles of circulating granulocyte-macrophage colony-stimulating factor and soluble cellular adhesion molecules in acute myocardial infarction. Contribution to post-infarction left ventricular dysfunction

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ABSTRACT. No *in vivo* data exist about the relationship of circulating granulocyte-macrophage colony stimulating factor (GM-CSF) and soluble adhesion molecules ICAM-1 and VCAM-1 (sICAM-1 and sVCAM-1) to the severity of acute myocardial infarction (AMI) and the pathophysiological events of post-infarction left ventricular dysfunction. We investigated the kinetics of these inflammatory mediators in the plasma of patients with AMI, and correlated the findings with the clinical severity of the disease during the first week of hospitalization as well as the degree of left ventricular dysfunction one month after the AMI.

Plasma levels of inflammatory markers were determined in 41 AMI patients (all received thrombolytic treatment) by ELISA assays, serially during the first week of hospitalization and one month after hospital admission. Patients ($n = 20$) with uncomplicated AMI (Killip class I) were classified as group A, patients ($n = 21$) with AMI complicated by heart failure manifestations (Killip classes II and III) were classified as group B, while 20 age- and sex-matched volunteers were used as healthy controls.

A sustained increase in GM-CSF, sICAM-1 and sVCAM-1 plasma concentrations was observed only in group B during the first week of the study. Patients from group B exhibited significantly higher levels of GM-CSF ($P < 0.01$), sICAM-1 ($P < 0.05$) and sVCAM-1 ($P < 0.01$) than patients from group A and the healthy controls ($P < 0.001$). In group B patients, significant correlations were observed between the peak of GM-CSF levels and the peak of serum creatine kinase-MB ($r = 0.42$; $P < 0.05$), white blood cell counts ($r = 0.67$; $P < 0.001$) and LVEF ($r = -0.51$; $P < 0.01$). At one month follow-up, patients ($n = 17$) with severe post-infarction left ventricular dysfunction (LVEF $\leq 35\%$) exhibited significantly higher levels of GM-CSF (21.8 ± 1.5 *versus* 11.7 ± 0.9 pg/mL, $P < 0.001$), sICAM-1 (331.4 ± 18.4 *versus* 201.3 ± 12.1 ng/mL, $P < 0.001$) and sVCAM-1 (748.4 ± 34.7 *versus* 512.9 ± 18.8 ng/mL, $P < 0.001$) than did the other patients ($n = 24$) without this condition (LVEF > 35%). Significant correlations were observed between GM-CSF levels and left ventricular end-diastolic volume index ($r = 0.55$; $P < 0.001$) or left ventricular end-systolic volume index ($r = 0.49$; $P = 0.001$).

We have found a significant elevation of plasma GM-CSF and soluble adhesion molecules during the course of AMI, with the highest values in patients with AMI complicated by heart failure manifestations and severe left ventricular dysfunction. These monocyte-related inflammatory mediators may actively contribute to the pathophysiology of the disease and post-infarction cardiac dysfunction.

Keywords: acute myocardial infarction, cardiac dysfunction, heart failure, inflammation, hemopoietic colony stimulating factors, adhesion molecules, cytokines

INTRODUCTION

Previous studies have shown an abnormal expression of pro-inflammation cytokines and chemokines during the course of acute myocardial infarction (AMI) [1, 2]. These factors may also play a pivotal role in cardiac inflammatory responses and the subsequent repair process after the AMI, mediating possibly the migration of mononuclear phagocytes into the injured myocardial tissue. Granulocyte-macrophage colony-stimulating factor is a cytokine that regulates the proliferation and differentiation of hemopoietic progenitor cells and may contribute to the pathophysiological events involved in atherosclerosis, acute coronary syndromes and chronic heart failure [3-7]. This inflammatory cell-growth factor is produced by a variety of cell types of hemopoietic and non-haemopoietic origin in response to antigens, hypoxia and cytokines (e.g. TNF-α) and its biological effects include macrophage

maturation, monocyte adhesion, superoxide anion generation and further enhancement or induction of other inflammatory mediator production [3, 4, 8, 9].

Furthermore, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are representative members of the immunoglobulin gene superfamily that are found on the surface of activated monocytes and endothelial cells, and mediate the monocyte-endothelial cell adhesive interaction process into cardiovascular system [10, 11].

The soluble forms of cell adhesion molecules may be shed from the surface of activated monocyte-endothelial cells and are increased in conditions in which expression on the cell membrane has also been shown to be increased, such as coronary artery disease and chronic heart failure [12, 13]. No *in vivo* data exist about the relationship of circulating GM-CSF and soluble adhesion molecules ICAM-1, and VCAM-1 (sICAM-1 and sVCAM-1) to the severity of AMI and the pathophysiologic events of post-infarction left ventricular dysfunction. We investigate the kinetics of these inflammatory mediators in plasma of patients with AMI and correlated the findings with the clinical severity of the disease during the first week of hospitalization as well as the degree of left ventricular dysfunction one month after the AMI.

PATIENTS AND METHODS

Study population

Forty-one patients who were admitted to our coronary care units within 6 hours (mean time: 2.7 ± 0.4 hours) after the onset of AMI (ST segment elevation ≥ 2 mm in two or more contiguous leads followed by significant enzyme release) and 20 sex-and age- matched control subjects with no evidence of cardiovascular disease, made up our study population. Patient population was separated into two groups according to the severity of AMI clinical manifestations. Patients ($n = 20$) with uncomplicated AMI and killip class I were classified as group A, while patients ($n = 21$) with AMI complicated by clinical manifestations of heart failure (Killip classes II, III), as group B. Patients were selected for this investigation if they met the following inclusion criteria: a) no previous AMI or percutaneous transluminal coronary angioplasty or coronary artery by pass graft surgery; b) no collagen diseases or malignancies or infections; c) no liver disease or renal failure; d) no patients had received any anti-inflammatory drugs or immunosuppressive agents within the preceding 2 weeks.

All AMI patients received thrombolytic treatment with rTPA or streptokinase as well as intravenous heparine for 48 hours and aspirin (300 mg orally on admission, followed by 160 mg/daily). Haemodynamics were measured with a Swan-Ganz catheter, placed after admission for at least 2 days in AMI patients. Left ventricular dimensions, volumes and ejection fraction were calculated by echocardiography according to modified Simpson's formula, during the first five days of hospitalization and one month after the onset of AMI.

Biochemical measurements

For the determination of circulating GM-CSF, sICAM-1 and sVCAM-1, peripheral venous blood samples were taken immediately after admission and at 12 hours, 24 hours, 36 hours, 48 hours, three days, four days, five days, one week and one month from the time of admission. The samples were centrifuged at 4 °C and aliquots of plasma were immediately stored at – 70 °C until analyzed. Plasma concentrations of inflammatory factors were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cytoscreen US, Biosource International for GM-CSF; R&D systems, Minneapolis, Minnesota for sICAM-1 and sVCAM-1) according to the manufacturer's instructions. All samples were run duplicate; the average value of two measurements is reported. The intra-assay and interassay coefficients of variation were < 8% for all ELISA assays in our laboratory.

Serum creatine kinase and myocardial band (MB) isoenzyme were also measured by an autoanalyzer and commercially available kits (Hybritech Inc San Diego, California, USA). C-reactive protein was measured by a high sensitive immunoassay system (Boehringer Mannheim, Germany). Finally, white blood cell counts were determined once every day of hospitalization, using standard techniques.

Statistical analysis

Plasma levels of inflammatory factors over the time course in the two groups of AMI patients were compared by analysis of variance for repeated measures with subsequent Bonferroni methods. Comparisons between two groups were done using an unpaired t-test, while differences among more than two groups were tested by one-way or two-way groups analysis of variance as appropriate. Coefficients of correlation (r) were calculated by the Pearson test. All values are given as mean ± SE (standard error), and a P value < 0.05 was considered statistically significant.

RESULTS

Demographics, clinical and laboratory characteristics, and medications of the two groups of AMI patients in first week of hospitalization are shown in *Table 1*. Group B patients had more extensive MIs, lower ejection fractions, higher wedge pressures and higher peaks of creatine kinase-MB, C-reactive protein and white blood cell counts than patients of group A *(Table 1)*.

A sustained increase of GM-CSF, sICAM-1 and sVCAM-1 plasma concentrations was observed only in group B during the first week of the study. On the other hand, a transient increase of plasma inflammatory factors was observed in group A during the first three days of hospitalization. Kinetics of inflammatory variables in the two groups of AMI patients and healthy controls are shown in *(Figure 1A, B and C)*. Peaks of plasma GM-CSF and soluble cellular adhesion molecules in AMI patients and healthy controls are summarized in *Table 1*. Group B patients exhibited significantly higher peaks of GM-CSF ($P < 0.01$), sICAM-1 ($P < 0.05$) and sVCAM-1 ($P < 0.01$) than group A patients and healthy controls ($P < 0.001$). Peaks of plasma inflammatory factors were also significantly higher in group A compared to control group *(Table 1, Figure 1)*.

In group B patients, significant correlations were observed between the peak of GM-CSF levels and the peak of serum creatine kinase-MB ($r = 0.42$; $P < 0.05$), white blood cell

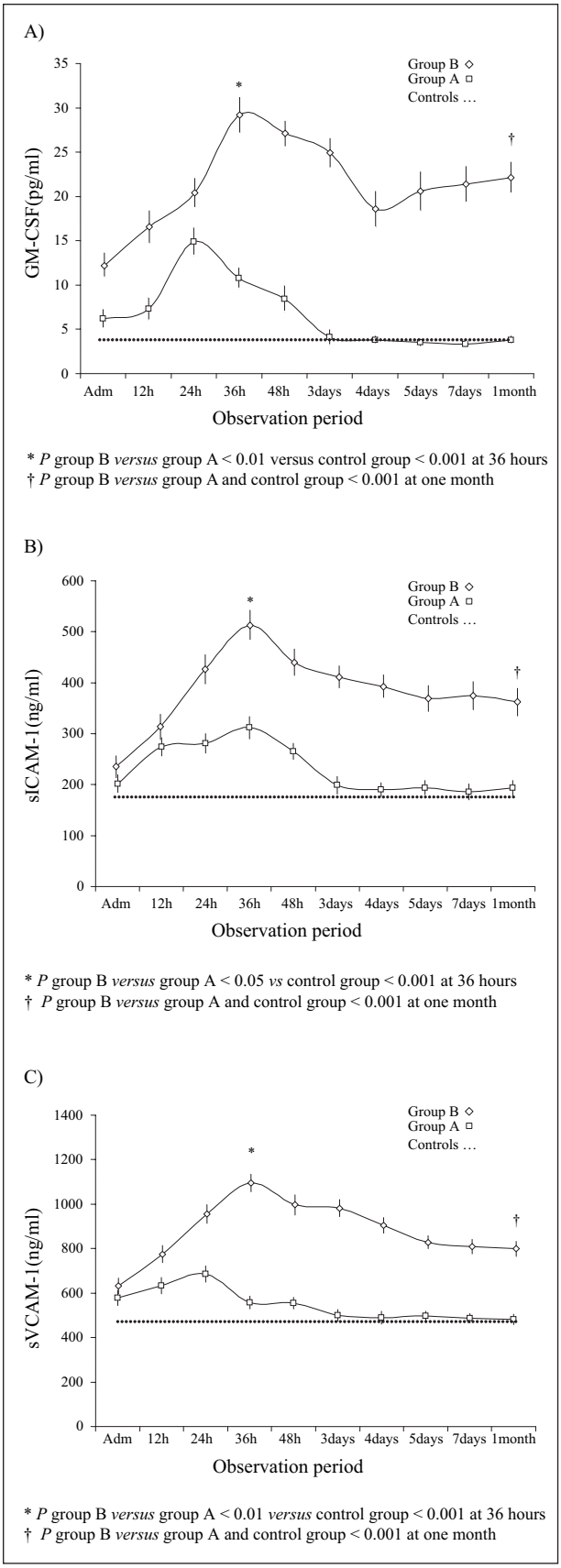

Figure 1
Curves showing the kinetics of plasma GM-CSF (A), sICAM-1 (B) and sVCAM-1 (C) in two groups of acute myocardial infarction patients (during the one-month observation period), and healthy controls.

counts ($r = 0.67$; $P < 0.001$) and LVEF ($r = -0.51$; $P < 0.01$). In the same AMI group, a moderate (non-significant) correlation of GM-CSF peak levels was also found with the peak of C-reactive protein ($r = 0.40$; $P = 0.055$) and sICAM-1 ($r = 0.42$; $P = 0.061$).

At one month follow-up, all patients included in the study had survived. Seventeen group B patients had severe cardiac dysfunction (LVEF ≤ 35%), and the other 24 AMI patients (20 from group A and 4 from group B) had LVEF > 35%. Patients with severe post-infarction left ventricular dysfunction exhibited significantly higher levels of GM-CSF, sICAM-1 and sVCAM-1 (all $P < 0.001$) than did the other patients without this condition *(Figure 2A, B and C)*. Significant correlations were observed between GM-CSF levels and left ventricular end-diastolic volume index ($r = 0.55$; $P < 0.001$) or left ventricular end-systolic volume index ($r = 0.49$; $P = 0.001$) *(Figure 3 a and B)*, as well as between GM-CSF levels and sICAM-1 values ($r = 0.67$; $P < 0.001$).

DISCUSSION

Experimental AMI is associated with the coordinated activation of a series of cytokine and adhesion molecule genes [1, 14]. Recent observations regarding the mechanisms which are responsible for cytokine cascade triggering in the infarcted myocardium indicated a role for preformed, inflammatory cell-derived, mediators in initiating the cytokine activation associated with cellular adhesion molecule induction in the infarcted regions [15, 16]. Leukocyte subpopulations such as neutrophils and mast-cells have been recognized as an important source of preformed and newly synthesized cytokines, chemokines and growth factors in the infarcted myocardium [14, 15].

Using a canine model of reperfused myocardial infarction, Kulielka *et al.* [17] found that ICAM-1 mRNA is overexpressed in ischemic myocardial segments as early as one hour after reperfusion, with marked elevations after longer time intervals. Furthermore, the area of induction of ICAM-1 mRNA in the ischemic/reperfused myocardium is the region where the most intense neutrophil margination and infiltration occur. Thus, it is reasonable to propose that ICAM-1 facilitates both the emigration of activated neutrophils in reperfused myocardium and their adherence-dependent cytotoxic behavior. ICAM-1 can be expressed by many cell types under certain circumstances [10, 11]. *In vitro* studies [18, 19] examined the potential mechanisms of neutrophil adhesion to isolated adult canine cardiac myocytes. Intercellular adhesion occurred only if the myocytes were stimulated with cytokines (i.e. IL-6, GM-CSF) inducing ICAM-1 expression, and when the neutrophils were stimulated to show Mac-1 activation. This inflammatory cell-cardiomyocyte adhesive interaction can lead to further enhancement of cardiac inflammatory responses, to increased oxidative stress and to subsequent myocardial injury. *In vivo* experiments [20, 21] with post-ischemic cardiac lymph have also demonstrated the appearance of late complement components present during the first hours of reperfusion, along with neutrophils showing upregulation of Mac-1 on their surface. Post-ischemic cardiac lymph also promotes ICAM-1 upregulation in cardiac myocytes mediated by pro-inflammatory cytokine overactivation.

Table 1
Demographic, clinical and laboratory characteristics of acute myocardial infarction patients (first week of hospitalization) and healthy controls (mean ± SE)

Characteristic	Acute myocardial infarction patients		
	Group A (n = 20)	Group B (n = 21)	Healthy controls (n = 20)
Age (years)	58 ± 7	56 ± 9	56 ± 11
Male/Female	14/6	16/5	15/5
Diabetes mellitus	10	11	–
Arterial hypertension	12	13	–
Acute myocardial infarction site			
Anterior/inferior	5/15	15/6	–
Thrombolytic treatment rTPA/Streptokinase	14/6	16/5	–
Successful reperfusion	16	15	
Co-medications:			
ACE inhibitors	17	19	–
Beta blockers	15	11	–
Statins	18	20	–
Aspirin	20	21	–
Killip class I/II/III	20/0/0	0/13/8	–
Left ventricular ejection fraction (%)*	44 ± 3	30 ± 3	62 ± 4
Pulmonary artery wedge pressure (mmHg)**	14 ± 2	26 ± 3	–
Cardiac index (L/min/m^2)**	3.2 ± 0.3	2.1 ± 0.1	
Serum creatinine (mg/dL)	0.9 ± 0.01	0.8 ± 0.01	0.7 ± 0.01
Peak of serum creatine kinase MB (ìg/dL)*	201 ± 11	318 ± 17	< 6
Peak of white blood cell counts (cells/mm^3)*	11.511 ± 787	16.321 ± 798	7.263 ± 750
Peak of C-reactive protein (mg/dL)*	2.9 ± 0.7	4.7 ± 0.9	< 0.8
Peak of GM-CSF (pg/mL)$^+$	14.9 ± 1.6	28.9 ± 2.3	3.7 ± 0.3
Peak of sICAM-1 (ng/mL)$^{++}$	312.7 ± 21.7	497.3 ± 29.1	187.1 ± 13.7
Peak of sVCAM-1 (ng/mL)$^{+++}$	685.5 ± 37.3	1120.1 ± 41.7	480.5 ± 33.2

* P group B *versus* A < 0.05
** P group B *versus* A < 0.01
$^+$ P group B *versus* A < 0.01, P group B *versus* controls < 0.001, P group A *versus* controls < 0.01
$^{++}$ P group B *versus* A < 0.05, P group B *versus* controls < 0.001, P group A *versus* controls < 0.001
$^{+++}$ P group B *versus* A < 0.01, P group B *versus* controls < 0.001, P group A *versus* controls < 0.05

GM-CSF is a pleiotropic cytokine that stimulates the growth and differentiation of granulocyte and macrophage precursor cells *in vitro*, it induces peripheral monocytosis, and prolongs the life-span of monocytes *via* the reduction of apoptosis [3, 4, 22]. Many studies have demonstrated that GM-CSF promotes the infiltration of monocytes and macrophages after tissue injury. Under the influence of chemoattractants such as MCP-1 that are produced in response to injury, GM-CSF enhances the effective recruitment of circulating monocytes to local inflammatory sites by attachment to the endothelium and upregulation of cellular adhesion molecules [23]. Furthermore, enhanced peripheral monocytosis, monocyte recruitment into the infracted myocardium and delayed collagen production by GM-CSF induction may lead to infarct expansion, and aggravated LV remodeling and dysfunction [24]. Recently, we have reported [7] that plasma GM-CSF levels were elevated in patients with ischemic dilated cardiomyopathy and that this increase was associated with the degree of LV dysfunction, while another study [25] demonstrated that GM-CSF administration in cancer patients reduced cardiac contractility. Thus, GM-CSF itself might directly influence the LV function during the course of AMI, as well in post-infarction period following extensive myocardial injury.

In this study, we investigated for the first time, the plasma activity of GM-CSF and soluble cellular adhesion molecules according to the clinical and hemodynamic severity of AMI during the acute and sub-acute phases, as well as the relationship of these plasma inflammatory markers to the degree of left ventricular dilatation and dysfunction one month later. During the acute and sub-acute phases, a sustained and significantly greater increase in inflammatory variables was observed only in the group of AMI patients with heart failure manifestations and worsening hemodynamic status. The significant correlation of plasma GM-CSF with markers of myocardial damage, as well GM-CSF-induced peripheral leukocytosis and upregulation of soluble ICAM-1, indicate that this hemopoietic factor is closely related with enhanced immune activation triggered by extended ischemic myocardial injury and targeted to infarct area repair early after the onset of AMI. Furthermore, GM-GSF may promote left ventricular dysfunction during the acute and sub-acute phases of AMI through the inhibition of leukocyte apoptosis, the enhancement of monocyte infiltration into ischemic myocardial areas, the enhancement of monocyte-endothelial cell adhesive interaction into injured tissues (mediated by overexpressed cellular adhesion molecules), and, finally, the induction of further pro-inflammatory cytokine production and superoxide anion release.

One month after the onset of AMI, we found that excessive activation of plasma GM-CSF and soluble adhesion molecules remained in patients with severe left ventricular

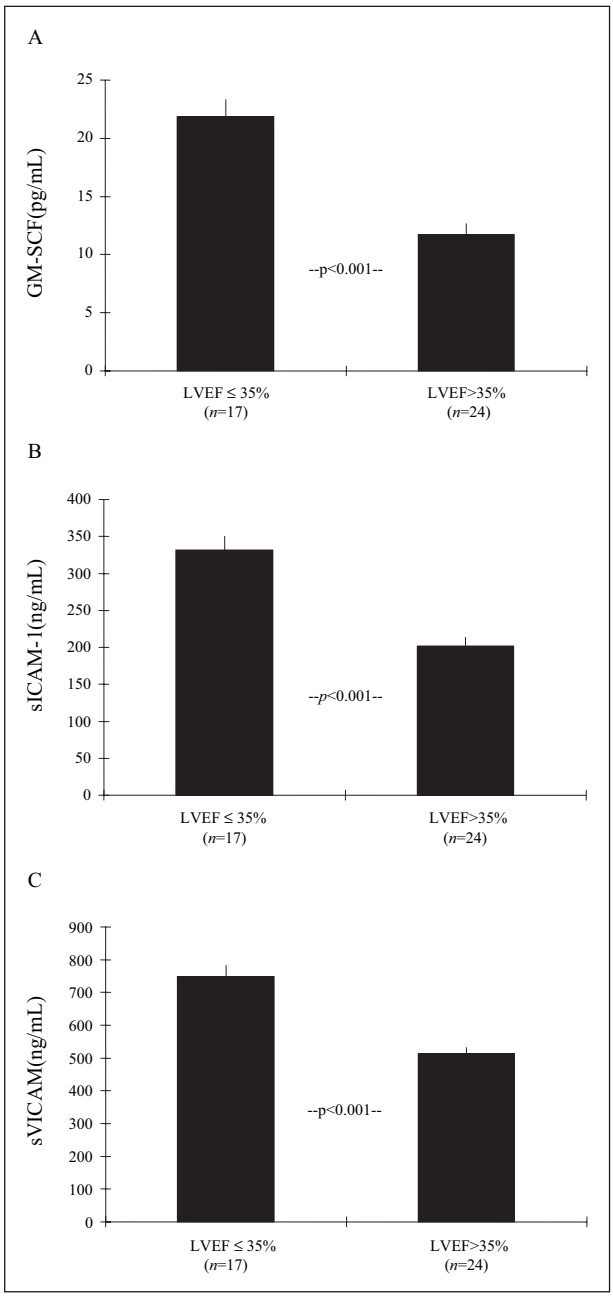

Figure 2
Plasma profiles of GM-CSF (A) and soluble adhesion molecules (B and C) according to left ventricular function in patients one month after the onset of acute myocardial infarction (mean ± SE).

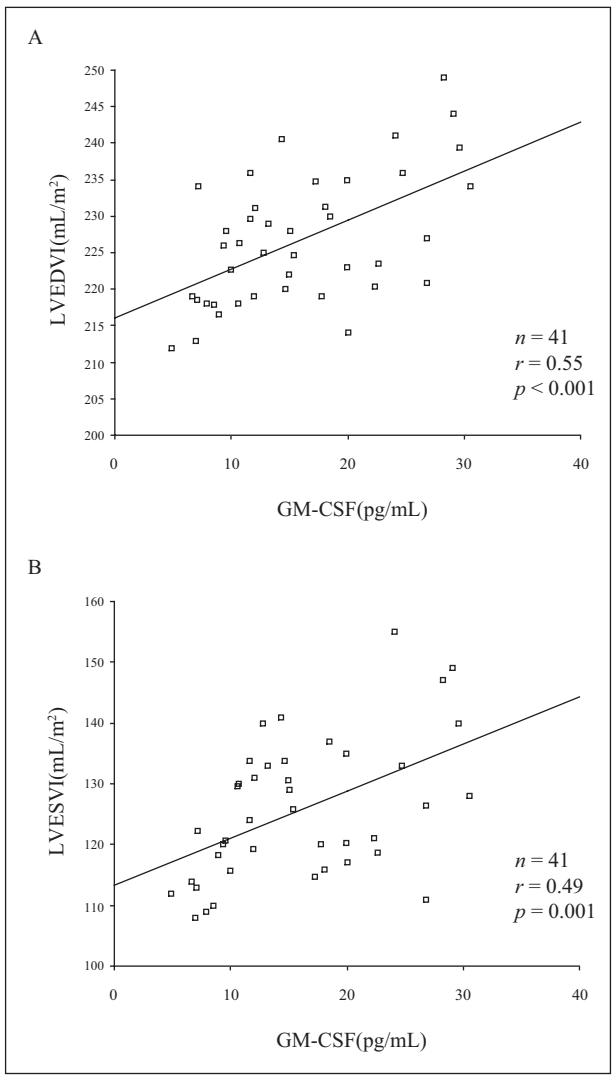

Figure 3
Graphs showing correlations between plasma concentrations of GM-CSF and left ventricular end-diastolic volume index (LVEDVI) (A), and end-systolic volume index (LVESVI) (B) in patients one month after the onset of acute myocardial infarction.

dysfunction, and correlated with the degree of left ventricular dilatation (end-diastolic and end-systolic volume indices). This observation is in keeping with recent experimental data that indicate that combined effects of pro-inflammatory cytokines and hemopoietic growth factors mediate infiltration and activation of mononuclear leukocytes into the injured and ischemic myocardium during the post-infarction period, thereby playing an important pathogenic role in left ventricular dysfunction and the development of the syndrome of chronic heart failure [26]. In this context, increased levels of circulating GM-CSF are a basal part of persistent inflammatory activity during the phase of cardiac repair that may promote cardiac dysfunction through the increased oxidative stress, activation of cardiomyocyte apoptosis and enhanced extracellular matrix degradation. Detrimental effects of enhanced immune activation during the course of AMI may be important pathophysiological events that lead to progressive cardiac dilatation and dysfunction in patients with more extensive and complicated infarcts, and their inhibition could be a potential therapeutic target for the prevention of left ventricular remodeling [16, 27].

STUDY LIMITATIONS

A larger patient population and a longer period of observation are required in order to clarify the exact role of these inflammatory mediators in the pathophysiology of post-infarction cardiac remodeling and to establish powerful correlations between the persistent inflammatory activity and progressive cardiac enlargement. Furthermore, elevated plasma levels of inflammatory mediators in patients with cardiac dysfunction may be due to multisite cytokine production (cardiac and extra-cardiac), and do not necessarily reflect their expression in the infarcted heart tissue. Thus, only indirect evidence suggests that the molecules

studied are associated with pathophysiological events of post-infarction cardiac dysfunction or dilatation. However, elevation of circulating inflammatory mediators may affect the clinical and hemodynamic status of AMI patients through their negative inotropic and cytotoxic effects on heart tissue [7, 9].

CONCLUSION

An abnormal activation of circulating GM-CSF and soluble adhesion molecules has been detected during the course of AMI as well as in the post-infarction period, with the highest levels in patients with severe left ventricular dilatation and dysfunction. This abnormal inflammatory response is closely related to post-infarction left ventricular dysfunction, and its attenuation may be a promising therapeutic approach in patients with AMI.

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