Elevated Monocyte Chemoattractant Protein-1 Serum Levels in Patients at Risk for Coronary Artery Disease

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Background  Monocyte chemoattractant protein-1 (MCP-1) is involved in the recruitment of monocytes into the arterial vessel wall as one of the major events leading to atherosclerotic vascular diseases, such as coronary artery disease (CAD).

Methods and Results  The study group comprised 263 volunteers aged between 18 and 85 years who were admitted to hospital or clinic for scheduled invasive and non-invasive diagnostic procedures. MCP-1 serum levels were determined using a sandwich-enzyme-linked immunosorbent assay. In each patient, the coronary risk factors (CRF), such as hypertension, high cholesterol, diabetes mellitus, obesity, positive family history, and smoking were evaluated. Low-density lipoprotein-cholesterol, lipoprotein(a), and hemoglobinA1c levels were determined. Patients with CAD proven by angiography had significantly increased MCP-1 levels. In patients without CAD, the increase in MCP-1 depended on the number of CRF. As a marker for endothelial activation the soluble adhesion molecules, soluble intercellular adhesion molecule and soluble E-selectin were measured and both markers were significantly elevated in patients with CAD or multiple CRF when compared with patients without CRF. Although this is not a direct proof, endothelial activation could contribute to elevated MCP-1 levels in atherosclerosis.

Conclusion  Elevated MCP-1 serum levels could serve as a direct marker of the inflammatory activity in patients at risk for coronary artery and other atherosclerotic vascular diseases. (Circ J 2005; 69: 1484–1489)

Key Words:  Atherosclerosis; Cardiovascular risk factors; Coronary artery disease; Inflammation

Monocyte chemoattractant protein-1 (MCP-1) is a potent specific chemoattractant for monocytes without chemotactic activity for neutrophils. In vitro it is secreted by cytokine-activated endothelial cells and vascular smooth muscle cells and its release seems to be induced by TWEAK, a member of the tumor necrosis factor superfamily. There is evidence that not only cytokine activation, but also interaction of activated platelets with endothelial cells or monocytes can induce MCP-1 secretion, depending on conditions.

Monocytes migrate into the intima following a MCP-1 concentration gradient formed by endothelial cell and monocyte activation. Once inside the intima, each monocyte amplifies the signal by synthesizing and secreting its own MCP-1. MCP-1 has been detected in macrophage-rich areas of rabbit and human atherosclerotic plaques and a MCP-1 antibody inhibited transmigration of monocytes through the endothelium into the intima. In genetically engineered mice that lack CCR2, the receptor for MCP-1, formation of atherosclerotic lesions was decreased, which implies a crucial role for MCP-1 in the pathogenesis of atherosclerosis. The ability of minimally modified low-density lipoprotein (LDL)-cholesterol (C) to activate MCP-1 production and secretion in cultured endothelial cells in vitro and in a rabbit model in vivo emphasizes its important role in atherogenesis. In addition, MCP-1 seems to induce angiogenesis; for example, in human cardiac myxoma.

It is still controversial whether markers of endothelial activation accompany risk factors for atherosclerotic diseases. Several studies have shown that soluble adhesion molecules are elevated in atherosclerotic and preatherosclerotic disease states there are also reports that show no difference in the serum levels of soluble intercellular adhesion molecule (sICAM)-1 and soluble E-selectin (sE-selectin) in patients with early atherosclerosis or those with manifest atherosclerotic diseases. Based on the evidence provided by experimental and clinical studies, endothelial cells that are activated by the inflammatory process might be a source of MCP-1 in these patients, but there has not yet been a clinical study performed to prove the role of MCP-1 serum levels in patients at risk for coronary artery disease (CAD).

Methods  The study subjects were recruited from patients of the outpatient clinic of the Cardiology Department of Mainz University. The outpatient clinic is a University of Mainz...
Hospital department that investigates patients with cardiovascular diseases or patients under suspicion of cardiovascular diseases. Healthy individuals without the suspicion of heart disease can also undergo routine check-ups. Each of the patients was carefully evaluated by physical examination performed by one of the authors, a laboratory investigation (see later), an ECG, and a physical exercise test. The medical history of each patient was evaluated. Patients with an acute coronary syndrome, such as unstable angina or myocardial infarction (MI), were excluded from the study and transferred to the coronary care unit. Other exclusion criteria are shown in Table 1.

**Study Protocol**

We enrolled 263 consecutive patients of the outpatient clinic who matched the inclusion criteria (Table 1) and their baseline characteristics are shown in Table 2. Peripheral venous blood was drawn in the morning between 08.00 and 10.00h, centrifuged and the serum frozen in aliquots for later analysis of MCP-1, sICAM-1 and sE-selectin. The determination of monocyte CD11b and L-selectin levels was performed immediately (see later). LDL-C, hemoglobin (Hb), blood cell count, creatine kinase, troponin I, creatinine and the liver parameters aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transpeptidase were determined in the University Hospital of Mainz Core Laboratory.

Using a questionnaire we evaluated medical treatment and cardiovascular risk factors (CRF) such as hypertension, high cholesterol, obesity, diabetes mellitus, family history (FH) for cardiovascular diseases and smoking.

Patients with a positive exercise test or who were otherwise under suspicion for CAD (eg, typical angina) were transferred to the catheterization department for coronary angiography. An exercise test was defined as positive if there was ST-segment depression of 0.1 mV in leads I, II, or III or of 0.15 mV in at least 2 of V1–6 or T-wave inversion of at least 0.15 mV in 2 leads. An atherosclerotic luminal obstruction of 50% and typical (CAD-like) vessel wall irregularities was diagnosed as CAD. The study was approved by the local ethics committee and all subjects gave written informed consent. Each patient was contacted 4 years after the initial investigation.

**Definition of CRF**

- **Hypertension** Hypertension was diagnosed according to international guidelines (WHO 1999). Antihypertensive medication was noted.
- **High Cholesterol** Patients with elevated LDL-C (upper limit: 155 mg/dl) were defined as hypercholesterolemic.
- **Smoking** If the patient smoked cigarettes or cigars regularly, that was defined as a current smoker.
- **Positive FH for Cardiovascular Diseases** Patients with a parent or sibling who had been previously diagnosed with CAD, MI, sudden cardiac death or stroke.
- **Diabetes Mellitus** Patients who were pre-diagnosed by a certified specialist and treated with a diabetic-specific diet, specific oral medication or insulin injections were defined as a diabetic.
- **Obesity** Patients with a body mass index exceeding 30 were defined as obese.

**Definition of “Control”** The ideal controls would be patients who do not have CRF or a history of cardiovascular diseases. However, in the present study we defined “controls” as healthy volunteers who did not have a history of CAD or other atherosclerotic diseases and did not have any CRF. Therefore, the controls for patients with multiple CRF were patients without CRF (Fig 1B) and the controls for patients with CAD were either those without CAD excluded by angiography (Fig 1A) or patients without

<table>
<thead>
<tr>
<th>Inclusion/Exclusion Criteria</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>Patient of the outpatient clinic of the Cardiology Department of the University of Mainz.</td>
<td></td>
<td>Elevated liver parameters, elevated creatinine, elevated leukocyte count</td>
</tr>
<tr>
<td>Patients aged 18–85 years, male and female</td>
<td>Acute coronary syndrome</td>
<td>Cancer</td>
</tr>
<tr>
<td>CK and troponin levels within normal range (CK &lt;60 U/L; troponin I &lt;0.1 U/L)</td>
<td>Infectious diseases</td>
<td>Significant valvular disease</td>
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<td>Written informed consent</td>
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**Table 1**

<table>
<thead>
<tr>
<th>CAD excluded</th>
<th>CRF</th>
<th>CAD proven</th>
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<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>M/F</td>
<td>19/21</td>
<td>34/27</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52±7.2</td>
<td>56±4.5</td>
</tr>
<tr>
<td>Smoking</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>High cholesterol</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Obesity</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Medications (% patients treated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE/AT1 antagonist</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>ß-blocker</td>
<td>64</td>
<td>56</td>
</tr>
<tr>
<td>ASS</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>Statin</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>32</td>
<td>11</td>
</tr>
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</table>

CAD, coronary artery disease; CRF, coronary risk factors; ACEI, angiotensin-converting enzyme inhibitor; AT1, angiotensin II type 1; ASS, aspirin.
suspicion of CAD (Fig 1C).

**Laboratory Methods** Serum levels of MCP-1, sICAM and sE-selectin were measured with commercially available enzyme-linked immunosorbent assay (ELISA) systems (R&D Systems, Wiesbaden, Germany) according to the manufacturers’ protocols. In some patients (n=40), the monocyte activation parameters CD11b and L-selectin were determined by flow cytometry using specific monoclonal antibodies (Serotec) gated for the monocyte window.

**Statistical Analysis**

Data are given as mean±SEM. Student’s t-test for unpaired data or ANOVA was applied where appropriate. A p-value less than 0.05 was considered significant. For Lp(a) and LDL-C, the correlation coefficient r with MCP-1 was calculated (Pearson correlation) and linear regression analysis was performed.

**Results**

The MCP-1 serum level of a healthy population aged 18–84 is 73.6±5.9 ng/ml (own observation). In the present study patients with either angiographically proven CAD or CRF showed significantly enhanced MCP-1 serum levels (CAD: 125.7±10.6 pg/ml; >3 CRF: 129.0±8.1 pg/ml; Fig 1A,B). The MCP-1 serum level directly correlated with the number of risk factors present (Fig 1B). In 40 patients CAD was excluded by angiography, and 19 of these did not have any CRF and their MCP-1 levels were almost identical to the levels in the group of healthy subjects without CRF who had not undergone angiography (control without CRF and angiography: MCP-1, 76.8±16 pg/ml; controls without CRF and without angiography: MCP-1, 73.6±5.9 pg/ml; NS).

The present findings support our hypothesis that a MCP-
1 level of approximately 75 pg/ml is the level usually found in healthy people without CRF or CAD. Their MCP-1 serum levels were as low as the control group that did not undergo angiography (“no suspicion of CAD”). In patients with only 1 risk factor, the MCP-1 levels were slightly increased (smoking: 105±31.7 pg/ml (NS); high cholesterol: 122.8±18.1 pg/ml; hypertension: 116.4±30.1 pg/ml; obesity: 122.5±16.2 pg/ml). The MCP-1 serum level increased with age (controls: <40 years, 69.8±9.7; 40–70 years, 74.7±8.4; >70 years, 79.0±18.2 pg/ml; CAD: 40–70 years, 121.8±10 pg/ml; >70 years: 126.5±13.1 pg/ml; Fig 1C).

Four years after the initial investigation, we contacted all patients; 8 patients had died, 6 of them had died of CAD, but 1 patient died from hemorrhage under therapy with phenprocoumon (similar to warfarin) and 1 from cancer. The MCP-1 levels of these patients were high (Fig 1A).

Patients with a high Lp(a) or LDL-C serum level had markedly higher MCP-1 serum levels than patients with low lipoprotein markers (Lp(a)-MCP-1 Pearson r=0.004, p=0.0278; LDL-MCP-1 Pearson r=0.0086, p=0.0299; Fig 2). Patients with elevated HbA1c (>6.1%) as an indicator of poorly treated diabetes also had increased MCP-1 levels (107.0±19.9; p<0.05 vs control). We did not find a significant difference in the MCP-1 levels between men or women with or without CAD. Taken together, these data demonstrate increased MCP-1 serum levels in patients with multiple CRF and CAD.

As monocyte activation parameters, we measured the...
surface expression of CD11b and CD62L. There was not a notable difference in either of these markers when patients with CAD or at least 3 CRF were compared with controls without CRF (relative fluorescence intensity (control vs patient): CD11b: 25.7±1.1 vs 25.8±3.8; CD62L: 41.6±3.8 vs 55.5±8.0 (n=17; NS).

As markers for endothelial activation and inflammation, sICAM-1 and sE-selectin were measured (n=122). Control subjects without CRF had an sE-selectin level of 23.4±2.2 pg/ml and an sICAM-1 level of 219.0±12.7 pg/ml. In patients with proven CAD the levels of both sE-selectin and sICAM-1 were markedly increased (sE-selectin: 30.8±3.6 pg/ml, p<0.05 vs control; sICAM-1: 260.0±11.9, p<0.01 vs control), as was also the case in patients with 3 or more CRF (sE-selectin: 31.9±3.1 pg/ml, p<0.05 vs control; sICAM-1: 280.6±19.2 pg/ml, p≤0.01 vs control, Fig 3).

Discussion

MCP-1 is a secreted chemokine that exhibits very powerful properties even at extremely low picomolar concentrations. MCP-1 mRNA and protein have been detected in rabbit and human atherosclerotic lesions5,16 but to date the clinical role of circulating MCP-1 and its relationship to human atherosclerotic diseases has not been well defined. In a small pre-study, we found elevated MCP-1 serum levels in patients after bicycle ergometry17 and these elevated MCP-1 levels were more evident in patients with multiple CRF. In the present study with more patients we again found elevated serum levels of MCP-1 in patients with multiple CRF and those with proven CAD. Regardless of which risk factors apply, the total sum of CRF such as smoking, hypertension, obesity, high cholesterol and positive FH seems to account more for high circulating levels of MCP-1 than the severity of a single CRF.

It has been reported that patients with acute MI have higher levels of MCP-1 within 24 h, and that interleukin-8, a chemoattractant for neutrophils, was also elevated when these patients were compared with patients suffering from angina only; healthy control subjects were not studied18. Those data suggest either an acute inflammatory response to tissue injury during myocardial ischemia or the occurrence of MI in patients with documented increased MCP-1 levels and monocyte/macrophage activation. Another study demonstrated that circulating levels of MCP-1 and tissue factor are both increased in patients with acute coronary syndromes compared with patients with stable exertional angina19. These studies investigated the effects of the acute atherosclerotic disease, but the purpose of our study was to identify MCP-1 as a direct indicator chemokine for chronic vascular inflammation.

Elevated serum levels of soluble endothelial adhesion molecules as markers of inflammatory activity of the endothelium are a controversial topic. Although studies have shown elevated levels of sICAM-1 and soluble vascular cell adhesion molecule (sVCAM-1)12,13 others have not proved a clear relationship between atherosclerotic diseases and endothelial activation.14,15 In hypercholesterolemic mice and rabbits endothelial intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 surface proteins and mRNA expressions were detected in intimal lesions of the aortic arch, but not E-selectin. However, other reports showed elevated E-selectin, ICAM and VCAM expressions in human subjects with atherosclerosis20–22. Kowalski et al23 investigated MCP-1 serum levels and the soluble adhesion molecules ICAM-1 and VCAM-1 in patients with high cholesterol and found a positive correlation of sICAM-1 with MCP-1 serum levels, although sVCAM levels remained unchanged. Their data support our hypothesis that MCP-1 is a marker of the extent of endothelial inflammation, leading to atherosclerosis.

We found significantly elevated levels of sICAM-1 and sE-selectin in patients with CAD or 3 or more CRF compared with healthy subjects. Together with the data from other groups our data suggest that endothelial inflammation and serum MCP-1 levels correlate with the clinical syndrome of atherosclerosis. The data do not essentially prove that elevated levels of serum MCP-1 directly derive from activated endothelium, but the endothelium is at least one important source of MCP-1. Although we also investigated the monocyte parameters CD62L and CD11b for monocyte activation, but we did not find any relevant differences in their expressions, so we do not believe that monocytes contribute significantly to elevated serum MCP-1 levels in patients with atherosclerosis. Monocyte MCP-1 might play a major role locally in the atherosclerotic lesion by amplifying the signal for monocyte invasion of the lesion.

Aging as a risk factor for atherosclerotic diseases in patients with elevated MCP-1 levels has been already investigated24 and those authors hypothesized that the increase in MCP-1 serum level with age might reflect the extent of atherosclerosis, although they did not find increased MCP-1 levels in patients with stroke or CAD. One possible explanation for the discrepancy in data is that they did not differentiate healthy subjects from subjects with one or multiple CRF.

Two other recently published studies support our hypothesis that circulating MCP-1 is a factor that contributes significantly to the development of atherosclerotic lesions25,26. Kitamoto et al developed a strategy against atherosclerosis and restenosis after coronary intervention using gene therapy that leads to the synthesis of an inactive transfecting mutant of smooth muscle cell-derived MCP-125. If circulating MCP-1 was not responsible for the development of atherosclerotic lesions and CAD, that approach would not work. In another study, the application of intravenous prostaglandin E1 reduced MCP-1 levels in peripheral arterial obstructive disease26 and furthermore, the MCP-1 levels seem to be elevated in the patients with peripheral arterial obstructive disease compared with healthy controls. The pathologic mechanism for the development of CAD and peripheral arterial obstructive disease is likely to be the same because both diseases have a high coincidence. Prostaglandin E1 inhibits platelet and neutrophil activity and may inhibit endothelial activation by impaired adhesion of blood cells and other mechanisms27.

Recently, Kusano et al28 found that the MCP-1 serum level and intima–media thickness correlated with each other in patients on chronic hemodialysis. They also found that MCP-1 was expressed in endothelial and smooth muscle cells in these patients. Their findings also support our hypothesis that the elevated MCP-1 levels in patients with CRF derive from the vasculature rather than from activated monocytes/macrophages.

Conclusion

This study is the first clinical report to link patients at risk for CAD to MCP-1, which is a key chemokine in the development of atherosclerotic lesions.
Acknowledgments
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References