Inflammatory Markers at the Site of Ruptured Plaque in Acute Myocardial Infarction
Locally Increased Interleukin-6 and Serum Amyloid A but Decreased C-Reactive Protein

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Background—Acute myocardial infarction (AMI) is associated with inflammation. However, it remains unclear whether it originates from the ruptured plaque or represents a systemic process.

Methods and Results—In 42 patients with AMI, a balloon-based embolization protection device and aspiration catheter (PercuSurge) were used during acute coronary interventions. Samples from the site of the ruptured plaque were taken under distal balloon occlusion. Systemic samples were taken from the aorta. Sera, plaques, and thrombi were analyzed for inflammatory markers and lipoproteins. Systemic levels of C-reactive protein (CRP), interleukin-6 (IL-6), and serum amyloid A (SAA) in the aorta amounted to 3.0 mg/L, 5.0 ng/L, and 22.1 mg/L, respectively (interquartile ranges [IQRs], 1.1 to 7.4 mg/L, 5.0 to 6.5 ng/L, and 13.9 to 27.0 mg/L, respectively). In blood surrounding ruptured plaques, local levels of IL-6 (8.9 ng/L; IQR, 5.0 to 16.9 ng/L) and SAA (24.3 mg/L; IQR, 16.3 to 44.0 mg/L) were significantly higher, whereas CRP levels (2.5 mg/L; IQR, 0.9 to 7.7 mg/L) were decreased compared with the aorta (all \( P<0.0001 \)). The coronary levels of IL-6 determined in vivo showed biological activity in vitro. Harvested thrombus contained CD68-positive monocytes expressing IL-6 and showed extracellularly and intracellularly positive staining for SAA, whereas CRP was found exclusively in the cytoplasm of phagocyting white blood cells.

Conclusions—Coronary levels of IL-6 and SAA at the site of plaque rupture were increased relative to the systemic circulation, indicating local production of biologically active inflammatory mediators. In contrast, CRP was locally decreased, at least in part by uptake by the phagocyting cells, suggesting a systemic origin of the protein. (Circulation. 2005;111:1355-1361.)

Key Words: inflammation ■ interleukins ■ myocardial infarction ■ plaque ■ thrombus

A cute myocardial infarction (AMI) resulting from plaque rupture represents the ultimate step in a chain of events arising from a combination of endothelial dysfunction and inflammation.1–7 In particular, the acute-phase reactant C-reactive protein (CRP) is a powerful predictor of cardiovascular events.8,9 It is still undetermined whether and to what extent markers of inflammation originate locally from the site of plaque rupture or reflect a systemic response. Moreover, the links between coronary plaque rupture and systemic inflammation are not clarified.

Primary percutaneous coronary intervention (PCI) is the current treatment of choice in AMI.10,11 The no-reflow phenomenon,12 observed after successful primary PCI, has initiated the design of distal protection devices to prevent embolization of plaque material into the microcirculation. Systems based on an occlusion balloon and an aspiration catheter like the PercuSurge GuardWire,13,14 for which clinical benefit has been documented at first in saphenous vein grafts,15 provide the unique opportunity to harvest not only plaque and thrombi but also blood from the site of coronary occlusion. Because no animal model of plaque rupture is available,16 our understanding of the local processes in humans is derived only from postmortem studies, which are obtained hours after the triggering event and therefore have marked limitations.6,7,17–19 Therefore, we hypothesized that in the aspirate of patients undergoing primary PCI for AMI,
local concentrations of selected inflammatory biomarkers, lipoproteins, and inflammatory cells contained in the thrombus material may allow delineation of local and systemic mechanisms during plaque rupture and intracoronary thrombosis.

**Methods**

**Patients and Procedures**

The study included 42 patients with AMI: 31 were ST-elevation myocardial infarctions (STEMIs), and 11 were non-STEMIs (NSTEMIs). All patients were scheduled for acute PCI. STEMI was defined as persisting chest pain at rest, together with new or presumed-new ST-segment elevation in ≥2 contiguous leads with a cutoff point ≥0.2 mV. NSTEMI was defined as prolonged chest pain with dynamic ST-segment changes and a troponin T level above the upper limit (0.1 μg/L). In all patients, myocardial damage was confirmed by an elevation of CK-MB (>2-fold the upper limit of normal) during the clinical course. Coronary angiography revealed ≥1 clearly identifiable culprit lesion. A culprit lesion was defined as an occluded coronary artery or a vessel with 90% to 99% stenosis by visual estimate and angiographic appearance of thrombus. Seventeen patients (40%) developed AMI after preceding discontinuous clinical symptoms for >24 hours, whereas 25 (60%) showed de novo symptoms. In 22 patients (52%), angiography revealed minor collaterals. The median time interval from the index event, defined as the onset of continuous chest pain, to the first balloon inflation was 263 minutes (range, 173 to 308 minutes).

Patients underwent coronary angiography according to standard techniques. On identification of the culprit lesion, 100 IU/kg heparin was administered, and anticoagulation was monitored and adapted, if necessary, according to the activated clotting time. The target activated clotting time was 250 to 300 seconds and ~200 seconds with glycoprotein IIb/IIIa receptor antagonists using the HemoTech system. Ten patients (24%) were treated with the glycoprotein IIb/IIIa receptor antagonist tirofiban (0.4 μg · kg⁻¹ · min⁻¹) for 30 minutes; maintenance dose, 0.1 μg · kg⁻¹ · min⁻¹; 29 (69%) were treated with abciximab (0.25 mg/kg bolus for 10 minutes; maintenance dose, 0.125 μg · kg⁻¹ · min⁻¹). Two of 3 patients (7%) not receiving glycoprotein IIb/IIIa receptor antagonists had undergone previous thrombolytic therapy.

The culprit artery was intubated with a 7F guiding catheter and wired with the 0.014-in PercuSurge GuardWire. The overall crossing profile of this system, including the part of the PercuSurge set. Heparinized blood was immediately processed at the Department of Clinical Chemistry, and resulting sera were deep-frozen and stored (~70°C) for subsequent analysis of specific parameters.

**Parameters**

The following markers were assessed: serum albumin, high sensitivity CRP (hs-CRP), serum amyloid A (SAA), interleukin-6 (IL-6), apolipoprotein (apo) A-I, apoB, and lipoprotein(a) [Lp(a)].

**Biochemical Assays**

Serum albumin and hs-CRP were assessed on a Roche-Hitachi Modular Clinical Chemistry analyzer using commercial tests from Roche Diagnostics GmbH with maximal coefficients of variation of 2.8% and 5.5% for the assays, respectively. SAA was analyzed with the SAA ELISA from Biosource with a maximal coefficient of variation of 10.6%; IL-6 was analyzed on an Immulite 2000 analyzer using chemiluminescence immunoassays from DPC (Bühlmann) with a maximal coefficient of variation of 11%. ApoA-I and apoB as Lp(a) were analyzed on a Roche-Integra analyzer using immunoturbidimetric tests from Roche Diagnostics GmbH with maximal coefficients of variation of 3.5%, 3.6%, and 3.6% respectively.

To exclude protein absorption in the Export catheter during aspiration, we performed in vitro controls (n=5). There was no difference in results compared with native blood exceeding the standard tolerance of the assays (data not shown).

**Immunohistochemistry**

The solid material was kept in buffered paraformaldehyde 4% for 4 to 6 hours and in 50% ethanol for 24 hours before paraffin embedding (Leica EG1160), xylol/ethanol fixation (Hypercenter XP, Shandon), and cutting with a microtome (Leica RM2035). The slides were treated with trypsin for 1 hour and blocked with 4% fat-free milk in Tris-HCl, pH 7.6, for 30 minutes. The following primary antibodies were used: murine anti-CD68 monoclonal antibodies (Clone PG-M1, Dako), murine anti-IL-6 monoclonal antibodies (Clone 6708, R&D), affinity-purified rabbit anti-CRP, goat anti-apoB polyclonal antibodies (both from Biotrend Chemikalien GmbH), and rat mab anti-human SAA (Biosource International).
After a 1-hour incubation at room temperature with the primary antibodies, the slides were washed and incubated for 30 minutes at room temperature with the appropriate HRP-conjugated secondary antibodies (anti-mouse, rabbit, goat, and rat Ig, Fc fragments, all from Dianova/Milan, La Roche). The staining was revealed with AEC Chormogen (Biogenex, Stehelin & Cie AG) as substrate.

**Biological Activity of IL-6 Concentrations at the Site of Plaque Rupture**

Vascular smooth muscle cells were isolated from human aorta by the explant method. Proliferation was assessed by determining the cell number over 4 days on stimulation with IL-6 (3·10 ng/L) compared with platelet-derived growth factor (10 μg/L) as control. Human aortic endothelial cells were obtained from Clonetics Corp. For determination of tissue factor expression, confluent endothelial cells were stimulated with IL-6 (3·10 ng/L) for 5 hours. Tissue factor expression was assessed by Western blot analysis with a murine monoclonal antibody (American Diagnostica Inc).

**Data Analysis and Statistics**

Patient safety during the intervention required some rinsing of the aspiration catheter between passages. Therefore, some minor dilution of the aspirate with 0.9% sodium chloride solution was unavoidable. To correct for this dilution and to exclude potential artifacts resulting from different dilution, all measurements were normalized mathematically for albumin concentrations. We have validated this procedure by measuring several inert parameters and normalizing them for albumin. Plasma activities of ALT, AST, CK, γGT, and LDH, as well as concentrations of total protein, cholesterol, triglycerides, and uric acid, showed 100% recovery between the local and systemic blood samples (n=15, data not shown).

Biochemical parameters were assessed for characteristics of distribution by use of the Kolmogorov-Smirnov test. Levels of inflammatory markers were identified as not normally distributed. Therefore, those variables were expressed as medians and interquartile range (IQR). For pairwise comparisons, the Wilcoxon signed-rank test was applied. For multiple comparisons within groups, the Friedman test was used. Comparisons between 2 different groups as appropriate. Values of P<0.05 were considered significant. Patient characteristics are given as mean±SD.

**Results**

**Patient Characteristics**

Characteristics of the 42 consecutive patients are summarized in the Table.

**Systemic Levels of Inflammatory Markers**

Systemic levels of hs-CRP, IL-6, and SAA in the aorta amounted to 3.0 mg/L (IQR, 1.1 to 7.4 mg/L), 5.0 ng/L (IQR, 5.0 to 6.5 ng/L), and 22.1 mg/L (IQR, 13.9 to 27.0 mg/L), respectively, in this population of patients with AMI.

**Inflammatory Markers and Lipoproteins at the Site of Coronary Occlusion**

Intracoronary levels of IL-6 (8.9 ng/L; IQR, 5.0 to 16.9 ng/L) and SAA (24.3 mg/L; IQR, 16.3 to 44.0 mg/L) obtained at the site of plaque rupture by the first aspiration under distal balloon occlusion of the culprit coronary artery were increased by 76% and 10%, respectively, compared with the aortic concentrations (IL-6, 5.0 ng/L; IQR, 5.0 to 6.5 ng/L; SAA, 22.1 mg/L; IQR, 13.9 to 27.0 mg/L; both P<0.0001; Figure 2). In contrast, hs-CRP was decreased by 16% compared with aortic levels (2.5 mg/L [IQR, 0.9 to 7.7 mg/L] versus 3.0 mg/L [IQR, 1.1 to 7.4 mg/L]; P<0.0001). Concentrations of Lp(a) were not different at the site of coronary occlusion compared with the aorta (111.4 mg/L [IQR, 38.3 to 679.7 mg/L] versus 113.5 mg/L [IQR, 39.0 to 644.0 mg/L]). Figure 2 summarizes the data for IL-6, SAA, hs-CRP, and Lp(a).

The concentrations of these parameters were again determined in a second control sample taken at the end of the PCI from the aorta. These were highly correlated to those found in the first control samples [r=0.95 for Il-6, SAA, hs-CRP, and Lp(a)]. ApoA-1 and apoB showed minimal concentration differences between local and systemic arterial blood samples of <3%. Consecutive samplings (second aspiration, after stenting) revealed that the levels of IL-6 and CRP returned to the concentrations observed in the aorta (P=NS versus aorta; data not shown), whereas SAA remained elevated (P<0.05).

**Heralded Versus Unheralded AMI**

Systemic levels of hs-CRP, IL-6, and SAA in patients with preceding symptoms were higher than in unheralded AMI (hs-CRP, 4.8 mg/L [IQR, 1.8 to 19.7] versus 2.2 mg/L [IQR,
In the coronary artery, patients with preceding symptoms again showed higher levels of inflammatory markers than those with unheralded AMI: IL-6, 11.0 ng/L (IQR, 7.8 to 38.3) versus 5.0 ng/L (IQR, 5.0 to 14.4 ng/L); SAA, 26.7 mg/L (IQR, 22.8 to 324.3 mg/L) versus 14.6 mg/L (IQR, 14.6 to 34.3 mg/L); and CRP, 5.7 mg/L (IQR, 1.7 to 19.1 mg/L) versus 1.7 mg/L (IQR, 0.7 to 4.0 mg/L) (all \( P < 0.05 \); Figure 3).

**Immunohistochemical Analysis of Thrombus**

Immunohistochemical staining of the thrombus material removed from the site of the ruptured plaque showed large amounts of CD68-positive monocytes (Figure 4A). Staining was also strongly positive for IL-6, which colocalized with macrophages (Figure 4B). SAA was detected widely, both freely floating in the thrombus and in the cytoplasm of white blood cells in all stains (n=5; Figure 4D). In contrast, immunohistochemistry for CRP revealed in 10 of 17 thrombi singular white blood cells with strong CRP-positive staining (but not outside the cells), indicating phagocytosis of the acute-phase reactant (Figure 4C).

**Biological Activity of Locally Assessed IL-6 Concentrations**

Concentrations of IL-6 corresponding to the levels measured at the site of coronary occlusion stimulated cell proliferation to about half of control stimulation with platelet-derived growth factor in cultured human vascular smooth muscle cells. Furthermore, in human endothelial cells in culture, these concentrations of IL-6 increased tissue factor expression (data not shown).

**Discussion**

This study analyzed inflammatory markers and lipoprotein levels at the site of ruptured plaques in patients with AMI and...
compared them with systemic levels. With the PercuSurge GuardWire system, designed to prevent embolization of plaque material into the microcirculation, a distally closed vascular compartment could be created. Therefore, fresh thrombus and blood aspirated from the site of the culprit lesion allowed us to analyze local inflammatory mediators involved in acute coronary occlusion. Among many potential candidates, the most clinically relevant inflammatory cytokines associated with clinical events, ie, CRP and SAA, were selected, together with IL-6, which is a major determinant of their production.

In the systemic circulation of our patients undergoing acute PCI for AMI, inflammatory markers such as CRP, SAA, and IL-6 were elevated, in line with work of others. At the site of the ruptured plaque, however, serum concentrations of the inflammatory markers CRP, IL-6, and SAA differed from those in the systemic circulation. Indeed, although the local coronary levels of IL-6 and SAA at the culprit lesion were markedly elevated compared with systemic levels, those of CRP were decreased. Because levels of Lp(a) remained unchanged, local dynamics of CRP, IL-6, and SAA reflect ongoing biological processes at the site of coronary occlusion. Any impact of the collateral circulation on the inflammatory reaction is unlikely. Although about half of the patients (52%) showed some collaterals at angiography, by definition (the patients presented with AMI and chest pain), the collateral circulation must have been functionally negligible. In an extension of the work of Liuzzo et al in the systemic circulation, the patients without preceding symptoms before AMI also exhibited lower local coronary levels of inflammatory markers compared with those with periods of transient unstable angina before the index event, suggesting that local mechanisms of coronary occlusion also may differ among those groups.

Increases in levels of IL-6 by >70% and SAA by ~10% in the culprit coronary artery relative to those in the aorta must be related to local release of these acute-phase proteins either from the arterial wall within the ruptured plaque or by blood cells trapped in the occluded coronary artery. Indeed, lipoproteins produced by the liver only were not increased locally. In agreement with the notion of local production of cytokines, immunohistochemistry demonstrated colocalization of IL-6 with monocytes in the thrombus occluding the culprit coronary artery (Figure 4), whereas apoB was not detectable. With directional coronary atherectomy, macrophage-rich areas could also be found in plaques from patients with unstable angina. Recently, CRP has been identified by immunohistochemistry in atherectomy specimens and was found to be elevated in the vessel wall of unstable compared with stable patients. Our results show that inflammatory mediators locally released within the culprit coronary artery are important in AMI. This is further supported by our in vitro findings in human vascular cells demonstrating biological activity of IL-6 at concentrations measured in the culprit artery.

The increase in local SAA blood levels in the culprit coronary artery is a novel and unexpected finding. With immunohistochemistry, SAA was detected both within the thrombus itself and in white blood cells contained therein. SAA, like CRP, is an acute-phase protein that, on induction by IL-6, is produced predominantly in the liver. Our observation of locally elevated levels indicates that SAA is also produced at the site of coronary occlusion either by cells of the atherosclerotic arterial wall or by the white blood cells trapped in the thrombus. In line with this interpretation, SAA mRNA and protein have been detected in human atherosclerotic lesions and cultured arterial smooth muscle cells. The importance of this finding is underscored by the fact that increased SAA plasma levels have also been identified as a predictor for adverse outcome in patients with unstable angina.

There is ongoing debate as to whether CRP is a marker of inflammation or an active mediator of the disease. Surprisingly and in contrast to IL-6 and SAA, we found CRP levels to be decreased in the blood obtained from the culprit lesion. Because we have ruled out the possibility that CRP was absorbed by the catheter, it must have undergone partial catabolism, phagocytosis, or uptake into the vessel wall while it was trapped. Indeed, CRP protein has been detected in human coronary plaques, and generation of CRP and complement has been reported in atherosclerotic plaque tissue. Immunohistochemical staining for CRP is increased in unstable plaques. In abdominal aortic aneurysms, 25% of the cases showed CRP mRNA in the explanted tissue, suggesting local production. Indeed, human coronary artery smooth muscle cells produce CRP in response to cytokines.

We would suggest that the amount of CRP produced locally must be rather small because its levels were decreased at the site of plaque rupture. Alternatively, because in AMI hepatic CRP production is markedly increased, one must also consider the possibility that the occluded culprit coronary artery acted as a closed compartment, thereby preventing systemically produced CRP from reaching the culprit lesion. However, because the systemic and local levels of Lp(a) were similar, this is unlikely. Thus, locally reduced CRP levels most likely reflect local uptake and catabolism of the protein. Most interestingly, immunohistochemistry of the thrombi showed the presence of CRP in phagocytizing white blood cells. Our data therefore indicate that CRP is taken up by phagocytizing white blood cells, although some influx into the vessel wall cannot be ruled out. Because CRP promotes recruitment of monocytes into the arterial wall and activates the complement system and foam cell formation, local uptake of CRP may play a pathogenic role in atherosclerotic lesion formation.

Recently, in the ostium of culprit coronary arteries in patients with acute coronary syndromes (ACS), elevated levels of IL-1 receptor antagonist and soluble CD40 ligand were found compared with the femoral artery, but no differences in CRP and IL-6 concentrations were seen. However, in the study by Agarwal et al, blood was obtained with a different sampling technique at the coronary ostium rather than at the site of plaque rupture. Indeed, given the high flow rate in the coronary arteries and the usually nonocclusive position of the guiding catheter, aspiration of blood from the ostium of a coronary artery is unlikely to deliver marker levels from remote culprit plaques. In our study, we have demonstrated cytokine gradients between the systemic circu-
fractional coronary occlusion, and the site of coronary occlusion, under conditions of distal outflow blockade and the use of an aspiration catheter reaching the culprit lesion. Our observations do not rule out that some degree of inflammation occurs in nonculprit coronary arteries; rather, they suggest that an important gradient exists between the site of plaque rupture and the aorta. The local cytokine gradient we observed disappeared rapidly after removal of thrombotic material and restoration of coronary flow by PCI. This can be explained by both removal of cytokine-producing monocytes trapped in the thrombus and dilution by systemic blood.

Limitations of the present in vivo analysis of local blood and thrombus obtained from culprit lesions in patients with AMI need to be considered. First, crossing the culprit lesion with a guidewire may result in partial restoration of flow and thus may change local marker concentrations. However, in most cases, we were able to cross the lesion directly with the PercuSurge guidewire without also using a more flexible conventional wire; thus, we avoided crossing the lesion twice. Furthermore, the distal occlusion was immediately installed. Although partial restoration of flow is an inherent limitation of this methodology, this phenomenon would work against our findings. Indeed, marked dilution with systemic blood would reduce rather than augment the differences observed. Second, the time interval from symptom onset to first balloon inflation was variable. Again, variability in the time of presentation is inherent in patients with ACS. Third, aspiration under distal protection cannot selectively harvest vessel wall or intact plaque; this is possible with directional atherectomy. However, it clearly contains the elements of ruptured plaque, which we have identified by microscopy and immunohistochemical staining. In particular, we have seen endothelial cells on the thrombi. This material provides immediate insight into freshly ruptured human coronary plaques. On the other hand, the use of directional atherectomy in the setting of acute PCI for AMI would clearly be against standard of care. Thus, particularly because a valid animal model for atherosclerotic coronary plaque rupture is currently not available,16 our data reflect a new in vivo approach to investigate the local processes in human AMI.

In summary, the striking differences in systemic and local coronary levels of inflammatory markers support the hypothesis that in AMI the inflammatory cytokine IL-6 and the acute-phase reactant SAA are produced at the site of coronary occlusion by macrophages and possibly vessel cells, whereas CRP is produced mainly in the liver and taken up locally by phagocytizing white blood cells. Because the amounts of produced IL-6 are biologically active in human endothelial and vascular cells, the observed changes are likely to contribute to the acute event.

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References


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