Elevated Levels of Shed Membrane Microparticles With Procoagulant Potential in the Peripheral Circulating Blood of Patients With Acute Coronary Syndromes

Ziad Mallat, MD, PhD; Hakim Benamer, MD; Bénédicte Hugel, PhD; Joëlle Benessiano, PhD; P. Gabriel Steg, MD, PhD; Jean-Marie Freysssinet, PhD; Alain Tedgui, PhD

**Background**—Apopotic microparticles are responsible for almost all tissue factor activity of the plaque lipid core. We hypothesized that elevated levels of procoagulant microparticles could also circulate in the peripheral blood of patients with recent clinical signs of plaque disruption and thrombosis.

**Methods and Results**—We studied 39 patients with coronary heart disease, including 12 patients with stable angina and 27 patients with acute coronary syndromes (ACS), and 12 patients with noncoronary heart disease. We isolated the circulating microparticles by capture with annexin V and determined their procoagulant potential with a prothrombinase assay. The cell origins of microparticles were determined in an additional 22 patients by antigenic capture with specific antibodies. The level of procoagulant microparticles did not differ between stable angina patients and noncoronary patients (10.1±1.6 nmol/L phosphatidylserine [PS] equivalent versus 9.9±1.6 nmol/L PS equivalent, respectively). However, procoagulant microparticles were significantly elevated in patients with ACS (22.2±2.7 nmol/L PS equivalent) compared with other coronary (P<0.01) or noncoronary (P<0.01) patients. Microparticles of endothelial origin were significantly elevated in patients with ACS (P<0.01), which suggests an important role for endothelial injury in inducing the procoagulant potential.

**Conclusions**—High levels of procoagulant endothelial microparticles are present in the circulating blood of patients with ACS and may contribute to the generation and perpetuation of intracoronary thrombi. *(Circulation. 2000;101:841-843.)*

**Key Words:** atherosclerosis • complications • thrombosis • microparticles • endothelium.
with SA (75%), 12 patients with UA (92%), and 14 patients with MI (100%).

Controls (9 men and 3 women, mean age 58±4 years) were patients with angiographic documentation of absence of CAD (4 patients with chest pain, 4 patients with valvular disease, and 4 patients with dilated cardiomyopathy).

To characterize the cell origins of the microparticles, we included 16 additional consecutive patients with angina and angiographic documentation of CAD (5 with SA, mean age 63±5 years, and 11 with ACS, mean age 62±4 years) and 6 noncoronary patients (5 with valvular disease and 1 with dilated cardiomyopathy, mean age 65±8 years).

**Isolation of the Circulating Microparticles and Determination of Their Procoagulant Potential**

Blood samples were collected before any mechanical intervention at admission (day 0), except in 6 patients with MI, in whom blood sampling was performed at day 8 after the coronary event. Microparticles were captured by immobilized annexin V, and the anionic phospholipid content was determined by a prothrombinase assay as previously described in detail.9,10 We verified that the method used for the capture of microparticles did not allow the capture of PS-containing lipoproteins.

**Determination of the Cell Origins of Circulating Microparticles**

Microparticles were captured by specific antibodies (anti-CD3, anti-CD11a, anti-CD31, anti-CD146, and anti-GP Ib).9,10 The morphology of circulating microparticles was recently published.11,12

**Statistical Analysis**

Results are expressed as mean±SEM. Comparisons between groups were made by a 1-way ANOVA. Simple regression analysis was performed to analyze the relation between values of microparticles captured with anti-CD31, anti-CD146, or anti-GP Ib antibodies. A value of P<0.05 was considered statistically significant.

**Results**

The level of circulating microparticles in patients admitted with a diagnosis of SA was not significantly different from that in patients admitted for noncoronary heart disease (10.1±1.6 versus 9.9±1.6 nmol/L PS equivalent, respectively). However, the level of procoagulant microparticles was significantly elevated in the circulating blood of patients with UA (18.6±1.9 nmol/L PS) or MI (25.6±4.8 nmol/L PS) compared with patients who presented with signs of SA (P<0.01) or with noncoronary patients (P<0.01). In the patients with ACS, no difference was found in the level of circulating microparticles between those with UA and those with MI (Figure).

Two patients experienced recurrent ischemic syndromes during hospitalization: 1 patient in the UA group developed recurrent documented myocardial ischemia, and 1 in the MI group had reocclusion of his stented culprit coronary artery. Interestingly, these 2 patients had very high baseline levels of circulating procoagulant microparticles (35.6 and 62.3 nmol/L PS, respectively).

Circulating microparticles captured with anti-CD146 or anti-CD31 antibody were elevated in patients with ACS (compared with both stable coronary and noncoronary patients), whereas those captured with anti-GP Ib, anti-CD3, or anti-CD11a were not (Table). The values obtained with anti-CD31 antibody were not correlated with those obtained with anti-GP Ib antibody but were highly correlated with those obtained with anti-CD146 antibody (P<0.001). Given that there were almost no CD31-bearing microparticles, these findings indicate that microparticles captured with anti-CD31 or anti-CD146 were most likely of endothelial origin.

**Discussion**

In the present study, the levels of circulating procoagulant microparticles in patients who presented with SA were not different from those found in noncoronary patients. However, we found significantly elevated levels of circulating procoagulant microparticles in the patients who presented with ACS in comparison with both SA patients and noncoronary patients. These results suggest that an increase in circulating microparticles more specifically reflects the complications of atherosclerosis (ie, thrombus formation occurring on the contact of a disrupted atheromatous plaque). Because only patients with ACS received heparin, it could be argued that differences in levels of microparticles reflect differences in medications. This is unlikely, however, because heparin has been reported either to have no effect or to decrease microparticle generation from thrombin-activated platelets.13

Soejima et al14 recently reported that plasma TF antigen levels are significantly elevated in patients with UA compared with those measured in patients with SA and are associated with a poor prognosis. However, these authors did not measure TF activity, nor did they determine the origin of the TF antigen. Our findings extend those of Soejima et al and suggest that the procoagulant potential of the circulating blood is, at least in part, related to the presence of elevated levels of circulating procoagulant microparticles. Moreover, the prevalence of microparticles bearing CD146 and CD31 suggests a potentially important role for acute endothelial injury in this process. This may reflect the endothelial erosion at the site of plaque disruption, the endothelial injury on exposure of plaque microvessels to inflammatory cells, and/or the endothelial injury associated with myocardial ischemia. It should be noted that our data do not exclude the possibility that a certain amount of circulating microparticles may also originate from other cell types, including smooth muscle cells and cardiomyocytes.

The detection of elevated levels of circulating procoagulant microparticles 8 days after the acute ischemic syndrome is in line with the observation of persistent intracoronary thrombi 24 hours to 30 days after the ischemic episode.15 In the
Antigenic Characterization of the Circulating Microparticles in Patients With SA, ACS or Noncoronary Heart Disease (NC)

<table>
<thead>
<tr>
<th>Capture Antibody</th>
<th>SA (n=5)</th>
<th>ACS (n=11)</th>
<th>NC (n=6)</th>
<th>P, 1-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD146</td>
<td>3.3±0.9</td>
<td>8.2±1.4</td>
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<td>&lt;0.01</td>
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<td>CD3</td>
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<td>GP Ib</td>
<td>2.6±0.5</td>
<td>6.9±1.8</td>
<td>6.5±1.5</td>
<td>NS</td>
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<td>0</td>
<td>0.2±0.2</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>CD11a</td>
<td>0.6±0.3</td>
<td>1.7±0.9</td>
<td>0.2±0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as nmol/L PS equivalent.

References

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