

## Enhanced Shear-Induced Platelet Aggregation in Acute Myocardial Infarction

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**Background**—Experiments under controlled flow conditions indicate that the binding of von Willebrand factor (vWF) to platelet glycoprotein (GP) Ib $\alpha$  and integrin  $\alpha_{IIb}\beta_3$  (GP IIb/IIIa complex) is crucial for aggregation at elevated shear rates. We have tested how the plasma of patients with acute myocardial infarction affects this process.

**Methods and Results**—Citrate plasma was obtained from 18 patients with acute myocardial infarction within 6 hours from the onset of symptoms and from 26 control subjects with chest pain syndrome without evidence of ischemia. Aggregation of normal platelets at high shear rates was significantly greater in the presence of patient than control plasma and was inhibited by both anti-GP Ib $\alpha$  and anti- $\alpha_{IIb}\beta_3$  monoclonal antibodies. The observed values (mean $\pm$ SD) were 47.6 $\pm$ 17.8% versus 30.1 $\pm$ 9.9% at 10 800 s $^{-1}$  ( $P<0.01$ ) and 32.9 $\pm$ 14.1% versus 17.5 $\pm$ 9.5% at 7200 s $^{-1}$  ( $P<0.01$ ), respectively, and were positively correlated with plasma vWF antigen levels and ristocetin cofactor activities. In contrast, at the lower shear rate of 1200 s $^{-1}$ , aggregation was similar in the presence of control or patient plasma and was not inhibited by the anti-GP Ib $\alpha$  antibody. Both vWF antigen and platelet aggregation decreased 2 weeks after the onset of myocardial infarction.

**Conclusions**—Shear-induced platelet aggregation is enhanced in plasma in the presence of acute myocardial infarction, apparently as a result of increased vWF concentration. This may contribute to the onset of acute coronary artery thrombosis and early reocclusion after reperfusion treatment. (*Circulation*. 1999;99:608-613.)

**Key Words:** platelets ■ von Willebrand factor ■ glycoproteins ■ thrombosis

Unstable angina and acute myocardial infarction are often associated with the development of fresh platelet-rich thrombi,<sup>1,2</sup> suggesting that platelet adhesion and aggregation, as well as fibrin deposition, are pathogenetic factors in ischemic heart disease. Moreover, reperfusion treatment by thrombolysis or angioplasty<sup>3-5</sup> may reduce in-hospital mortality and preserve left ventricular pump function in patients with acute myocardial infarction, but its efficacy is limited by the occurrence of thrombotic reocclusion after revascularization.<sup>6</sup> Animal studies and clinical observations suggest that increased thrombin activity and platelet aggregation, both amenable to control by pharmacological agents, may be involved in the occurrence of reocclusion.<sup>7-9</sup> Thus, understanding the mechanisms that regulate platelet reactivity may lead to better prevention and treatment of ischemic heart disease. Fluid dynamic forces may be relevant in this regard, because blood flow through stenotic atherosclerotic arteries results in extremely elevated shear rates.<sup>10</sup> Under such hemodynamic conditions in ex vivo experiments, the adhesive protein von Willebrand factor (vWF) and its 2 membrane receptors, glycoprotein (GP) Ib $\alpha$  and integrin  $\alpha_{IIb}\beta_3$  (GP IIb/IIIa), are crucial for platelet aggregation.<sup>11-13</sup>

Moreover, epidemiological studies have shown a positive correlation between plasma vWF levels and incidence of heart disease caused by arterial thrombosis.<sup>14,15</sup> All these results suggest that vWF may be involved in the onset of coronary events, but whether this depends on its effects on the thrombogenic potential of platelets in flowing blood remains to be established.

In the present study, we have found that the plasma of patients with acute myocardial infarction significantly augments the aggregation of platelets exposed to high shear rates.<sup>16</sup> Our results suggest that increased vWF concentration, possibly in association with other humoral factors, may be responsible for this phenomenon and imply that vWF-dependent shear-induced platelet aggregation may have a causative role in the onset of acute coronary thrombosis leading to myocardial infarction or reocclusion after reperfusion treatment.

### Methods

#### Patient Selection

Eighteen patients admitted to Tokai University Hospital in Kanagawa, Japan, within 6 hours of the onset of acute myocardial

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**TABLE 1. Clinical Characteristics of Enrolled Patients**

Case Patient	Age, y	Location and Severity of Coronary Artery Disease
1	66	LAD #7, 100%
2	49	LAD #7, 99%
3	87	LMT #5, 99%
4	82	LAD #7, 99%
5	47	RCA #2, 100%
6	39	LAD #7, 100%
7	62	LAD #7, 100%
8	54	LAD #6, 100%
9	64	RCA #2, 100%
10	59	RCA #2, 100%
11	39	RCA #1, 100%
12	56	LCx #13, 100%
13	58	RCA #1, 100%
14	39	LAD #7, 100%
15	57	RCA #1, 100%
16	22	LAD #6, 100%
17	59	RCA #2, 100%
18	56	LCx #13, 100%

LAD indicates left anterior descending artery; LMT, left main trunk; RCA, right coronary artery; and LCx, left circumflex coronary artery.

Complete occlusion of coronary artery is indicated as 100%; >90% stenosis in luminal diameter with delay in coronary arterial opacification<sup>17</sup> is indicated as 99%.

infarction symptoms, who had not received medications known to interfere with platelet function, gave written informed consent to enter the study. The characteristics of their coronary artery disease<sup>17</sup> are summarized in Table 1. The diagnosis was based on typical chest pain resistant to nitroglycerin, ECG ST-segment elevation in  $\geq 2$  leads with or without Q-wave formation, and significant increase in plasma cardiac enzymes, including creatine phosphokinase (CPK) and lactate dehydrogenase. The MB isozyme of CPK, a relatively specific indicator of myocardial damage, was elevated by >5% in all enrolled patients. Emergency coronary angiography and subsequent revascularization by balloon angioplasty were performed in all cases.

### Sample Preparation

Blood samples were drawn from the femoral vein with a 19-gauge needle before any medical treatment and transferred from a plastic syringe into a tube containing 10% vol of 3.8% trisodium citrate (pH 7.4). Platelet-poor plasma was separated from blood cells by centrifugation at 1500g for 20 minutes and stored at  $-80^{\circ}\text{C}$  until used. In 7 cases (patients 11, 12, 14, 15, 16, 17, and 18 in Table 1), plasma for measuring vWF antigen concentration was obtained immediately after reperfusion treatment and then 7 and 14 days after the diagnosis of myocardial infarction. Plasma from normal donors who had not taken drugs known to interfere with platelet function was prepared and stored in the same manner. Plasma vWF antigen was also determined in 26 patients (aged  $53.1 \pm 8.2$  years; 18 men and 8 women) who presented with chest pain without evidence of ischemia induced by treadmill exercise. Ten of the 26 control samples were randomly selected for additional determination of the ristocetin cofactor activity of vWF (vWF RCA) and shear-induced platelet aggregation.

### Shear-Induced Platelet Aggregation

The influence of patient and control plasma on shear-induced aggregation of normal platelets in the absence of exogenous agonists was tested in an optically modified cone-and-plate viscometer.<sup>18</sup>

Platelets were prepared before each experiment from the same 36-year-old normal male volunteer with blood group O. Platelet-rich plasma (PRP) was prepared by centrifugation at 120g for 15 minutes and adjusted to a platelet count of  $4 \times 10^5/\mu\text{L}$  by addition of homologous platelet-poor plasma obtained after further centrifugation of blood at 1500g for 15 minutes. The effect of acute myocardial infarction plasma on platelet aggregation was tested in mixtures of 200  $\mu\text{L}$  of normal PRP and 200  $\mu\text{L}$  of patient or control platelet-poor plasma. The effect of exogenously added purified human vWF was tested in the mixture of 200  $\mu\text{L}$  of normal PRP, 180  $\mu\text{L}$  of control platelet-poor plasma, and 20  $\mu\text{L}$  of HEPES-NaCl solution (pH 7.4) with or without vWF to achieve a final concentration of 15 or 30  $\mu\text{g/mL}$ . Aggregation was evaluated at 10 800  $\text{s}^{-1}$  (108 dyne/cm<sup>2</sup>) and 7200  $\text{s}^{-1}$  (72 dyne/cm<sup>2</sup>), shear rates at which it is dependent on vWF, as well as at 1200  $\text{s}^{-1}$  (12 dyne/cm<sup>2</sup>), in which case it is mediated by fibrinogen.<sup>18</sup> Shear stress was calculated assuming a viscosity of 1 centipoise because no red cells were present in the experimental mixtures. We calculated the extent of platelet aggregation from the changes in light transmittance through the sample, recorded at 1-second intervals, using the Lambert-Beer equation.<sup>12,18</sup>

### Monoclonal Antibodies

LJ-Ib1, an anti-GP Ib $\alpha$  murine IgG1, inhibits vWF binding<sup>19</sup> and platelet aggregation at high shear rates but not agonist-induced aggregation at lower shear rates.<sup>18</sup> LJ-CP8, a complex specific anti- $\alpha_{\text{IIb}}\beta_3$  murine IgG1, inhibits fibrinogen and vWF binding to activated platelets as well as shear-induced and agonist-induced platelet aggregation.<sup>18,20</sup>

### Plasma Antigen Level, RCA, and Multimeric Distribution of vWF

The plasma concentration of vWF antigen was measured by enzyme immunoassay<sup>21</sup> in 18 acute myocardial infarction and 26 control plasma samples. RCA was measured by a fixed platelet ELISA method<sup>22</sup> in all myocardial infarction and in 10 randomly selected control plasma samples. The multimeric distribution of vWF was analyzed by SDS-agarose gel electrophoresis<sup>23</sup> in 10 myocardial infarction (patients 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 in Table 1) and 2 control samples. vWF antigen level and RCA were also measured serially in 7 selected cases of myocardial infarction, as described above.

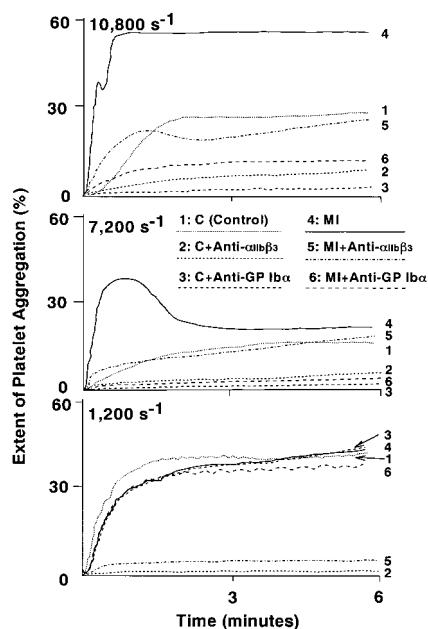
### Statistics

The null hypothesis between control and patient results was evaluated by use of the Student *t* test for unpaired samples. Serial data were evaluated by ANOVA with the Newman-Keuls multiple comparison procedure. A *P* value of <0.05 was considered statistically significant.

## Results

### Platelet Aggregation Under Shearing Flow

Platelet aggregation induced by shear rates of 10 800 and 7200  $\text{s}^{-1}$  but not 1200  $\text{s}^{-1}$  was enhanced in the presence of plasma from several patients with acute myocardial infarction compared with control plasma (Figure 1). The mean  $\pm$  SD of the maximum extent of aggregation at 10 800  $\text{s}^{-1}$  was  $47.6 \pm 17.8\%$  with patient plasma and  $30.1 \pm 9.9\%$  with control plasma; at 7200  $\text{s}^{-1}$ , the respective values were  $32.9 \pm 14.1\%$  and  $17.5 \pm 9.5\%$ . The difference was significant at the 2 higher shear rates ( $P < 0.01$ ) but not at 1200  $\text{s}^{-1}$  (Table 2). In spite of the clear group separation, some individual control and patient values overlapped (Figure 2). Not only the extent but also the maximum rate of aggregation at the higher shear rates was significantly greater in the presence of patient than control plasma, more so at 7200 than at 10 800  $\text{s}^{-1}$  (Table 2). In fact, the maximum rate of aggregation at 7200

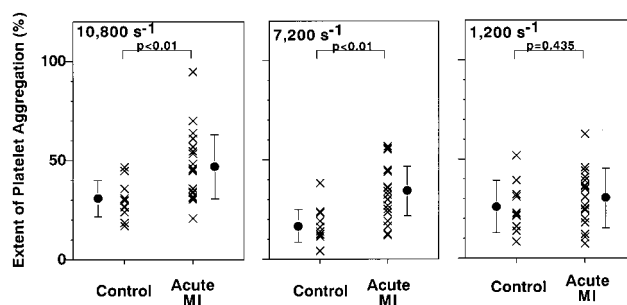


**Figure 1.** Representative tracing of shear-induced platelet aggregation in the presence of plasma from either a case of acute myocardial infarction (MI) or a control. Extent of platelet aggregation shown here was obtained at 3 indicated shear rates, without or with the addition of monoclonal antibodies specifically inhibiting GP Ib $\alpha$  or  $\alpha$ IIb $\beta$ <sub>3</sub>.

$s^{-1}$  was  $\approx 33\%$  of that at  $10\,800\,s^{-1}$  with control plasma but essentially identical with acute myocardial infarction plasma (Table 2). These results indicate that the latter condition lowered the threshold for rapid platelet response to high shear stress. At both  $10\,800$  and  $7\,200\,s^{-1}$ , aggregation with either control or patient plasma was inhibited by antibody LJ-Ib1 (Figure 1), which is in agreement with the known role of vWF binding to GP Ib $\alpha$  in the initiation of shear-induced aggregation. Inhibition, although less pronounced, was also observed with the anti- $\alpha$ IIb $\beta$ <sub>3</sub> antibody LJ-CP8 (Figure 1). In contrast, LJ-Ib1 had no effect at  $1\,200\,s^{-1}$ , whereas LJ-CP8 caused complete inhibition (Figure 1), which supports the concept that aggregation induced by this relatively low shear is mediated by fibrinogen binding to  $\alpha$ IIb $\beta$ <sub>3</sub> and is independent of vWF interaction with GP Ib $\alpha$ .<sup>12,18</sup>

### Plasma Concentration of vWF

Concentrations of vWF antigen and RCA were significantly higher in patients with acute myocardial infarction than in normal controls (Figure 3). Larger vWF multimers were relatively increased in 3 patients (patients 1, 7, and 8) but not in the remaining 7 (data not shown). The extent of aggrega-



**Figure 2.** Individual values of shear-induced platelet aggregation in the presence of acute myocardial infarction or control plasma. Each symbol indicates extent of aggregation at indicated shear rates observed after mixing normal platelets with individual plasma samples from patients with myocardial infarction or controls. Also shown are corresponding mean values (filled circles)  $\pm$  SD (bars) in each group.

tion at  $10\,800$  and  $7\,200\,s^{-1}$  but not at  $1\,200\,s^{-1}$  was significantly correlated with the plasma levels of vWF antigen and RCA in the patients (Figure 4), which suggests a causative role for vWF in the observed phenomenon. Accordingly, platelet aggregation at  $10\,800$  and  $7\,200\,s^{-1}$  but not at  $1\,200\,s^{-1}$  was enhanced by the addition of purified human vWF to normal PRP (Figure 5). Plasma vWF antigen (Figure 6) and shear-induced aggregation (Figure 7) did not change significantly after successful revascularization, which suggests that these parameters are not strictly a reflection of platelet activation or endothelial cell damage in the affected coronary arteries. However, a significant ( $P=0.02$ ) difference in serial measurements of vWF antigen was found in the patients, with lower values at 14 days after the onset of myocardial infarction than before or immediately after PTCA (Figure 6;  $P<0.01$  in either case). Platelet aggregation at high shear rate (Figure 7) was also decreased by 14 days after the onset of acute myocardial infarction.

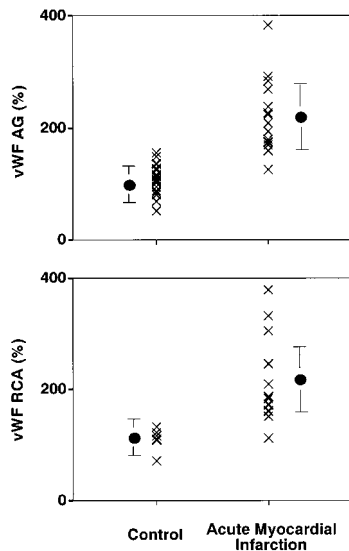
### Discussion

Our results indicate that the plasma of patients with acute myocardial infarction can enhance shear-induced platelet aggregation, and vWF appears to be the relevant if not essential determinant of this response, possibly contributing to the onset of coronary thrombosis and early reocclusion of recanalized arteries. Elevation of plasma vWF from any cause may be accompanied by heightened shear-induced aggregation, as shown in the present study by the addition of purified vWF to normal plasma or in patients with effort angina after treadmill exercise.<sup>24</sup> However, plasma vWF levels may become a risk factor for arterial thrombosis only when altered

**TABLE 2. Maximum Extent and Maximum Rate of Platelet Aggregation Induced by Different Shear Rates**

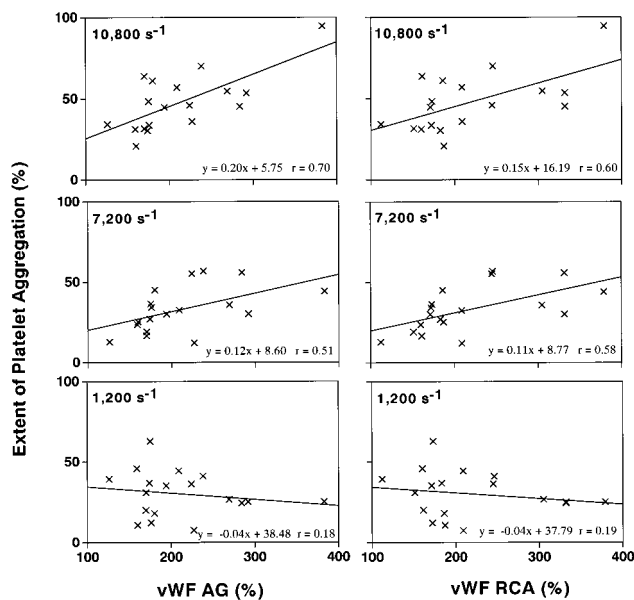
	Aggregation, %			Rate (% Aggregation/Second)		
	$10\,800\,s^{-1}$	$7\,200\,s^{-1}$	$1\,200\,s^{-1}$	$10\,800\,s^{-1}$	$7\,200\,s^{-1}$	$1\,200\,s^{-1}$
Control	$30.1 \pm 9.9$	$17.5 \pm 9.4$	$25.8 \pm 13.0$	$0.82 \pm 0.36$	$0.28 \pm 0.15$	$1.02 \pm 1.12$
Acute MI	$47.6 \pm 17.8$	$32.9 \pm 14.1$	$30.1 \pm 14.1$	$1.77 \pm 1.74$	$1.51 \pm 1.48$	$0.98 \pm 0.88$
P	$<0.01$	$<0.01$	0.435	$<0.05$	$<0.01$	0.526

MI indicates myocardial infarction.

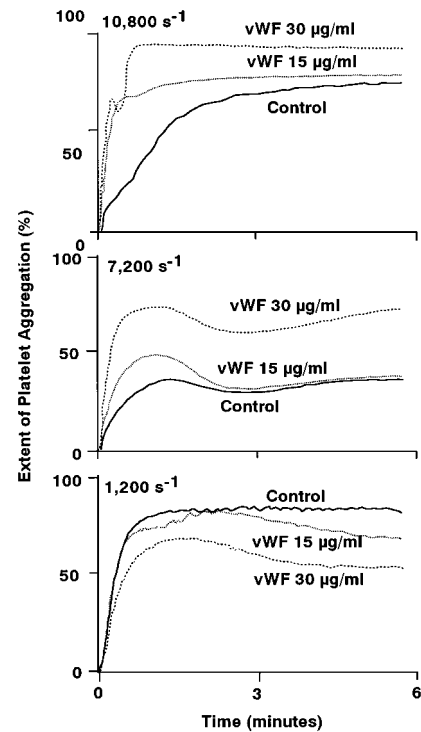


**Figure 3.** Concentration of vWF antigen (vWF AG) and RCA in acute myocardial infarction and normal donor plasma. Each symbol indicates the concentration of vWF antigen or RCA in an individual plasma sample. vWF antigen level was measured in all enrolled patients with acute myocardial infarction and all 26 controls, whereas vWF RCA was measured only in 10 randomly selected controls. Mean  $\pm$  SD of each group is shown with a circle and bars on the side of the corresponding individual values. Both vWF antigen and RCA were significantly higher in plasma from patients in the acute phase of myocardial infarction than in plasma from normal donors ( $P < 0.01$ ).

hemodynamic conditions at the site of severe coronary artery stenosis result in abnormally high shear rates.<sup>25</sup> Such conditions may arise suddenly from the progression of atherosclerotic plaques restricting the arterial lumen beyond a critical

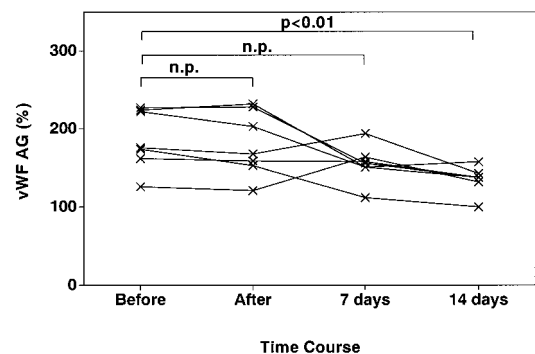


**Figure 4.** Correlation between plasma levels of vWF antigen (vWF AG) or RCA and extent of shear-induced platelet aggregation. The different shear rate values used in these studies are shown in the upper left corner of each panel. In patients, the extent of aggregation occurring at the higher shear rates of 10 800  $s^{-1}$  and 7200  $s^{-1}$  but not at the lower shear rate of 1200  $s^{-1}$  was significantly correlated with plasma levels of vWF AG and RCA.



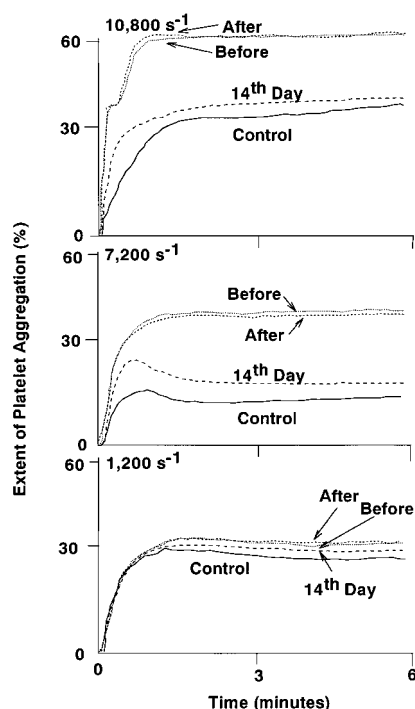
**Figure 5.** Effect of exogenously added purified human vWF on shear-induced platelet aggregation. Exogenously added purified human vWF enhanced in a dose-dependent manner both the extent and maximal rate of platelet aggregation occurring at the higher shear rates of 10 800 and 7200  $s^{-1}$ . In contrast, the apparent inhibitory effect of vWF at 1200  $s^{-1}$  was likely due to competition with fibrinogen for binding to integrin  $\alpha_{IIb}\beta_3$ , because fibrinogen is known to be a more effective ligand than vWF for aggregation at lower shear rates. The figure shows representative results of 1 of 3 different experiments.

point, possibly with the contribution of vasospasm, and may be precipitated by the exposure of thrombogenic surfaces after plaque rupture.<sup>1,2</sup> Thus, enhanced vWF-dependent platelet aggregation is only 1 of several possible coincidental factors involved in the onset of myocardial infarction, but it may be a prevailing cause of acute thrombotic occlusion in the context of chronic coronary artery disease. The evidence provided herein that abnormally increased vWF concentra-



**Figure 6.** Serial determinations of plasma vWF antigen (vWF AG) after acute myocardial infarction. Each line links the symbols corresponding to the plasma vWF antigen concentration in individual patients with acute myocardial infarction before and at different times after successful revascularization treatment by PTCA. n.p. indicates  $P = NS$ .





**Figure 7.** Shear-induced platelet aggregation in presence of plasma from a patient with acute myocardial infarction before and at different times after reperfusion treatment with PTCA. Aggregation occurring at shear rates of 10 800 and 7200  $\text{s}^{-1}$  but not at 1200  $\text{s}^{-1}$  was enhanced in presence of acute myocardial infarction plasma compared with control, and there was no difference whether the sample had been obtained before or immediately after successful reperfusion treatment. Aggregation was lower in patients 14 days after onset of myocardial infarction than in the acute phase. Figure shows results of 1 experiment with patient 11 (Table 1), but the findings were similar in 2 additional patients (patients 14 and 16), except that with plasma from patient 16, the extent of aggregation 14 days after onset of myocardial infarction was even less than with control plasma.

tions further enhance shear-induced platelet aggregation may explain why elevated plasma levels of this adhesive protein constitute a risk factor for ischemic heart disease.<sup>14,15</sup>

The effects on aggregation at high shear rates were observed when normal platelets and plasma from patients with acute myocardial infarction were mixed, thus ruling out the possibility that acquired platelet abnormalities could play a major role in the phenomenon.<sup>26</sup> Plasma vWF remained unchanged after successful reperfusion treatment, which suggests that the reasons for its increase were not simply related to acute platelet or endothelial cell release at the site of coronary thrombosis. Increased plasma vWF concentrations persisted for 1 week after the onset of symptoms but significantly decreased by the end of the second week, which indicates that this change is more likely related to the onset of acute myocardial infarction than to an associated chronic abnormality such as atherosclerosis. Although the causative mechanism remains to be elucidated, our findings may be explained in part by the occurrence of pulmonary congestion secondary to acute myocardial infarction, because it has previously been pointed out that plasma vWF concentrations are increased in patients with heart failure regardless of the underlying heart disease.<sup>27–30</sup> In accordance with this hypoth-

esis, plasma vWF levels have been shown to be correlated with pulmonary capillary wedge pressure.<sup>31</sup> In the present study, the highest vWF plasma levels were demonstrated in patients with delayed blood flow in the main trunk of the left coronary artery, resulting in severe pulmonary edema. Thus, the clinical observation that myocardial infarction recurs more frequently in patients with heart failure<sup>32</sup> may be explained by increased vWF concentrations and subsequent enhanced risk of vWF-dependent platelet thrombus formation.

We have previously shown that low concentrations of epinephrine, equivalent to those induced by strenuous sympathetic stimulation, augment vWF-dependent shear-induced platelet activation and aggregation, as well as aggregation at a low shear rate.<sup>33,34</sup> Because the present results are seen only at higher shear rates, it appears that the levels of secreted epinephrine in plasma from patients with acute myocardial infarction are not sufficiently elevated to enhance platelet aggregation after being mixed in equal proportion with normal plasma. In vivo, however, epinephrine release may contribute to increased risk of arterial thrombosis, possibly through an effect on platelets distinct from that produced by elevated plasma vWF levels.<sup>16</sup>

Recently developed specific antiplatelet agents, including the chimeric 7E3 antibody, have proven effective in the prevention of acute coronary artery occlusion after angioplasty.<sup>9,35</sup> These agents inhibit not only fibrinogen but also vWF binding to activated  $\alpha_{\text{IIb}}\beta_3$  and subsequent shear-induced platelet thrombus growth.<sup>36</sup> Although it is generally maintained that fibrinogen is the exclusive mediator of platelet aggregation initiated by exogenous chemical agonists,<sup>37</sup> a more careful consideration of the effects of fluid dynamic forces has revealed that vWF may provide a major contribution to interplatelet cohesion during thrombus formation.<sup>10–12,38,39</sup> Such a conclusion is supported by epidemiological studies that have linked vWF, as well as fibrinogen, to the risk of developing acute coronary events.<sup>14,15</sup> Thus, it seems reasonable to propose that vWF may be 1 of the ligands involved in mediating platelet aggregation in blood flowing with high shear rates. Experimental evidence in this regard has been obtained with the demonstration that even after strong activation by exogenous chemical agonists, fibrinogen binding by itself is not sufficient to support interplatelet contacts with adequate adhesive strength to oppose high shear stress.<sup>40</sup> In such conditions, which may be of paramount importance in the arterial circulation, particularly in the presence of stenosis, the role of vWF in mediating optimal platelet aggregation appears to be essential, notably with the involvement not only of GP  $\text{Ib}\alpha$  but also of  $\alpha_{\text{IIb}}\beta_3$ .<sup>40</sup> Consequently, the clinical effects of pharmacological inhibitors of  $\alpha_{\text{IIb}}\beta_3$  function may depend on blocking platelet binding of vWF as well as fibrinogen. A conclusive evaluation of this hypothesis in the clinical setting must await the availability of drugs that selectively inhibit the interaction of vWF with platelets.

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