

Increased Platelet Reactivity and Circulating Monocyte-Platelet Aggregates in Patients With Stable Coronary Artery Disease

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Objectives. We sought to examine whether patients with stable coronary artery disease (CAD) have increased platelet reactivity and an enhanced propensity to form monocyte-platelet aggregates.

Background. Platelet-dependent thrombosis and leukocyte infiltration into the vessel wall are characteristic cellular events seen in atherosclerosis.

Methods. Anticoagulated peripheral venous blood from 19 patients with stable CAD and 19 normal control subjects was incubated with or without various platelet agonists and analyzed by whole blood flow cytometry.

Results. Circulating degranulated platelets were increased in patients with CAD compared with control subjects (mean [\pm SEM] percent P-selectin-positive platelets: 2.1 ± 0.2 vs. 1.5 ± 0.2 , $p < 0.01$) and were more reactive to stimulation with $1 \mu\text{mol/liter}$ of adenosine diphosphate (ADP) (28.7 ± 3.9 vs. 16.1 ± 2.2 , $p < 0.01$), $1 \mu\text{mol/liter}$ of ADP/epinephrine (51.4 ± 4.6

vs. 37.5 ± 3.8 , $p < 0.05$) or $5 \mu\text{mol/liter}$ of thrombin receptor agonist peptide (TRAP) (65.7 ± 6.8 vs. 20.2 ± 5.1 , $p < 0.01$). Patients with stable CAD also had increased circulating monocyte-platelet aggregates compared with control subjects (percent platelet-positive monocytes: 15.3 ± 3.0 vs. 6.3 ± 0.9 , $p < 0.01$). Furthermore, patients with stable CAD formed more monocyte-platelet aggregates than did control subjects when their whole blood was stimulated with $1 \mu\text{mol/liter}$ of ADP (50.4 ± 4.5 vs. 28.1 ± 5.3 , $p < 0.01$), $1 \mu\text{mol/liter}$ of ADP/epinephrine (60.7 ± 4.3 vs. 48.0 ± 4.8 , $p < 0.05$) or $5 \mu\text{mol/liter}$ of TRAP (67.6 ± 5.7 vs. 34.3 ± 7.0 , $p < 0.01$).

Conclusions. Patients with stable CAD have circulating activated platelets, circulating monocyte-platelet aggregates, increased platelet reactivity and an increased propensity to form monocyte-platelet aggregates.

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The pathophysiology of atherosclerosis involves thrombosis and inflammation (1-3), conditions in which cellular activation plays a predominant role. Traditionally these two cellular processes have been studied independently of each other. However, the heterotypic interaction between platelets and leukocytes has recently become a focus of attention (4-10).

It is well established that serologic markers of platelet activation are present in acute coronary artery syndromes (2,11-13). However, direct evidence of hyperreactive or

"primed" platelets in stable coronary artery disease (CAD) is lacking. Furthermore, recent evidence points to a major role for leukocytes (14,15) and humoral inflammatory mediators in the pathogenesis of coronary atherosclerosis (1,16-18).

P-selectin (also known as CD62P, previously known as GMP-140 and PADGEM protein), a component of the platelet alpha granule membrane, is translocated to the surface membrane on platelet activation (19,20). P-selectin mediates the binding of platelets to leukocytes (21,22). Leukocytes have been associated with platelets at sites of hemorrhage (23) and during atherogenesis (1). P-selectin-mediated circulating leukocyte-platelet aggregates have been noted after exposure to oxidized low density lipoprotein (24) and cigarette smoke (25), both of which are known risk factors for the development of atherosclerosis. Recent reports have suggested an increase in leukocyte-platelet interactions in patients with unstable angina (10) and in those who have had percutaneous transluminal coronary angioplasty (9).

In this study, we used whole blood flow cytometry to investigate platelet activation and leukocyte-platelet aggregation in patients with stable CAD. Whole blood flow cytometry has the advantage of directly analyzing individual cells in their native milieu with minimal artifactual cellular activation (26).

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Abbreviations and Acronyms

ADP	= adenosine diphosphate
CAD	= coronary artery disease
FITC	= fluorescein isothiocyanate
GP	= glycoprotein
Ig	= immunoglobulin
RANTES	= regulated on activation normal T cell expressed presumed secreted
TRAP	= thrombin receptor activating peptide

Methods

Study group. The protocol was approved by the Committee for the Protection of Human Subjects in Research at the University of Massachusetts Medical Center. The study group consisted of patients undergoing outpatient cardiac catheterization at the University of Massachusetts Medical Center for symptomatic stable angina. This group of patients was chosen to ensure documentation by angiography of the extent of CAD. No subject had rest pain or a myocardial infarction within 3 months of the study. Sampling occurred before administration of heparin or contrast agents. The characteristics of the study group are displayed in Table 1. The control group consisted of healthy nonsmoking adult volunteers (10 men and 9 women) not taking any medication and who had not taken any antiplatelet agent for at least 10 days before sampling. Coronary artery disease was excluded in the control group by a self-reported lack of symptoms suggestive of CAD or a documented cardiac event.

Monoclonal antibodies. S12 and G1 (Centacor) are directed against different epitopes on P-selectin (22). S12 was biotinylated as previously described (27). Y2/51 (DAKO) is directed against glycoprotein (GP) IIIa (28) and was purchased conjugated to fluorescein isothiocyanate (FITC). 7E3 (courtesy of Dr. Barry Coller, Mt. Sinai Medical School, New York, New York) is directed against the GPIIb-IIIa complex (29) and was FITC-conjugated with QUICK TAG Kit (Boehringer Mannheim). BEAR1 (Immunotech) is directed against the M chain of CD11b (MAC-1) (30) on leukocytes and was purchased FITC-conjugated. S12, G1, Y2/51, 7E3 and BEAR1 are all immunoglobulin (Ig) G murine antibodies.

Whole blood flow cytometry. Platelet activation state. The method has been previously described in detail (31). There were no centrifugation, gel filtration, vortexing or stirring steps that could artifactually activate platelets. Blood from patients and control subjects was drawn into a sodium citrate Vacutainer (Becton Dickinson). Within 15 min of drawing, the blood was diluted 1:4 in modified HEPES-Tyrode's buffer (137 mmol/liter NaCl, 2.8 mmol/liter KCl, 1 mmol/liter MgCl₂, 12 mmol/liter NaHCO₃, 0.4 mmol/liter Na₂HPO₄, 0.35% bovine serum albumin, 10 mmol/liter HEPES, 5.5 mmol/liter glucose), pH 7.4, containing the peptide glycyl-L-prolyl-L-arginyl-L-proline (Calbiochem) at a concentration of 2.5 mmol/liter to prevent fibrin polymerization (27) and either 1) no agonist; 2) adenosine diphosphate (ADP), 1 μmol/liter or 10 μmol/liter

(BIO/DATA); 3) ADP, 1 μmol/liter with epinephrine, 1 μmol/liter (BIO/DATA) or ADP, 10 μmol/liter with epinephrine, 10 μmol/liter; 4) thrombin receptor activating peptide (TRAP), 5 μmol/liter or 30 μmol/liter (Calbiochem); or 5) purified human alpha-thrombin (provided by Dr. John W. Fenton II, New York Department of Health, Albany, New York), 2 U/ml.

After 10 min, all samples were fixed at 22°C for 20 min with 1% formaldehyde (final concentration). After fixation, samples were diluted 10-fold in modified Tyrode's buffer, pH 7.4. The samples were then incubated at 22°C for 20 min with a near-saturating concentration of FITC-conjugated monoclonal antibody Y2/51 and a saturating concentration of biotinylated monoclonal antibody S12, followed by incubation at 22°C for 20 min with 30 μg/ml of phycoerythrin-streptavidin (Jackson ImmunoResearch). The platelet surface expression of P-selectin is calcium-independent (32).

As previously described (27), samples were analyzed in an EPICS Profile flow cytometer (Coulter Cytometry). Analysis was performed within 4 h of fixation, at which time the epitopes are still stable (26). The flow cytometer was equipped with a 500 mW argon laser (Cyomics) operated at 15 mW with an emission wavelength of 488 nm. The fluorescence of FITC and phycoerythrin was detected using 525-nm and 575-nm bandpass filters, respectively. After identification of platelets by gating on both Y2/51-FITC positivity (i.e., GPIIIa positivity) and their characteristic light scatter, binding of the biotinylated monoclonal antibody S12 was determined by analyzing 5,000 individual platelets for phycoerythrin fluorescence. The percentage of P-selectin-positive platelets before and after platelet activation was defined as the percentage of platelets that had a phycoerythrin fluorescence greater than a threshold determined by 99% of platelets incubated with purified biotin-

Table 1. Patient Characteristics

Age (yr)	62 ± 3
Male	13 (68%)
Hypertension	17 (89%)
Diabetes	6 (32%)
Current smoker	0
Hypercholesterolemia (>230 mg/dl)	6 (32%)
Family history of CAD	4 (21%)
Previous MI	6 (32%)
Previous PTCA	5 (26%)
LVEF (%)	55 ± 2.3
Extent of CAD	
Single vessel	16 (84%)
Double vessel	3 (16%)
Medications	
Aspirin	19 (100%)
Beta-blocker	19 (100%)
Calcium channel blocker	14 (79%)
Oral nitrates	11 (58%)
ACE inhibitor	6 (32%)

Data presented are mean value ± SEM or number (%) of patients. ACE = angiotensin-converting enzyme; CAD = coronary artery disease; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

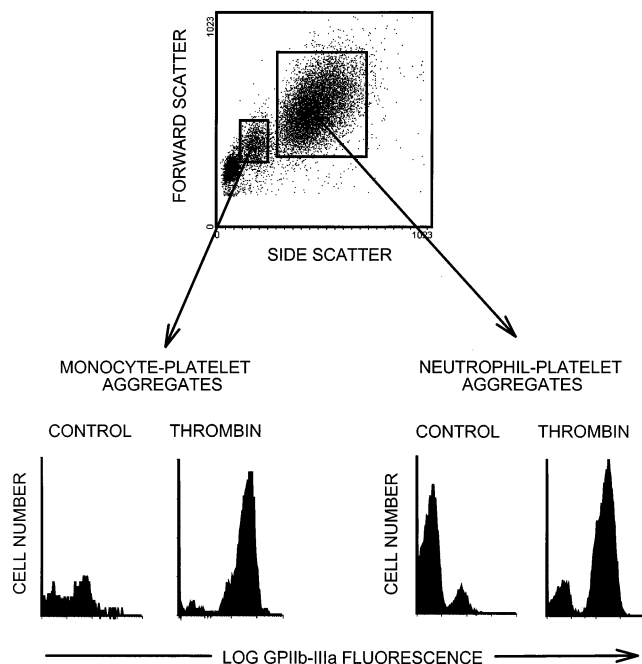


Figure 1. Whole blood flow cytometric analysis of monocyte-platelet and neutrophil-platelet aggregates in normal donors. Monocytes and neutrophils were identified by their characteristic light scatter properties (**upper panel**). Platelet-positive monocytes and platelet-positive neutrophils were identified by binding of FITC-conjugated GPIIb-IIIa-specific monoclonal antibody 7E3 (**lower panels**). Addition of 2 U/ml of thrombin in the presence of 2.5 mmol/liter glycyl-L-prolyl-L-arginyl-L-proline (an inhibitor of fibrin polymerization) resulted in platelet binding to most monocytes and neutrophils.

ylated mouse IgG (Boehringer Mannheim) rather than biotinylated S12.

Monocyte-platelet aggregates/neutrophil-platelet aggregates. Blood was diluted 1:4 in modified HEPES-Tyrode's buffer, pH 7.4, containing the FITC-conjugated monoclonal antibody 7E3, 2 $\mu\text{g}/\text{ml}$, and either 1) no agonist; 2) ADP, 1 $\mu\text{mol}/\text{liter}$ or 10 $\mu\text{mol}/\text{liter}$; 3) ADP, 1 $\mu\text{mol}/\text{liter}$ with epinephrine, 1 $\mu\text{mol}/\text{liter}$, or ADP, 10 $\mu\text{mol}/\text{liter}$ with epinephrine, 10 $\mu\text{mol}/\text{liter}$; 4) TRAP, 5 $\mu\text{mol}/\text{liter}$ or 30 $\mu\text{mol}/\text{liter}$; or 5) purified human alpha-thrombin, 2 U/ml. After 10 min, the samples were fixed at 22°C for 10 min with 1.1% formaldehyde and 1.4 \times Hanks balanced saline solution (GIBCO), then diluted 4.6-fold with distilled water.

The samples were then analyzed in an EPICS Profile flow cytometer. Analysis was performed within 4 h of fixation. The fluorescence of FITC was detected using a 525-nm bandpass filter. Monocytes and neutrophils were identified by their characteristic light scatter properties (Fig. 1, upper panel). Platelet-positive monocytes and neutrophils before and after ex vivo activation were identified by binding of the FITC-conjugated GPIIb-IIIa-specific monoclonal antibody 7E3 (Fig. 1, lower panel). The percentage of platelet-positive monocytes and neutrophils before and after ex vivo activation was defined as the percentage of monocytes and neutrophils that had FITC fluorescence greater than a threshold determined by 99% of

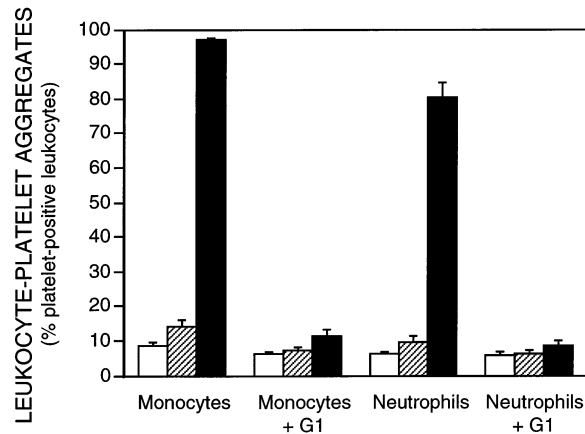


Figure 2. Monocyte-platelet and neutrophil-platelet aggregates in peripheral blood of normal control subjects incubated with or without TRAP (5 $\mu\text{mol}/\text{liter}$ [hatched bars] or 30 $\mu\text{mol}/\text{liter}$ [solid bars] in the presence or absence (open bars) of the P-selectin-specific monoclonal antibody G1. Data shown are mean value \pm SEM, n = 4.

monocytes and neutrophils incubated with FITC-mouse IgG (Boehringer Mannheim) rather than FITC-7E3. One thousand monocytes and 5,000 neutrophils were counted.

The monocyte-platelet and neutrophil-platelet aggregates were P-selectin-dependent because, consistent with previous studies (22), the formation of these aggregates was completely blocked by 7.5 $\mu\text{g}/\text{ml}$ of the P-selectin-specific monoclonal antibody G1 (Fig. 2).

Monocyte activation. Blood was diluted 1:4 in modified HEPES-Tyrode's buffer, pH 7.4, containing a saturating concentration of the FITC-conjugated CD11b-specific monoclonal antibody BEAR1 and fixed at 22°C for 10 min with 1% formaldehyde. The samples were then analyzed in an EPICS Profile flow cytometer. Monocytes were identified by their characteristic light scatter properties. The fluorescence of FITC-BEAR1 was detected using a 525-nm bandpass filter. Background binding, obtained from parallel samples run with FITC-mouse IgG, was subtracted from each test sample.

Statistics. Data were analyzed using the paired *t* test.

Results

Circulating activated platelets and circulating leukocyte-platelet aggregates. Unstimulated peripheral blood from patients with stable CAD contained increased numbers of surface P-selectin-positive platelets compared with peripheral blood from normal control subjects (mean [\pm SEM] 2.1 \pm 0.2 vs. 1.4 \pm 0.2, $p < 0.01$) (Fig. 3). Furthermore, peripheral blood from patients with stable CAD as compared with normal control subjects contained increased numbers of circulating monocyte-platelet aggregates (15.3 \pm 3.0 vs. 6.3 \pm 0.9, $p < 0.01$) (Fig. 3), but no significant increase in the presence of circulating neutrophil-platelet aggregates (6.8 \pm 1.2 vs. 5.6 \pm 1.0, $p = 0.10$) (Fig. 3).

Platelet reactivity. As determined by the platelet surface expression of P-selectin, platelets from patients with stable

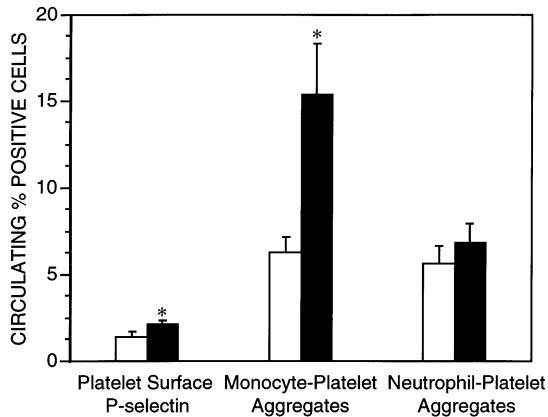


Figure 3. Platelet surface P-selectin, monocyte-platelet aggregates and neutrophil-platelet aggregates circulating in the peripheral blood of patients with stable CAD (solid bars) and control subjects (open bars), as determined by whole blood flow cytometry. * $p < 0.01$. Data shown are mean value \pm SEM, $n = 19$.

CAD were significantly more reactive to ex vivo agonist stimulation than were platelets from normal control subjects (Fig. 4). Platelets from patients with stable CAD as compared with those from control subjects were more likely to degranulate in response to stimulation with a low concentration of ADP (1 $\mu\text{mol/liter}$) (28.7 ± 3.9 vs. 16.1 ± 2.2 , $p < 0.01$), a low concentration of ADP (1 $\mu\text{mol/liter}$) and concomitant epinephrine (1 $\mu\text{mol/liter}$) (51.4 ± 4.6 vs. 37.5 ± 3.8 , $p < 0.05$) and a low concentration of TRAP (5 $\mu\text{mol/liter}$) (65.7 ± 6.8 vs. 20.2 ± 5.1 , $p < 0.01$) (Fig. 4). No significant differences in the platelet surface expression of P-selectin were observed between patient and control platelets stimulated with maximal concentrations of agonists (ADP, 10 $\mu\text{mol/liter}$; ADP, 10 $\mu\text{mol/liter}$, with epinephrine, 10 $\mu\text{mol/liter}$; TRAP, 30 $\mu\text{mol/liter}$) (Fig. 4), demonstrating that the differences observed with the use of low agonist concentrations were not due to an increased amount of available platelet P-selectin in the patients.

Figure 4. Platelet reactivity of patients with stable CAD (solid bars) and control subjects (open bars), as determined by platelet surface expression of P-selectin in response to exogenous agonist stimulation. Epi = epinephrine. * $p < 0.01$. † $p < 0.05$. Data shown are mean value \pm SEM, $n = 19$.

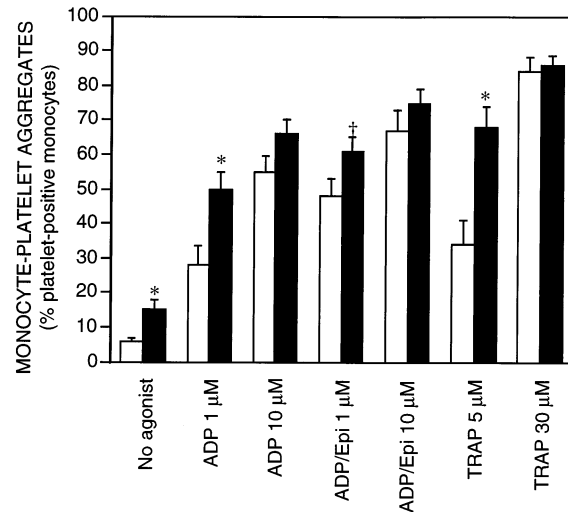
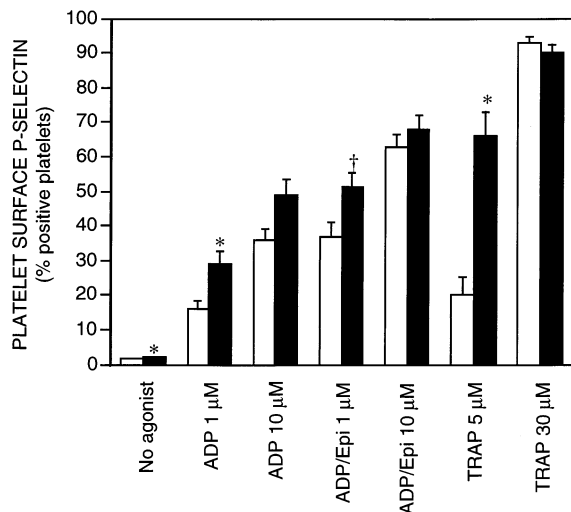


Figure 5. Monocyte-platelet aggregates in the blood of patients with stable CAD (solid bars) and control subjects (open bars), as determined by whole blood flow cytometry after ex vivo agonist stimulation. Epi = epinephrine. * $p < 0.01$. † $p < 0.05$. Data shown are mean value \pm SEM, $n = 19$.

As was observed with platelet reactivity (Fig. 4), no significant differences between patients with stable CAD and control subjects were seen in the propensity to form monocyte-platelet aggregates when high concentrations of agonists (ADP, 10 $\mu\text{mol/liter}$; ADP, 10 $\mu\text{mol/liter}$ with epinephrine, 10 $\mu\text{mol/liter}$; TRAP, 30 $\mu\text{mol/liter}$) were used (Fig. 5).

Propensity to form leukocyte-platelet aggregates. In addition to the increased monocyte-platelet aggregates present in the peripheral circulation of patients with stable CAD, monocyte-platelet aggregates were more likely to form in patients with stable CAD than in control subjects in response to ex vivo agonist stimulation (Fig. 5). Stimulation of peripheral whole blood from patients with stable CAD as compared with control subjects by a low concentration of ADP (1 $\mu\text{mol/liter}$) resulted in significantly more monocyte-platelet aggregates (50.4 ± 4.5 vs. 28.1 ± 5.3 , $p < 0.01$). Similar results were seen when peripheral whole blood from patients with stable CAD and normal control subjects was stimulated with a low concentration of ADP (1 $\mu\text{mol/liter}$) and concomitant epinephrine (1 $\mu\text{mol/liter}$) (60.7 ± 4.3 vs. 48.0 ± 4.8 , $p < 0.05$) or a low concentration of TRAP (5 $\mu\text{mol/liter}$) (67.6 ± 5.7 vs. 34.3 ± 7.0 , $p < 0.01$) (Fig. 5). As was observed with platelet reactivity (Fig. 4), no significant differences between patients with stable CAD and control subjects were seen in the propensity to form monocyte-platelet aggregates when high concentrations of agonists (ADP, 10 $\mu\text{mol/liter}$; ADP, 10 $\mu\text{mol/liter}$ with epinephrine, 10 $\mu\text{mol/liter}$; TRAP, 30 $\mu\text{mol/liter}$) were used (Fig. 5).

No significant differences between patients and control subjects were noted in the propensity to form neutrophil-platelet aggregates in response to stimulation with any of the following agonists: 1) ADP, 1 $\mu\text{mol/liter}$; 2) ADP, 10 $\mu\text{mol/liter}$; 3) ADP, 1 $\mu\text{mol/liter}$ with epinephrine, 1 $\mu\text{mol/liter}$; 4) ADP, 10 $\mu\text{mol/liter}$ with epinephrine, 10 $\mu\text{mol/liter}$; 5) TRAP, 5 $\mu\text{mol/liter}$; 6) TRAP, 30 $\mu\text{mol/liter}$ (Fig. 6).

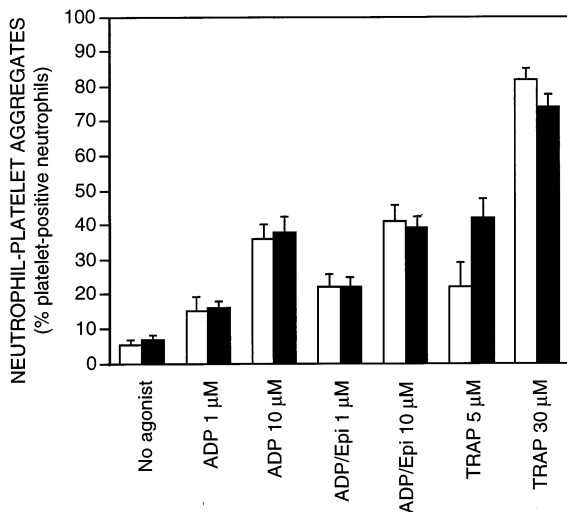


Figure 6. Neutrophil-platelet aggregates in the blood of patients with stable CAD (solid bars) and control subjects (open bars), as determined by whole blood flow cytometry after ex vivo agonist stimulation. Epi = epinephrine. $p = NS$. Data shown are mean value \pm SEM, $n = 19$.

Monocyte activation. There was no significant difference in the binding of the activation-dependent anti-CD11b monoclonal antibody BEAR1 to the surface of monocytes from patients with stable CAD compared with control subjects (Fig. 7).

Subgroup analysis. There were no significant differences between patients with or without hyperlipidemia or between patients with or without diabetes with respect to 1) circulating activated platelets; 2) platelet reactivity; 3) circulating monocyte-platelet aggregates; 4) circulating neutrophil-platelet aggregates; 5) propensity to form monocyte-platelet aggregates; and 6) propensity to form neutrophil-platelet aggregates.

Discussion

Platelet degranulation in patients with stable CAD. This study directly demonstrates circulating degranulated platelets and circulating monocyte-platelet aggregates in patients with stable CAD. Previous studies of platelet function in patients with coronary atherosclerosis have focused on patients with acute coronary artery syndromes and active ischemia presumably due to thrombosis within the coronary vessels (11,33-35). These studies measured platelet secretory products and the assays used were prone to artifactual platelet activation (36). Flow cytometry is a direct method for the measurement of platelet function, avoids in vitro artifactual platelet activation and assesses the activation state of individual platelets (26,31). Flow cytometry has been used to study platelets from patients with CAD undergoing angioplasty (37-40) and to a limited extent in patients with unstable angina (41). The present study supports the usefulness of flow cytometry in studying platelets from patients with stable CAD.

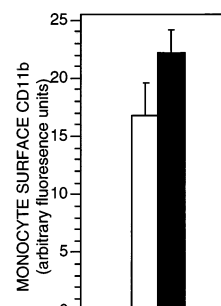
Our study not only demonstrates the presence of circulating

degranulated platelets in patients with stable CAD, but also provides evidence of enhanced platelet reactivity as demonstrated by the increased platelet surface expression of P-selectin in response to stimulation with low concentrations of agonists (Fig. 4). In patients with stable CAD, full stimulation with ADP, ADP with epinephrine or TRAP resulted in a similar number of P-selectin-positive platelets as those found in control subjects. Thus, the increased platelet reactivity in patients with stable CAD is not due to an increased amount of available platelet P-selectin, but to an alteration in the intracellular platelet environment, which makes them more responsive to agonist stimulation. The cellular mechanisms responsible for these "primed" platelets remain unknown. Although alterations in platelet nitric oxide production are associated with CAD (42), the use of oral nitrates in 58% of the subjects in this study makes decreased nitric oxide production an unlikely explanation for the increased platelet responsiveness. Furthermore, alterations in platelet surface receptors are unlikely to be involved in the mechanism because similar results were obtained with ADP, epinephrine and TRAP, each of which is known to stimulate platelets through a different receptor.

P-selectin is rapidly cleared from the surface of circulating degranulated platelets (43). Thus, the detection of circulating P-selectin-positive platelets in patients with stable CAD must be due to a process of ongoing platelet degranulation because the sampling of the patients' blood was not temporally related to an ischemic event, nor did it occur immediately distal to an injured vessel.

In a group of patients with large-vessel peripheral vascular disease, Davi et al. (44) reported that diabetes, hypercholesterolemia and hypertension, but not vascular disease per se, are associated with platelet activation in vivo. Although it did not reach statistical significance, a trend for increased thromboxane A_2 biosynthesis was noted in Davi's study for patients with peripheral artery disease but without diabetes, hypercholesterolemia or hypertension. Davi et al. used a radioimmunoassay of a major metabolite of thromboxane A_2 , 11-dehydrothromboxane B_2 , to detect platelet activation, an assay system different from the whole blood flow cytometric assay used in the present study. Furthermore, their study group consisted of

Figure 7. Monocyte surface CD11b in patients with stable CAD (solid bars) and control subjects (open bars), as determined by whole blood flow cytometry. $p = NS$. Data shown are mean value \pm SEM, $n = 19$.



patients with mild to moderate large-vessel peripheral artery disease in contrast to the patients in the current study with angiographically documented significant stenoses of moderate sized coronary arteries. Fitzgerald et al. (11) report normal thromboxane A₂ synthesis in a group of patients with stable CAD, a group of patients similar to the group of patients in the present study.

Monocyte-platelet aggregates in patients with stable CAD.

In addition to identifying increased circulating degranulated platelets and increased platelet reactivity in patients with stable CAD, we demonstrate that this patient group has increased circulating monocyte-platelet aggregates and an increased propensity to form monocyte-platelet aggregates (Fig. 3 and 5). Our assay used the monoclonal antibody 7E3 to identify platelets adherent to leukocytes. It has been reported that 7E3 binds to CD11b/CD18 (MAC-1) on the surface of monocytes (45,46). However, our assay does not detect significant binding of 7E3 to monocytes, as demonstrated by the almost complete inhibition of monocyte-platelet aggregates by the P-selectin-specific monoclonal antibody G1 (Fig. 2).

Platelet adhesion to monocytes and neutrophils is mediated by the platelet surface expression of P-selectin (21,22), which binds to P-selectin glycoprotein ligand-1 on leukocytes (47-49). Thus, the increased circulating monocyte-platelet aggregates observed in this study may simply reflect the presently described increased platelet degranulation in patients with stable CAD. Leukocyte-platelet aggregates have also been identified in the whole blood of patients undergoing cardiopulmonary bypass (8) or hemodialysis (50). More recently, neutrophil-platelet aggregates have been identified in the peripheral blood of patients with unstable angina (10) and monocyte-platelet and neutrophil-platelet aggregates in the coronary sinus of patients undergoing coronary angioplasty (9).

The exact physiologic significance of monocyte-platelet aggregation is unknown, but it may represent targeting of both cell types to appropriate inflammatory or hemostatic sites (6). Functional changes in one cell type by another have also been demonstrated. For example, platelets have been shown to supply cholesterol to monocytes, which may then mature into lipid-laden macrophages (51). P-selectin on the surface of activated platelets induces the expression of tissue factor in monocytes (52). The binding of P-selectin to monocytes in the area of vascular injury may be an initiator of thrombosis (52). Thus, Palabrica et al. (53) have demonstrated that P-selectin-mediated platelet adhesion to leukocytes is important in promoting fibrin deposition within a growing thrombus. More recently, Weyrich et al. (54) have shown that platelet surface P-selectin can regulate monocyte chemokine synthesis in concert with the platelet chemokine RANTES (regulated on activation normal T cell expressed presumed secreted). Our study did not demonstrate enhanced formation of neutrophil-platelet aggregates in patients with stable CAD, a finding consistent with recent reports that monocyte-platelet aggregates form more easily than neutrophil-platelet aggregates (6-8).

Study limitations. A limitation of this study is that platelets and leukocyte-platelet aggregates were sampled in the peripheral circulation, not at the diseased vessel wall where cellular activation presumably occurs. Thus, the circulating degranulated platelets, circulating monocyte-platelet aggregates, increased platelet reactivity and increased propensity to form monocyte-platelet aggregates may not be wholly reflective of local events within the coronary vasculature. Furthermore, our control group consisted of healthy adults without significant cardiac risk factors. Therefore, the differences in platelet function and monocyte-platelet aggregation between the patients with stable CAD and the control subjects may not be caused by the presence of atherosclerosis in the patients, but may be due in part to the presence of hypertension, hyperlipidemia or diabetes or the use of beta-blockers, angiotensin-converting enzyme inhibitors or calcium channel blockers. In our laboratory, aspirin does not affect the platelet surface expression of P-selectin or P-selectin-dependent monocyte-platelet aggregates and neutrophil-platelet aggregates in response to activation with TRAP or thrombin in normal volunteers (data not shown). Our data are consistent with previous reports (55,56) that aspirin does not affect the platelet surface expression of P-selectin, because alpha granule secretion is independent of the arachidonic acid pathway (55). Although aspirin may affect platelets from patients with CAD differently from those of normal control subjects, its use by patients in this study would then have resulted in an underestimation of the presently described difference between normal control subjects and patients with stable CAD.

Conclusions. In this study we demonstrate that patients with stable CAD have circulating degranulated platelets, circulating monocyte-platelet aggregates, increased platelet reactivity and an increased propensity to form monocyte-platelet aggregates. The question as to whether the degree of increased platelet reactivity and monocyte-platelet aggregation correlates with specific clinical events in these patients is currently being addressed in a large prospective study.

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