

# Pharmacodynamic Study of F(ab')<sub>2</sub> Fragments of Murine Monoclonal Antibody 7E3 Directed against Human Platelet Glycoprotein IIb/IIIa in Patients with Unstable Angina Pectoris

H. K. Gold, L. W. Gimple, T. Yasuda, R. C. Leinbach, W. Werner, R. Holt, R. Jordan, H. Berger, D. Collen, and B. S. Coller  
Cardiac Division, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114; Department of Hematology, State University of New York at Stony Brook, Stony Brook, New York; and Centocor, Inc., Malvern, Pennsylvania 19355

## Abstract

The pharmacodynamics of intravenous bolus injections of 0.05, 0.10, 0.15, and 0.20 mg/kg of F(ab')<sub>2</sub> fragments of the murine monoclonal antibody 7E3, 7E3-F(ab')<sub>2</sub>, directed against the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor of human platelets, were studied in groups of four patients with unstable angina pectoris. With 0.20 mg/kg, the template bleeding time prolonged from 6.3±1.9 (mean±SD) to > 30 min; it subsequently decreased to 13±7.8 min after 12 h and to 8.3±1.5 min after 24 h. The number of unblocked GPIIb/IIIa receptors (preinfusion value, 32,000±3,000 per platelet) decreased to 13±7% of the preinfusion value 1 h after infusion, and then increased to 33±10% at 12 h, 44±8% at 24 h and 67±7% at 72 h. The logarithm of the bleeding time was inversely proportional with the residual GPIIb/IIIa receptors ( $r = 0.73$ ,  $P < 0.0001$ ). ADP-induced platelet aggregation (measured by changes in light transmittance in percent) decreased from 60±5% before infusion to 1.5±3% 1 h after infusion; it then increased to 29±3% after 24 h and 39±6% after 72 h. Platelet counts decreased by 16% at 1 h and returned to control values within 24 h. Proportionally smaller effects were seen at lower doses of 7E3-F(ab')<sub>2</sub>. Antibody injection did not induce spontaneous bleeding. Angina was not observed during the first 12 h when the bleeding time was significantly prolonged, but occurred in 6 of the 16 patients within the next 3 d. 2 of the 16 patients developed low titers of IgG antibodies specific for 7E3-F(ab')<sub>2</sub>. Thus 7E3-F(ab')<sub>2</sub> induces dose-related inhibition of platelet function; at a dose of 0.20 mg/kg, it causes profound inhibition of platelet aggregation and prolongation of the bleeding time, but no spontaneous bleeding. (*J. Clin. Invest.* 1990; 86:651-659.) Key words: anti-platelet glycoprotein IIb/IIIa antibody • coronary artery thrombosis • bleeding time • platelet aggregation inhibition • ischemic heart disease

## Introduction

Platelet thrombus formation is thought to contribute to the vaso-occlusive and thromboembolic disorders in the coronary and cerebral arteries that collectively constitute the most common cause of death in the United States (1). Aspirin, the most

frequently used antiplatelet agent, has shown some efficacy in treating and preventing these disorders (2), raising the possibility that more potent inhibitors of platelet function may be more beneficial. Recent advances in the understanding of platelet physiology have identified a unique role for the platelet glycoprotein IIb/IIIa (GPIIb/IIIa)<sup>1</sup> receptor in mediating platelet aggregation (3, 4), which most likely contributes to vaso-occlusive and thromboembolic phenomena. Therefore, we and others have been studying the potential of monoclonal antibodies to this receptor as therapeutic agents.

The F(ab')<sub>2</sub> fragment of the murine monoclonal antibody 7E3, 7E3-F(ab')<sub>2</sub>, is more potent than aspirin in inhibiting platelet aggregation ex vivo and platelet thrombus formation in vivo in experimental models designed to simulate unstable angina and transient ischemic attacks (5-7). It can also reduce both the time to reperfusion and the dose of recombinant tissue-type plasminogen activator needed to achieve reperfusion in dog models of acute myocardial infarction (8, 9), and protect against acute reocclusion (8-10). Other preliminary evidence indicates that the antibody can also prevent both experimental coronary artery thrombosis (11) and acute reocclusion after experimental coronary artery angioplasty-induced injury (12). These data, together with toxicological studies in primates that did not identify serious acute toxicity (13-15), encouraged us to embark on a more extensive Phase I study in patients with unstable angina. We now report on the pharmacodynamics of increasing doses of 7E3-F(ab')<sub>2</sub> in this patient population.

## Methods

**Patients.** 16 patients aged 38-73 yr with unstable angina were studied. Diagnostic criteria were angina at rest for < 30 min in duration during the last 6 d before entry into the study and confirmation that the pain was of cardiac origin with electrocardiographic changes (ST segment elevations or depression, or T wave pseudonormalization or inversion) and angiographic evidence of at least 50% diameter stenosis in the ischemia-related artery. Patients with angiographically demonstrable intraluminal filling defects were considered candidates for rt-PA therapy (16) and so were not included in this study. Patients were excluded from participation if they had (a) high-grade stenosis supplying two myocardial zones that were inaccessible to angioplasty, (b) left main coronary disease with > 50% diameter stenosis, (c) a history of a hemorrhagic diathesis, (d) systolic blood pressure > 160 mm Hg or diastolic pressure > 90 mm Hg, (e) surgery within 8 wk of enrollment, (f) a recent arterial puncture in a noncompressible site, (g) diabetes mellitus for more than 5 yr, (h) prior exposure to a murine monoclonal

Address reprint requests to Dr. Gold, Cardiac Unit, ACC 480, Massachusetts General Hospital, 15 Parkman Street, Boston, MA 02114.

Received for publication 15 December 1989 and in revised form 27 March 1990.

*J. Clin. Invest.*

© The American Society for Clinical Investigation, Inc.

0021-9738/90/08/0651/09 \$2.00

Volume 86, August 1990, 651-659

1. Abbreviations used in this paper: GPIIb/IIIa, glycoprotein IIb/IIIa; HAMA, human anti-mouse IgG antibodies; 7E3, murine monoclonal antibody directed against the human glycoprotein IIb/IIIa platelet receptor.

antibody, or (i) abnormal baseline blood counts, blood chemistry measurements, or coagulation assays. The patients were given standard antianginal therapy including  $\beta$ -adrenergic receptor blocking agents, intravenous nitroglycerin, and calcium antagonists, but were not treated with antiplatelet agents or heparin. The protocol was approved by the Institutional Review Board of the Massachusetts General Hospital and conducted under the Investigational New Drug procedures established by the Food and Drug Administration.

*F(ab')<sub>2</sub> fragment of monoclonal anti-platelet GPIIb/IIIa antibody 7E3.* The F(ab')<sub>2</sub> fragment of the 7E3 antibody was prepared by Centocor, Inc. (Malvern, PA). After the enzymatic digestion of the cell culture-derived 7E3 IgG1 antibody with pepsin, the F(ab')<sub>2</sub> fragment was extensively purified to ensure the absence of residual IgG or Fc fragments employing modifications of previously published methods (6, 7, 10). The final F(ab')<sub>2</sub> concentrate was supplied as a sterile 2 mg/ml solution in 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2, and conformed to the Food and Drug Administration's guidelines for a biopharmaceutical product under the rules governing investigational new drugs.

*Study protocol.* Between 24 and 36 h before study admission, coronary angiography was performed to establish eligibility. A baseline bleeding time was obtained and blood samples were drawn into the following: 0.013 M EDTA final concentration for blood counts; 0.01 vol 40% trisodium citrate for platelet aggregation, the antibody binding assay, fibrinogen determination, prothrombin time, and activated partial thromboplastin time; a combination of thrombin and trasylol for determining fibrin(ogen) degradation products; and a glass tube to prepare serum for determination of IgG antibodies to 7E3-F(ab')<sub>2</sub>. Antibody at doses of 0.05, 0.10, 0.15, or 0.20 mg/kg was then infused through an intravenous catheter over a 5-min period in four sequential groups of four patients. Blood samples were obtained 1, 12, 24, 48, and 72 h after the infusion for repeat assays. Bleeding times were repeated at the same time points up to 24 h or until the bleeding time returned to the normal range. Repeat serum samples were obtained at 2, 4, and 6 wk to test for anti-7E3-F(ab')<sub>2</sub> IgG antibodies.

Bleeding times were performed with a spring-loaded device (Surgicutt, International Technidyne Corp., Edison, NJ) designed to produce a 5 × 1-mm incision. A blood pressure cuff was inflated to 40 mm Hg before making the incision and maintained at this pressure throughout. Blood coming from the wound, but not the wound itself, was blotted with filter paper every 30 s until the wound was completely dry. If the bleeding did not stop within 30 min, the test was terminated and the result recorded as > 30 min.

The binding of <sup>125</sup>I-7E3 to platelets was measured by incubating 0.2 ml of the citrated platelet-rich plasma at  $3.0 \pm 0.1 \times 10^8$  platelets/ml with 20  $\mu$ l of <sup>125</sup>I-labeled 7E3 at a final concentration of 20  $\mu$ g/ml, which was previously shown to be a near-saturating dose (5). After 55 min at 22°C, duplicate 0.1-ml aliquots of the samples were layered over 0.1 ml of 30% sucrose contained in 400- $\mu$ l microcentrifuge tubes and centrifuged at 12,000 g for 5 min at 22°C. The radioactivity in both the platelet pellet and the supernatant fluid was then determined and the total number of molecules of <sup>125</sup>I-7E3 bound was calculated from the antibody added, the percentage of radioactivity in the pellet, and the platelet count. The number of GPIIb/IIIa receptors blocked by in vivo infusion of unlabeled 7E3-F(ab')<sub>2</sub> was defined as the reduction in the number of <sup>125</sup>I-7E3 molecules that could bind per platelet after the infusion.

The percentage of injected antibody that became bound to platelets at 1 h was calculated by first multiplying the blood volume (estimated at 72 ml/kg) by the platelet count (per milliliter of blood) in the preinfusion sample, the number of GPIIb/IIIa receptors per platelet in the preinfusion sample, the percentage of GPIIb/IIIa receptors blocked at 1 h, and the factor 1.5 (to correct for splenic pooling of one third of the platelets) (17); the resulting product was then divided by the number of molecules of 7E3-F(ab')<sub>2</sub> injected per kilogram.

Platelet aggregation was performed on citrated platelet-rich plasma adjusted to  $3 \times 10^8$  platelets/ml using a dual-channel aggregometer (Chrono-Log Corp., Havertown, PA). ADP (Sigma Diagnostic Re-

agent, Sigma Chemical Co., St. Louis, MO; 100  $\mu$ M stock solution in water) was tested against the preinfusion samples at final concentrations of 1.1–3.8  $\mu$ M to establish the minimal dose giving a full aggregation response. This concentration was then used for all subsequent samples. Collagen (Sigma Diagnostic Reagent, 2 mg/ml stock solution in water) was used at a final concentration of 22  $\mu$ g/ml for all but one of the patients (no. I-1), who responded poorly to this dose, but responded well to 85  $\mu$ g/ml. Ristocetin (Sigma Diagnostic Reagent, 15 mg/ml stock solution in water) was employed at 1.5 mg/ml final concentration for all samples. An attempt was made to maintain the pH of the platelet-rich plasma within the range 7.7–8.0 by carefully capping the samples, and 82% of the samples fell within this range. Quantitative analysis of aggregation induced by ADP, collagen, and ristocetin was performed by measuring the maximum change in light transmission (i) after adding the agonist; the results were compared to the value obtained with the preinfusion sample and expressed as a percent of the control value ( $\Delta T$  in percent). A similar analysis was performed for the slope of ADP-induced platelet aggregation.

Human anti-mouse IgG antibodies (HAMA) in the serum of patients given the 7E3 F(ab')<sub>2</sub> were assessed at Centocor, Inc. as follows. Microtiter plates (polystyrene, Corning Glass Works, Corning, New York) were coated for 16–20 h at 4°C with 10  $\mu$ g/ml (0.5  $\mu$ g/well) 7E3 F(ab')<sub>2</sub> in 0.1 M glycine, 5 mM EDTA, pH 8.5. Plates were washed with phosphate-buffered saline (PBS) containing 0.5% Tween-20 and 0.1% chloroacetamide, pH 7.2, blocked by incubation for 1 h at 37°C with PBS containing 1% bovine serum albumin (BSA, Sigma Chemical Co., Fraction V powder), and washed again with the PBS-Tween buffer. Human serum samples were diluted 1:20 in 0.1 M Tris, 0.15 M sodium chloride, pH 7.5, containing 45% goat serum, 30% calf serum, and 0.1% sodium azide. Triplicate 50- $\mu$ l aliquots of the diluted test sera, or of positive or negative control sera, were incubated in the microtiter wells at 45°C for 1 h. After washing with the PBS-Tween buffer, 50  $\mu$ l of horseradish peroxidase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:100,000 in PBS containing 50% fetal calf serum, 1% goat serum, 0.01% thimerosal, and 0.01% chloramphenicol, was added to the wells and incubated for 1 h at 45°C. The plates were washed with PBS-Tween buffer and 200  $\mu$ l of 3 mg/ml *O*-phenylenediamine (Sigma Chemical Co., St. Louis, MO) in 0.05 M sodium phosphate, 0.025 M sodium citrate, 0.03% hydrogen peroxide, 0.1% chloroacetamide, pH 4.8, was added to the wells. After incubation for 30 min at room temperature, the reaction was terminated by addition of 50  $\mu$ l of 4 M sulfuric acid and the resultant color was reported as the difference in absorbance at 490 and 650 nm on an enzyme immunoassay plate spectrophotometer ( $V_{\max}$  kinetic microplate reader, Molecular Devices, Palo Alto, CA).

A sample was considered to be reactive in the assay if it yielded an absorbance reading > 0.30 optical density (OD) units. The 0.30 OD cutoff was determined from a statistical analysis of the distribution of normal human sera ( $n = 181$ ) under these specific assay conditions. All reactive samples were tested for reproducibility and to ensure a lack of reactivity on non-7E3-coated, BSA-blocked, microtiter plates. Confirmation as a positive response was established by inhibition experiments in which the HAMA-positive samples were preincubated (1 h, 45°C) with fluid-phase 7E3 F(ab')<sub>2</sub> (0.1 mg/kg). Neutralization of sample reactivity in the presence of fluid-phase 7E3 is a requirement for consideration as a positive HAMA response. Inhibition experiments were also conducted with 0.1 mg/ml normal, intact mouse IgG (Jackson ImmunoResearch Laboratories) to further assess the specificity of the immune reaction.

Samples determined to be positive for HAMA were serially diluted to establish the maximum serum dilution (titer) which yielded an optical density value > 0.30. A sample was considered negative (titer < 1:20) if it yielded an OD < 0.30 at the 1:20 dilution.

Fibrinogen was assayed by a chromometric thrombin clotting assay (18) and fibrin(ogen) degradation products by a commercial latex agglutination assay (Thrombo-Wellco test, Wellcome Diagnostics, Dartford, England).

*Statistical analysis.* The data are expressed as mean  $\pm$  SD unless

indicated otherwise. Differences between groups were analyzed by Student's *t* test for paired or unpaired values and *P* values > 0.05 were considered not to be significant. The correlation between bleeding time, percent residual GPIIb/IIIa receptors, dose of 7E3-F(ab')<sub>2</sub>, and  $\Delta T$  of ADP-induced platelet aggregation was analyzed by stepwise multiple linear regression (RS/1 Program, BBN Software Products Corp., Cambridge, MA), based on data obtained 1, 12, and 24 h after 7E3-F(ab')<sub>2</sub> injection.

## Results

**Acute adverse reactions.** None of the 16 patients demonstrated changes in vital signs during or after antibody administration. One patient (no. I-2), given the lowest dose of 7E3-F(ab')<sub>2</sub>, had an episode of bleeding from hemorrhoids on day 5, which was most likely unrelated to the 7E3-F(ab')<sub>2</sub> infusion. No other side effects were observed.

**Bleeding times.** There was a dose-dependent increase in bleeding times at 1 h, with all four patients treated with 0.20 mg/kg having bleeding times in excess of 30 min (Table I). With the 0.15 mg/kg dose, all of the patients developed bleeding time prolongations (from 6.8±2.2 to 16±9.2 min), but only one time was > 30 min. The lower doses did not produce significant prolongations. By 12 h the bleeding times reverted back toward normal, and by 24 h, only one patient (no. III-2) had a bleeding time in excess of 10 min.

**Platelet counts and GPIIb/IIIa receptor blockade.** Changes

in platelet counts are summarized in Table II. At 1 h there was a modest decrease in platelet count, with the highest dose (0.20 mg/kg) producing a 16±10% (mean±SD) reduction. The platelet counts returned toward normal over the first 24 h with one exception, a patient given 0.1 mg/kg (no. II-4) whose platelet count decreased from 2.4 × 10<sup>8</sup>/ml preinfusion to 0.94 × 10<sup>8</sup>/ml at 12 h and then increased to 1.5 × 10<sup>8</sup>/ml at 48 h and 1.9 × 10<sup>8</sup>/ml at 72 h. The percentage of GPIIb/IIIa receptors blocked by the infusion of 7E3-F(ab')<sub>2</sub> also demonstrated a dose-response pattern with the highest dose (0.20 mg/kg) producing 87±7% blockade at 1 h (Table III). Thus, in this group, the number of unoccupied GPIIb/IIIa receptors per platelet decreased from 32,000±3,000 to 4,300±2,500 at this time point. Thereafter, there was a return toward the original number of unblocked receptors, with 23,000±5,000 unblocked receptors present at 72 h. The time course of the return of unoccupied GPIIb/IIIa receptors after injection of 7E3-F(ab')<sub>2</sub> is illustrated in Fig. 1.

The percentage of injected antibody bound to circulating platelets, calculated as described in Methods amounted to 76±25, 97±17, 70±21, and 62±8% in groups I-IV, respectively. This calculation relies on the assumption that intrasplenic platelets are coated with 7E3-F(ab')<sub>2</sub> to the same extent as circulating platelets. As expected, the percentage bound decreased at the higher doses, but even at 0.20 mg/kg, a dose that produced nearly quantitative blockade of the GPIIb/IIIa receptors, the mean value was > 60%. Even if the intrasplenic pool is excluded from the analysis, > 40% of the injected antibody bound to the circulating platelets. Three patients had values in excess of 100%. One possible explanation for this might be an overestimate of the splenic component owing to obesity, since in obese individuals the percentage of platelets in the spleen is likely to be smaller than in thin individuals. In fact, the patient with the highest value (120%) weighed 106 kg.

**Platelet aggregation.** In general, there was a dose-dependent increase in inhibition of platelet aggregation induced by both ADP and collagen, with much less, or no inhibition of ristocetin-induced aggregation (Table IV and Fig. 2). All five patients whose bleeding times rose to > 30 min at the 1-h time point had marked or total inhibition of platelet aggregation in response to both ADP and collagen, but only minor changes in ristocetin-induced aggregation, indicating that despite high-grade GPIIb/IIIa receptor blockade, the GPIb receptor remained available for binding of von Willebrand factor. The inhibition of ADP- and collagen-induced aggregation returned toward normal over 48–72 h, but some remaining inhibition was apparent, even at these time points (Fig. 2).

There was a correlation between inhibition of ADP-induced platelet aggregation and the percentage of GP IIB/IIIa receptors blocked. There were, however, some anomalous results that require additional comment. Patient I-1 given a dose of 0.05 mg/kg, resulting in a 28% decrease in GPIIb/IIIa receptors and no prolongation of the bleeding time, had a markedly reduced platelet aggregation by all of the agents, including ristocetin. Technical problems probably accounted for the apparent decrease in aggregation responses. One patient given 0.05 mg/kg (I-3) had a decrease in ADP-induced platelet aggregation that was greater than expected on the basis of GPIIb/IIIa receptor blockade and changes in bleeding time. We suspect that this patient may have had a transient increase in platelet sensitivity at the beginning of the study, as has been reported for some patients with unstable angina, because the

Table I. Template Bleeding Times after 7E3-F(ab')<sub>2</sub> Injection

Patient group	Dose	Patient number	Time after injection of 7E3-F(ab') <sub>2</sub>			
			Control	h		
	mg/kg			min		
I	0.05	1	7.5	8.0	14.0	5.0
		2	9.0	8.0	3.5	7.0
		3	4.5	5.5	3.0	5.0
		4	7.0	8.0	8.0	8.0
		Mean±SD	7.0±1.9	7.4±1.3	7.1±5.1	6.3±1.5
II	0.10	1	4.5	6.0	5.0	5.0
		2	6.0	8.0	6.5	4.5
		3	5.0	8.0	7.5	5.5
		4	6.0	6.0	6.5	7.0
		Mean±SD	5.4±0.8	7.0±1.2	6.4±1.0	5.5±1.1
III	0.15	1	9.5	13.5	>30	9.5
		2	7.5	>30	10.5	15.0
		3	5.0	10.0	8.5	6.5
		4	5.0	12.0	6.5	7.5
		Mean±SD	6.8±2.2	16±9.2*	14±11*	9.6±3.8
IV	0.20	1	9.0	>30	24.0	9.0
		2	5.5	>30	6.0	7.0
		3	4.5	>30	10.0	7.0
		4	6.0	>30	10.5	10.0
		Mean±SD	6.3±1.9	>30	13±7.8	8.3±1.5

\* Bleeding times > 30 min are given a 30-min value for calculation of mean±SD.

Table II. Platelet Counts after 7E3-F(ab')<sub>2</sub> Injection

Patient group	Dose	Patient number	Baseline	Time after injection of 7E3-F(ab') <sub>2</sub>			
				h			
	mg/kg			1	12	24	72
				10 <sup>9</sup> /ml			
I	0.05	1	1.4	1.4	1.4	1.5	1.4
		2	1.7	1.7	1.8	1.6	2.0
		3	2.5	—	2.7	3.0	—
		4	1.7	1.5	1.6	1.5	1.7
		Mean±SD	1.8±0.5	1.5±0.2	1.9±0.6	1.9±0.7	1.7±0.3
II	0.10	1	2.8	—	2.6	2.8	3.2
		2	3.5	2.8	3.0	3.0	3.3
		3	2.0	—	—	1.7	1.5
		4	2.4	1.8	0.94	0.97	1.9
		Mean±SD	2.7±0.6	2.3±0.7	2.2±1.1	2.1±1.0	2.5±0.9
III	0.15	1	3.5	2.9	3.1	3.1	3.2
		2	1.5	1.6	1.5	1.6	1.8
		3	1.9	1.2	1.6	—	1.8
		4	4.5	4.5	4.5	3.7	4.5
		Mean±SD	2.9±1.4	2.6±1.5	2.7±1.4	2.8±1.1	2.8±1.3
IV	0.20	1	2.0	1.6	1.8	1.8	2.0
		2	2.9	2.1	2.5	2.7	2.5
		3	2.1	1.7	1.6	2.0	1.7
		4	2.9	2.8	2.8	2.6	—
		Mean±SD	2.5±0.5	2.1±0.5	2.2±0.6	2.3±0.4	2.1±0.4

patient required the lowest dose of ADP to achieve aggregation. Loss of this unusual sensitivity could then have resulted in an apparent inhibition of platelet aggregation by the antibody. In support of this, the "inhibition" in this patient did not return to normal, even after 24 h. The inhibition of aggregation produced by 0.1 mg/kg in patient II-2 at 1 h (68%) also appeared to be out of proportion to the percentage of GPIIb/IIIa receptors blocked (31%), but the reason for this was not clear.

**Correlation between GPIIb/IIIa receptor blockade and platelet function parameters.** Stepwise multiple linear regression analysis of the correlation between bleeding time (BT), percent residual GPIIb/IIIa receptors (% GPIIb/IIIa), dose of 7E3-F(ab')<sub>2</sub>, and  $\Delta T$  yielded the following equation:  $\log(BT) = 1.4 - 0.0078(\% \text{ GPIIb/IIIa})$  with  $r = 0.73$ ,  $P < 0.0001$ . This indicates that bleeding time prolongation is primarily determined by the residual GPIIb/IIIa receptor density, as illustrated in Fig. 3. In addition, a more complex relationship was observed between percent residual GPIIb/IIIa, and platelet function parameters and bleeding time:  $\log(\% \text{ GPIIb/IIIa}) = 1.1 - 1.6(\text{dose}) - 0.005(\text{slope}) - 0.22 \log(BT) + 0.11 \log(\Delta T) + 0.68 \log(\text{slope})$  with  $r = 0.95$ .

Prolongation of the bleeding time to > 15 min required reduction of the unblocked GPIIb/IIIa receptors to < 10,000 per platelet (7/8 values), whereas substantial inhibition of platelet aggregation occurred when the number of free GPIIb/IIIa receptors was < 20,000 per platelet (Tables I–IV).

**Effect of 7E3-F(ab')<sub>2</sub> on angina pectoris.** 10 of the 16 pa-

tients were free of pain for the 24-h period before the antibody infusion and for at least 72 h thereafter. Three patients (nos. II-2, II-4, and IV-2) had chest pain during the 24 h before the infusion. All three were free of pain during the 12 h period immediately after infusion. Pain returned in patient IV-2 after 23 h. The bleeding time in this patient was > 30 min at 1 h, 6 min at 12 h, and 7 min at 24 h, and the number of unblocked GPIIb/IIIa receptors was 35,000 before antibody injection, 8,200 at 1 h, 15,000 at 12 h, and 17,000 at 24 h. Patients II-2 and II-4 had return of pain between 24 and 72 h; neither had a prolonged bleeding time or inhibition of platelet aggregation induced by ADP or collagen at those time points. Three patients (nos. II-1, III-1, and IV-3) did not have chest pain for the 24-h time period before infusion, but developed chest pain after the infusion. The onset of pain occurred at 12 h in patient II-1 and after 24 h in patient III-1; neither had a prolonged bleeding time when the pain occurred. Patient IV-3, who sustained chest pain 11 h after the infusion, had a 1-h bleeding time of > 30 min, but at 12 h this had decreased to 10 min, and at 24 h it was 7 min (Table I). The number of unblocked GPIIb/IIIa receptors per platelet in this patient was 33,000 before injection of antibodies, 3,500 at 1 h, 13,000 at 12 h, and 17,000 at 24 h.

**IgG antibodies against 7E3-F(ab')<sub>2</sub>.** The preinfusion serum sample from one of the 16 patients (no. IV-2) gave an elevated value in the ELISA assay (OD = 0.82). This response appeared to be specific for 7E3-F(ab')<sub>2</sub> because it could be reduced by > 90% when the assay was performed in the presence of fluid-

Table III. Residual Unblocked GPIIb/IIIa Receptors per Platelet after 7E3-F(ab')<sub>2</sub> Injection

Patient group	Dose	Patient number	Preinfusion GPIIb/IIIa receptors per platelet	Time after 7E3-F(ab') <sub>2</sub> injection			
				h			
				1	12	24	72
	mg/kg		$\times 10^{-3}$				
I	0.05	1	46	72	53	99	100
		2	39	54	83	86	—
		3	38	85	92	100	—
		4	52	76	76	85	86
		Mean $\pm$ SD	43 $\pm$ 7	72 $\pm$ 13	76 $\pm$ 17	93 $\pm$ 8	93 $\pm$ 7
II	0.10	1	43	60	76	75	89
		2	41	69	61	64	—
		3	58	44	66	63	—
		4	43	44	54	58	72
		Mean $\pm$ SD	46 $\pm$ 8	54 $\pm$ 12	64 $\pm$ 9	65 $\pm$ 7	81 $\pm$ 12
III	0.15	1	44	54	67	70	76
		2	37	15	4	47	71
		3	38	46	60	64	—
		4	30	44	59	65	72
		Mean $\pm$ SD	37 $\pm$ 6	40 $\pm$ 17	48 $\pm$ 29	62 $\pm$ 10	73 $\pm$ 3
IV	0.20	1	33	9	21	33	—
		2	35	23	44	49	62
		3	33	10	37	51	72
		4	28	10	30	42	55
		Mean $\pm$ SD	32 $\pm$ 3	13 $\pm$ 7	33 $\pm$ 10	44 $\pm$ 8	63 $\pm$ 9

phase 7E3-F(ab')<sub>2</sub> but not when the assay was performed in the presence of normal, intact mouse IgG. There were no obvious differences in this patient's response to the 7E3-F(ab')<sub>2</sub> infusion with regard to changes in platelet count (28% decrease at 1

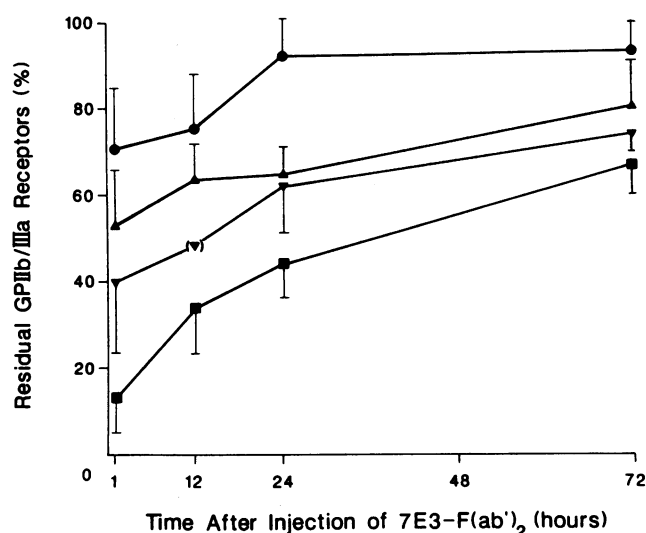


Figure 1. Time course of the return of unoccupied GPIIb/IIIa receptor after injection of 7E3-F(ab')<sub>2</sub>. (●) 0.05 mg/kg; (▲) 0.1 mg/kg; (▼) 0.15 mg/kg; (■) 0.2 mg/kg; [(▼)] the SD for this value is 29%.

h, 14% decrease at 12 h, 7% decrease at 24 h) or bleeding time prolongation. This patient did not show any evidence of an anamnestic response in the 7E3-F(ab')<sub>2</sub> infusion, with repeat assays at 14, 28, and 42 d after the infusion having titers of 1:160 (OD = 0.84), 1:160 (OD = 0.75), and 1:160 (OD = 0.57), in the enzyme immunoassay, respectively.

Of the remaining 15 patients, 13 had no increase in HAMA assay reactivity on repeat testing, with all but one patient having samples obtained on at least two occasions sometime between 2 and 7 wk postinfusion. The remaining two patients (nos. III-2 and III-3), both of whom received a dose of 0.15 mg/kg, developed detectable HAMA titers after 7E3-F(ab')<sub>2</sub> infusion. Patient III-2, whose control value at the 1:20 dilution was OD = 0.024, was tested at 2 wk (OD = 1.5, titer = 1:320), 4 wk (OD = 1.5, titer = 1:160), and 6 wk (OD = 0.84, titer = 1:160). Patient III-3, whose preinfusion value was OD = 0.042, was only available for blood sampling after 4 wk, and the assay on that sample yielded an OD = 0.86 (titer = 1:320).

**Hematological assays.** The fibrinogen concentrations in 13 patients tested before and 24 h after 7E3-F(ab')<sub>2</sub> injection showed only modest variations. Thus, there was a < 10% difference between the control value and the 24-h value in eight of the patients, including five of the seven patients given 0.15 and 0.20 mg/kg. Four of the remaining patients in this group had an increase of < 35 percent and the other had a 35% decrease in fibrinogen level. The fibrin(ogen) degradation product titers were either 1:2 or 1:4 for all of the patients both in the preinfusion sample and the 24-h sample (data not

Table IV. Platelet Aggregation after 7E3-F(ab')<sub>2</sub> Injection

Patient group	Dose	Patient number	ADP-induced platelet aggregation						Collagen			Ristocetin		
			Dose	$\Delta T$					$\Delta T$			$\Delta T$		
				Pre	h				Pre	h		Pre	h	
					1	12	24	48		1	72		1	72
	mg/kg		$\mu M$			%				%			%	
I	0.05	1	2.2	61	33	38	41	21	56	38	39	76	49	44
		2	2.6	52	50	53	43	55	39	46	41	54	61	78
		3	1.1	78	4	15	16	—	93	73	45	93	89	49
		4	3.4	53	51	45	70	—	74	56	75	53	100	83
		Mean	2.3	61	35	38	43	38	66	53	50	69	74	64
		SD	1.0	12	22	16	22	24	23	15	17	19	24	20
II	0.10	1	1.1	60	46	16	20	24	56	66	63	64	70	68
		2	1.1	68	21	—	73	78	73	55	—	69	55	85
		3	2.2	64	41	44	53	69	46	38	—	76	73	79
		4	2.6	50	63	48	35	49	58	66	54	60	75	59
		Mean	1.8	61	43	36	45	55	58	56	58	67	68	73
		SD	0.8	8	17	17	23	24	11	13	6	7	9	12
III	0.15	1	3.9	53	51	51	56	61	31	26	65	88	93	78
		2	2.2	62	0	0	20	35	49	55	73	63	81	76
		3	1.3	64	35	71	69	—	79	81	—	78	93	—
		4	3.2	63	25	65	64	71	73	56	—	78	85	75
		Mean	2.7	61	27	47	52	56	58	55	69	77	88	76
		SD	1.1	5	21	32	22	19	22	23	6	10	6	2
IV	0.20	1	2.6	63	0	13	24	33	63	0	—	78	78	73
		2	3.0	54	6	24	30	39	—	0	—	75	79	83
		3	2.2	65	0	10	30	46	59	0	61	75	74	64
		4	3.4	58	0	13	30	36	34	0	—	80	72	74
		Mean	2.8	60	1.5	15	29	39	52	0	—	77	76	74
		SD	0.5	5	3	6	3	6	16			3	3	8

shown). The hemoglobin and hematocrit values did not change significantly after injection of the antibody (Table V).

## Discussion

This Phase I study demonstrates that infusion of 7E3-F(ab')<sub>2</sub> at doses of 0.20 mg/kg to patients with unstable angina can produce high-grade blockade of GPIIb/IIIa receptors (to 13±7% of baseline) and marked inhibition of platelet aggregation induced by ADP and collagen. A marked prolongation of the bleeding time (to > 30 min) with normalization within 24 h was observed but this was not associated with spontaneous hemorrhage or hemodynamic compromise. Platelet count reductions were mild and transient except in one patient who sustained a more significant decrease to a nadir of  $0.9 \times 10^8$  ml, with a return toward normal over the next 2 d; there was no obvious explanation for this patient's response, including a negative evaluation for preexisting IgG antibodies to 7E3-F(ab')<sub>2</sub>.

One patient had low levels of preexisting IgG anti-7E3-F(ab')<sub>2</sub>, but the presence of this IgG did not seem to alter the patient's response to the infusion, nor did the patient develop an anamnestic response despite the fact that the inhibition assays suggested that the IgG was specific for 7E3-F(ab')<sub>2</sub>. The

significance of this finding is unclear. Two other patients developed positive assays for IgG to 7E3-F(ab')<sub>2</sub> several weeks after the infusion, indicating that the material is potentially immunogenic.

A significant correlation was observed between the log bleeding time and the residual GPIIb/IIIa receptor density ( $r = 0.73$ ,  $P = 0.0001$ ) indicating that the receptor density is an important determinant of the bleeding time.

The return of free GPIIb/IIIa receptors with time after infusion of 0.20 mg/kg 7E3-F(ab')<sub>2</sub> was relatively rapid, increasing from 13±7% of the baseline value 1 h after injection of the antibody, to 33±10% at 12 h, 44±8% at 24 h, and 67±7% at 72 h. It is possible that clearance of antibody-coated platelets is more rapid than normal, and provided platelet production matches the increased clearance, a more rapid return of free receptors would be expected. Direct platelet survival measurements will be required to address this issue. Similarly, if the cohort of platelets in the spleen does not become coated with 7E3-F(ab')<sub>2</sub> to the same extent as peripheral blood platelets during the first hour, then the emergence of these less-coated platelets into the circulation would produce a more rapid return of free GPIIb/IIIa receptors. The intraplatelet pool(s) of GPIIb/IIIa furnish another potential source of free GPIIb/IIIa,

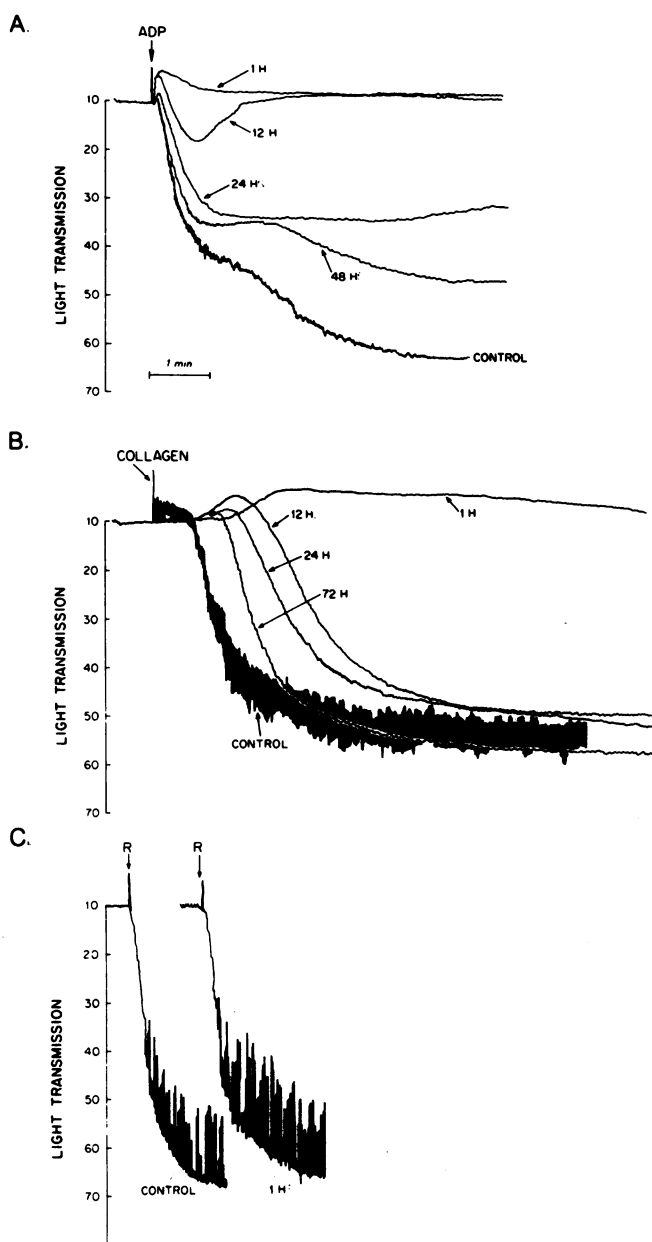


Figure 2. Aggregometer tracings of platelet aggregation with (A) ADP, (B) collagen, or (C) ristocetin before and at different time intervals after injection of 0.20 mg/kg 7E3-F(ab')<sub>2</sub>. R, ristocetin.

and recent preliminary evidence indicates that GPIIb/IIIa receptors may cycle between the pools (19). Strategies to prolong the receptor blockade may be aided by additional information on the mechanism(s) of the rapid return of free receptors.

In group IV given 0.20 mg/kg of 7E3-F(ab')<sub>2</sub>, all patients had extensive GPIIb/IIIa receptor blockade and prolongation of the bleeding time to > 30 min at 1 h. Anginal pain did not occur within the first hours after injection of antibody in these patients, but was documented at 11 h in patient IV-3 and at 23 h in patient IV-2. The onset of pain in patient IV-3 occurred at a time when the bleeding time was 10 min and the GPIIb/IIIa receptor density 37%; in patient IV-2 pain occurred at a time when the bleeding time was 7.0 min and the GPIIb/IIIa receptor density 49%. These observations suggest either that the

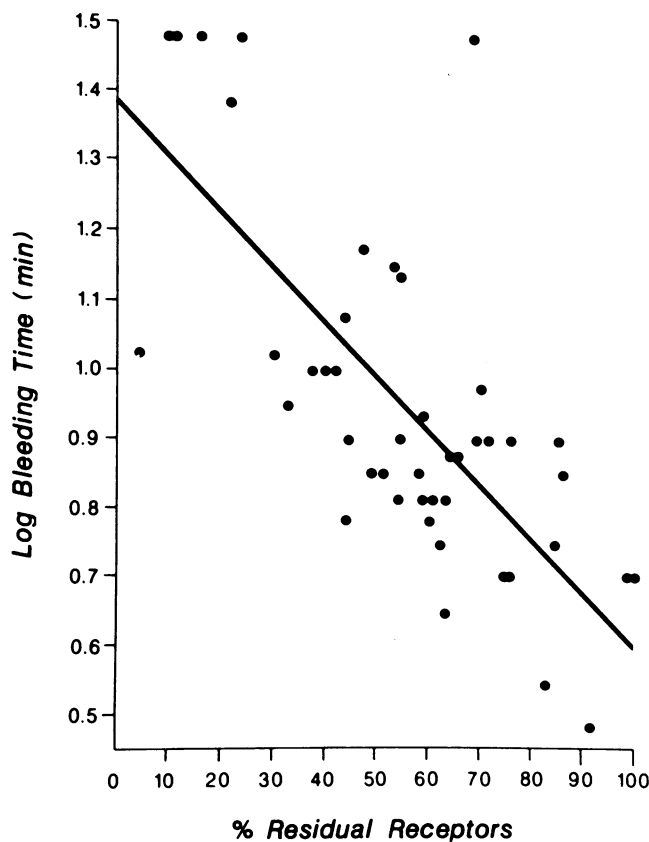


Figure 3. Correlation between the log bleeding time and the percent residual GPIIb/IIIa receptors, based on data obtained in all patients at 1, 12, and 24 h after injection of 7E3-F(ab')<sub>2</sub>.

mechanism of ischemic pain in these patients was not platelet mediated, or that very high grade blockade of GPIIb/IIIa receptors is required to prevent platelet thrombus formation and precipitation of ischemic pain. In view of the known pathophysiological relationship between atherosclerotic plaque rupture, platelet-rich localized thrombus formation contiguous to the site of rupture (20, 21), and ischemic coronary pain (22), we favor the latter hypothesis. This would entail that thrombogenicity of the vessel wall is either recurrent or persistent beyond the time zone of sufficient platelet inhibition with a single bolus injection of 7E3-F(ab')<sub>2</sub>. Further studies will indicate whether higher doses of the antibody fragment or repetitive injections of the antibody over a 24–48-h period might protect patients with unstable angina from recurrent ischemia until passivation of the thrombogenicity of the vessel wall has occurred.

Analysis of the present study, our previous study in a human (15), and data obtained by us (6, 8, 10, 13, 23) and others (24, 25) in animals given monoclonal antibodies to platelet GPIIb/IIIa, permits us to make some tentative correlations between GPIIb/IIIa levels and platelet function. Decreasing the number of GPIIb/IIIa receptors from the normal values of ~ 30,000–50,000 to ~ 20,000 has little effect on platelet function other than to decrease platelet aggregation mildly in response to ADP. This is also in accord with clinical observations on individuals who are obligatory heterozygotes for Glanzmann thrombasthenia, since these individuals are asymptomatic and have essentially normal platelet function

Table V. Hematological Parameters after 7E3-F(ab')<sub>2</sub> Injection

Patient group	Dose	Patient number	Hemoglobin			Hematocrit		
			Baseline	24 h	72-120 h	Baseline	24 h	72-120 h
	mg/kg		g/dl			%		
I	0.05	1	13	13	12	38	38	33
		2	13	13	14	37	36	37
		3	13	14	—	36	42	—
		4	15	13	14	42	38	38
		Mean	14	13	13	38	39	36
		SD	1	1	1	3	3	3
II	0.10	1	14	12	12	41	36	35
		2	12	13	13	35	37	35
		3	13	12	11	35	35	33
		4	13	16	8	37	48	37
		Mean	13	13	11	37	39	35
		SD	1	2	2	3	6	2
III	0.15	1	11	—	13	45	—	38
		2	14	14	—	42	41	—
		3	15	—	15	43	—	43
		4	14	14	—	41	40	—
		Mean	14	14	14	43	41	41
		SD	2		1	2	1	4
IV	0.20	1	17	16	—	49	47	—
		2	17	16	15	50	44	42
		3	14	14	12	40	40	35
		4	15	14	—	45	43	—
		Mean	16	15	14	46	44	39
		SD	2	1	2	5	3	5

even though they have ~ 40% fewer GPIIb/IIIa receptors than normal individuals (26). Reduction of the number of GPIIb/IIIa receptors to between 10,000 and 20,000 has a more profound effect on ADP-induced platelet aggregation, with nearly total abolition at ~ 10,000 residual receptors (23). The effect on the bleeding time, however, is only modest, unless the number of GPIIb/IIIa receptors is reduced to < 10,000. Interestingly, in the model developed by Folts to assess platelet thrombus formation in moderately stenosed (~70%) and damaged arteries, we found that platelet thrombus formation could be abolished in many animals by decreasing the number of residual GPIIb/IIIa receptors to between 10,000 and 20,000, a dose that did not completely inhibit platelet aggregation and rarely increased the bleeding time to > 10 min (23).

In contrast, in our previously described model designed to study thrombolysis of an acute coronary artery thrombus in severely stenosed (> 90%) blood vessels, in general, doses that would be expected to decrease the number of GPIIb/IIIa receptors to < 10,000 were required to obtain favorable results in speeding reperfusion, permitting recombinant tissue-type plasminogen activator dose reductions, and preventing reocclusion. In view of the greater stenosis in this model compared to the Folts model, the presence of an initial thrombus, and the likelihood of residual thrombogenic material remaining after thrombolysis in this model, it is perhaps not surprising that

higher-grade GPIIb/IIIa receptor blockade is required to have a favorable effect. In a preliminary report (27), Takami et al. found that 1-1.5 mg/kg of a murine monoclonal antibody to pig platelet GPIIb/IIIa produced only modest increases in bleeding time but complete inhibition of ADP-induced platelet aggregation. They indicated that the plasma level of the Fab' reached a near saturating level (> 95%) after the infusion, but they did not measure the number of blocked receptors directly. Since it is possible that equilibrium had not been established by 5 min, it may be that the extent of blockade was in fact < 95%. In addition, our data indicate that even when platelet aggregation has been abolished, additional 7E3-F(ab')<sub>2</sub> can increase the bleeding time (23). Moreover, patients with Glanzmann thrombasthenia who have very few or no functional GPIIb/IIIa receptors, do have prolonged bleeding times. Thus, although it is possible that there are species differences or differences related to the monoclonal antibodies, if the receptor blockade in this study was actually approximately 80%, we believe their data are consistent with ours. The dose response studies conducted by Hanson et al. (24) in baboons using the murine monoclonal antibody AP-2 are also consistent with our data since they found that at 0.4 mg/kg, a dose that only increased the bleeding from ~ 5 min to < 10 min, there was a 77-82% decrease in ADP-induced platelet aggregation and 100% inhibition of collagen-induced aggregation. At a higher dose of AP-2 (1.0 mg/kg) the bleeding time rose to nearly 20 min. Moreover, a very high dose (10 mg/kg) of another monoclonal antibody (LJ-CP8) prolonged the bleeding time > 30 min while essentially eliminating platelet aggregation to ADP and collagen. All doses of AP-2 down to 0.2 mg/kg, which did not increase the bleeding time at all, significantly reduced platelet deposition on Dacron vascular grafts (0.2 mg/kg = 41%, 0.4 mg/kg = 51%, 1.0 mg/kg = 73%), demonstrating an antithrombotic effect at antibody concentrations that only had a modest effect on the bleeding time. Platelet GPIIb/IIIa receptors were not measured directly in this study. In another study (25) using LJ-CP8, 10 mg/kg of the antibody did not prevent occlusion of a 4-mm expanded polytetra fluoroethylene (Gore-Tex, W. L. Gore and Associates, Inc., Flagstaff, AZ) graft placed in the femoral artery of baboons despite the antibody abolishing platelet aggregation and increasing the bleeding time to > 30 min. Although we have not tested our antibody in a similar system, it is possible that thrombosis in this model cannot be prevented by even high-grade GPIIb/IIIa blockade.

In summary, this study demonstrates that infusion of 7E3-F(ab')<sub>2</sub> at 0.20 mg/kg transiently produces profound inhibition of platelet function without causing spontaneous hemorrhage. Additional studies will be required to assess whether this approach may be efficacious in the treatment of ischemic coronary syndromes.

## Acknowledgments

The authors are grateful to Mr. J. Newell, Cardiac Computer Center, Massachusetts General Hospital for the statistical analysis and to Ms. Missy Stanton for excellent secretarial assistance.

This study was supported in part by National Institutes of Health Ischemia SCOR grant HL-26215, by National Heart, Blood and Lung Institute grant HL-19278, and by a sponsored research agreement between Massachusetts General Hospital and Centocor, Inc.



## References

1. Health, United States 1981. U.S. Department of Health and Human Services, National Center for Health Statistics, Hyattsville, MD. DHHS Publication No. (PHS) 82-1232.
2. Antiplatelet Trialists' Collaboration. 1988. Secondary prevention of vascular disease by prolonged antiplatelet treatment. *Br. Med. J.* 296:320-331.
3. Collier, B. S. 1989. Activation-specific platelet antigens. In *Platelet Immunobiology: Molecular and Clinical Aspects*. T. J. Kunicki and J. George, editors. J.P. Lippincott Co., Philadelphia. 166-169.
4. Plow, E. F., and M. H. Ginsberg. 1988. Cellular adhesion: GPIIb/IIIa as a prototypic adhesion receptor. *Prog. Hemostasis Thromb.* 9:117-156.
5. Collier, B. S. 1985. A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. *J. Clin. Invest.* 76:101-108.
6. Collier, B. S., and L. E. Scudder. 1985. Inhibition of dog platelet function by in vivo infusion of F(ab')<sub>2</sub> fragments of a monoclonal antibody. *Blood.* 66:1456-1450.
7. Collier, B. S., J. D. Folts, L. E. Scudder, and S. R. Smith. 1986. Antithrombotic effect of a monoclonal antibody to the platelet glycoprotein IIb/IIIa receptor in an experimental animal model. *Blood.* 68:783-786.
8. Gold, H. K., B. S. Collier, T. Yasuda, T. Saito, J. T. Fallon, J. L. Guerrero, R. C. Leinbach, A. A. Ziskind, and D. Collen. 1988. Rapid and sustained coronary artery recanalization with combined bolus injection of recombinant tissue-type plasminogen activator and monoclonal anti-platelet GPIIb/IIIa antibody in a dog model. *Circulation.* 77:670-677.
9. Fitzgerald, D. F., F. Wright, and G. A. Fitzgerald. 1989. Increased thromboxane biosynthesis during coronary thrombolysis: evidence that platelet activation and thromboxane A<sub>2</sub> modulate the response to tissue-type plasminogen activator in vivo. *Circ. Res.* 65:83-94.
10. Yasuda, T., H. K. Gold, J. T. Fallon, R. C. Leinbach, J. L. Guerrero, L. E. Scudder, M. Kanke, D. Shealy, M. J. Ross, D. Collen, et al. 1988. A monoclonal antibody against the platelet GPIIb/IIIa receptor prevents coronary artery reocclusion following reperfusion with recombinant tissue-type plasminogen activator in dogs. *J. Clin. Invest.* 81:1284-1291.
11. Mickelson, J. K., P. J. Simpson, and B. R. Lucchesi. 1989. Antiplatelet monoclonal F(ab')<sub>2</sub> antibody directed against the platelet GPIIb/IIIa receptor complex prevents coronary artery thrombosis in the canine heart. *J. Mol. Cell. Cardiol.* 21:393-405.
12. Bates, E. R., M. J. McGillem, J. K. Mickelson, B. Pitt, and G. B. J. Mancini. 1988. A monoclonal antibody to the platelet receptor GPIIb/IIIa (7E3) prevents acute thrombosis in a canine model of coronary angioplasty. *Circulation.* 78:II?--189. (Abstr.)
13. Cavagnaro, J., M. A. Serabian, D. J. Shealy, B. S. Collier, and J. D. Iulucci. 1987. Pharmacokinetic analysis of murine monoclonal antibody 7E3 F(ab')<sub>2</sub> in monkeys. *Blood.* 70(Suppl. 1):349a. (Abstr.)
14. Cavagnaro, J., M. A. Serabian, B. S. Collier, and J. D. Iulucci. 1987. Long term toxicologic evaluation of anti-platelet monoclonal antibody 7E3 F(ab')<sub>2</sub> in monkeys. *Blood.* 70:337a. (Abstr.)
15. Collier, B. S., L. E. Scudder, H. J. Berger, and J. D. Iulucci. 1988. Inhibition of human platelet function in vivo with a monoclonal antibody: with observations on the newly dead as experimental subjects. *Ann. Intern. Med.* 109:635-638.
16. Gold, H. K., J. A. Johns, R. C. Leinbach, T. Yasuda, E. Grossbard, R. Zusman, and D. Collen. 1987. A randomized, blinded, placebo-controlled trial of recombinant human tissue-type plasminogen activator in patients with unstable angina pectoris. *Circulation.* 75:1192-1199.
17. Shulman, N. R., and J. V. Jordan. 1987. Platelet kinetics. In *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, 2nd Edition. R. W. Colman, J. Hirsh, V. J. Marder, and E. W. Salzman, editors. J.B. Lippincott Co., Philadelphia. 431-451.
18. Clauss, A. 1957. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Haematol.* 17:237-245.
19. Wencel-Drake, J. D. 1990. Plasma membrane GPIIb/IIIa: evidence for a cycling receptor pool. *Am. J. Pathol.* 136:61-70.
20. Friedman, M. 1971. The coronary thrombus: its origin and fate. *Hum. Pathol.* 2:81-128.
21. Davies, M. J., and A. C. Thomas. 1985. Plaque fissuring: the cause of acute myocardial infarction, sudden ischemic death, and crescendo angina. *Br. Heart J.* 53:363-373.
22. Swahn, E., and L. Wallentin. 1987. Platelet reactivity in unstable coronary artery disease. *Thromb. Haemostasis.* 57:302-305.
23. Collier, B. S., J. D. Folts, S. R. Smith, L. E. Scudder, and R. Jordan. 1989. Abolition of in vivo platelet thrombus formation in primates with monoclonal antibodies to the platelet GPIIb/IIIa receptor. *Circulation.* 80:1766-1774.
24. Hanson, S. R., F. I. Pareti, Z. M. Fuggeri, U. M. Marzac, J. J. Kunicki, P. R. Montgomery, T. S. Zimmerman, and L. Harker. 1988. Effects of monoclonal antibodies against the platelet glycoprotein IIb/IIIa complex on thrombosis and hemostasis in the baboon. *J. Clin. Invest.* 81:149-158.
25. Torem, S., P. A. Schneider, and S. R. Hanson. 1988. Monoclonal antibody-induced inhibition of platelet function: effects on hemostasis and vascular graft thrombosis in baboons. *J. Vasc. Surg.* 7:172-180.
26. Collier, B. S., U. Seligsohn, A. Zivelin, E. Zwang, A. Lusky, and M. Modan. 1986. Immunologic and biochemical characterization of homozygous and heterozygous Glanzmann's thrombasthenia in Iraqi-Jewish and Arab populations of Israel: comparison of techniques for carrier detection. *Br. J. Haematol.* 62:723-735.
27. Takami, H., W. L. Nichols, S. E. Kaese, and J. W. Bowie. 1987. Infusion study of monoclonal antibodies against porcine platelet membrane GPIIb and GPIIb/IIIa: differential effect on platelet function and bleeding time. *Blood.* 70(Suppl. 1):344a. (Abstr.)