Abolition of In Vivo Platelet Thrombus Formation in Primates With Monoclonal Antibodies to the Platelet GPIIb/IIIa Receptor

Correlation With Bleeding Time, Platelet Aggregation, and Blockade of GPIIb/IIIa Receptors

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We studied the dose-response effects of the F(ab')2 fragments of murine monoclonal antibodies to the platelet GPIIb/IIIa receptor (7E3 and 10E5) on in vivo platelet thrombus formation in a well-characterized monkey model in which the carotid artery is stenosed and thrombus formation is provoked and augmented by intimal damage and the infusion of subaggregating doses of epinephrine. Both antibodies abolished thrombus formation with a mean dose of ~0.2 mg/kg. Ex vivo platelet aggregation was not always abolished at doses that abolished thrombus formation; similarly, bleeding times were only moderately prolonged (9.1 ± 1.4 minutes) at these doses. Increasing the dose above that required to abolish thrombus formation consistently produced abolition of ex vivo platelet aggregation, marked prolongation of the bleeding time (14.2 ± 1.5 minutes), and nearly quantitative blockade of GPIIb/IIIa receptors. We conclude that in a significant percentage of animals, the extent of GPIIb/IIIa blockade required to prevent vasoocclusive thrombus formation in this model is less than that required for abolition of platelet aggregation, and that the preservation of only a minority of functional GPIIb/IIIa receptors might be adequate to maintain a nearly normal bleeding time. (Circulation 1989;80:1766–1774)

Platelet thrombus formation is believed to contribute to many vaso-occlusive and thromboembolic disorders that collectively constitute the most common cause of death in the United States.1,2 Currently available antiplatelet agents have shown some efficacy in treating these disorders, but the results have fallen short of expectations, leading to the continued search for more effective antiplatelet drugs.3

One of us (J.F.) has designed a quantitative animal model of platelet thrombus formation involving partially stenosed coronary arteries in the dog and carotid arteries in the monkey.3–5 In this model, platelet thrombi form in the stenosed segment after intimal damage and then embolize, either spontaneously or with mechanical dislodgment, producing cyclical flow reductions (CFRs) in the blood flow pattern that can be monitored with an electromagnetic flow probe. The model is designed to simulate thrombus formation on diseased arteries that are partially stenosed as a result of underlying atherosclerosis and then have superimposed intimal damage. Support for the validity of such a model comes from a variety of data, with the most recent anatomic evidence coming from angioscopic studies of patients with unstable angina showing active, nonocclusive thrombus formation.6 Additional physiologic evidence comes from both animal models and the human experience of cyclical thrombus formation as a harbinger of reocclusion of coronary arteries after thrombolytic reperfusion by tissue plasminogen activator despite full heparin therapy.7–9

We previously showed that a dose of 0.8 mg/kg of the F(ab')2 fragment of a murine monoclonal antibody (7E3) directed against the platelet GPIIb/IIIa

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**Laboratory Investigation**

*Abstract*

We studied the dose-response effects of the F(ab')2 fragments of murine monoclonal antibodies to the platelet GPIIb/IIIa receptor (7E3 and 10E5) on in vivo platelet thrombus formation in a well-characterized monkey model in which the carotid artery is stenosed and thrombus formation is provoked and augmented by intimal damage and the infusion of subaggregating doses of epinephrine. Both antibodies abolished thrombus formation with a mean dose of ~0.2 mg/kg. Ex vivo platelet aggregation was not always abolished at doses that abolished thrombus formation; similarly, bleeding times were only moderately prolonged (9.1 ± 1.4 minutes) at these doses. Increasing the dose above that required to abolish thrombus formation consistently produced abolition of ex vivo platelet aggregation, marked prolongation of the bleeding time (14.2 ± 1.5 minutes), and nearly quantitative blockade of GPIIb/IIIa receptors. We conclude that in a significant percentage of animals, the extent of GPIIb/IIIa blockade required to prevent vasoocclusive thrombus formation in this model is less than that required for abolition of platelet aggregation, and that the preservation of only a minority of functional GPIIb/IIIa receptors might be adequate to maintain a nearly normal bleeding time. (Circulation 1989;80:1766–1774)

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complex, which acts as a receptor for fibrinogen, von Willebrand factor, and fibronectin when platelets are appropriately activated, was the most effective antiplatelet agent tested thus far in this model.\(^5\) Not only did it stop the periodic thrombus formation and CFRs, but in all eight animals (four dogs and four monkeys), it also protected against the restoration of the CFRs by several provocative maneuvers that augment thrombus formation (infusion of subaggregating doses of epinephrine, hemostat-induced increased intimal damage to the vessel, and passing an electric current through the vessel). These provocations, alone or in combination, can usually restore thrombus formation in this model when conventional antiplatelet agents such as aspirin have abolished the initial cycles of thrombus formation.\(^10\)^\(^1^1\)

The dose of 7E3-F(ab')\(_2\) antibody used in the previous study was chosen because a preliminary series of experiments demonstrated its capacity to completely block ex vivo platelet aggregation induced by ADP when injected intravenously into dogs.\(^12\) The current study was designed to investigate the minimum dose of 7E3-F(ab')\(_2\)\(^13\) and another antibody directed at the GPIIb/IIIa receptor [10E5-F(ab')\(_2\)]\(^14\) required to abolish platelet thrombus formation in monkeys and to correlate this response with the bleeding time, platelet aggregation, and blockade of GPIIb/IIIa receptors.

### Methods

The in vivo, coronary artery, platelet thrombus model has been described previously in detail.\(^3\)^\(^4\) A variant of this model, conducted on the carotid arteries of 20 cynomolgus monkeys was used in the present study. The monkeys were premedicated with 5 mg/kg ketamine followed by increments of sodium thiamylal given to maintain general anesthesia. The right femoral artery and vein were dissected out and cannulated for blood pressure measurement and for giving intravenous fluids and drugs. The left carotid artery was dissected out for a length of 2–3 cm and an electromagnetic flow probe placed on it to continuously measure blood flow. Distal to the probe, the artery was clamped several times with a hemostat to produce moderate intimal and medial damage. A plastic constricting cylinder was then placed around the damaged artery to produce an external stenosis of 70–75%. We and others have shown that in the dog model, acute platelet thrombus formation occurs periodically, causing blood flow reductions that are terminated abruptly when distal embolization occurs.\(^3\)^\(^5\)^\(^13\)^\(^2\)\(^15\)^\(^16\) Occasionally, embolization does not occur spontaneously, and under these circumstances, the thrombus is dislodged by gently shaking the cylinder. These cyclical flow reductions nearly always continue unabated for hours. In this study, similar cyclical flow reductions were produced in the carotid arteries of the monkeys and allowed to proceed for 30 minutes before administering the antibodies to be tested.

Bleeding times were performed in duplicate on the lateral aspects of the tongues of the anesthetized animals using a Mielke template device (Hemakit, Malden, Massachusetts) adjusted to deliver an approximately 1.5-mm deep incision by grinding the template to make it thinner by approximately 0.5 mm; the same template was used for all of the animals. We previously showed that this technique is reproducible, with control values in 15 normal dogs equal to 3.3±0.5 minutes (mean±SD).\(^17\) Platelet aggregation was measured in citrated platelet-rich plasma (~300,000/µl) as previously described,\(^14\) with the following reagents: ADP (5 and 10 µM), epinephrine (11 µM) followed 30 seconds later by ADP (5 µM), and collagen (6 µg/ml) (ChronoLog, Havertown, Pennsylvania). Ex vivo binding of near-saturating concentrations (~20 µg/ml) of \(125I\)-10E5 and \(125I\)-7E3 to platelets in citrated platelet-rich plasma was measured by incubating the platelets with the radiolabeled antibodies for 30 and 60 minutes, respectively, and then separating the platelet-bound antibody from free antibody by centrifugation through 30% sucrose. The number of molecules bound per platelet was then calculated from the bound radioactivity.\(^13\) The decrease in GPIIb/IIIa sites available for binding radiolabeled antibody ex vivo after F(ab')\(_2\) injection was taken as the number of sites blocked in vivo by the unlabeled F(ab')\(_2\) fragments. The above studies were performed 20 minutes after each dose of antibody.

Antibodies 7E3, 10E5, and a control monoclonal antibody directed at an ovarian carcinoma antigen (OC-125)\(^18\) were purified from cell culture supernatants, digested to F(ab')\(_2\) fragments with pepsin and then repurified, with minor modifications of the method previously described.\(^5\) The resulting sterile 0.9% NaCl solutions were low in pyrogens (<1 EU/mg).

Antibody was administered as one or more intravenous bolus injections spaced approximately 30–45 minutes apart until the spontaneous cycles of platelet thrombi were abolished. Attempts were then made to restore the thrombi by increasing the intimal damage by repeated clamping with a hemostat and infusing epinephrine at 0.4 and 0.8 µg/kg/min for 15 minutes. If thrombi returned, additional antibody was given and the provocations repeated. In some experiments, additional antibody was then administered to assess the effect of higher doses on the bleeding time, platelet aggregation, and blockade of GPIIb/IIIa receptors. The first 15 animals (phase 1) were treated with varying doses of 7E3 and 10E5 in an attempt to identify the range of minimum inhibitory doses. The last five animals (phase 2) were treated with a uniform protocol, wherein 7E3-F(ab')\(_2\) was administered at cumulative doses of 0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg. In three of these monkeys, control F(ab')\(_2\) was
administered at 0.2 mg/kg before beginning the 7E3-F(ab')2 infusions.

Results

Control Antibody

Control antibody was administered to eight monkeys at doses of 0.2 mg/kg (four monkeys) and 0.4 mg/kg (four monkeys). In seven of the eight animals, there was no inhibition of thrombus formation (Figure 1). In addition, in these seven animals, there was no significant effect on the bleeding, the binding of 125I-7E3, or platelet aggregation. The data on three of these animals given the control antibody before 7E3-F(ab')2 are shown in Figure 2. One monkey given 0.4 mg/kg of the control antibody at a time when thrombus formation had just begun, stopped forming thrombi approximately 15–20 minutes after the infusion, a time span considerably more than the 3–5 minutes required for the specific antibodies to affect thrombus formation.5 125I-7E3 binding studies performed on this animal’s platelets showed only a minimal decrease in the number of molecules bound (13%), and platelet aggregation studies showed no decrease in the initial slope of aggregation induced by ADP or the combination of epinephrine and ADP; there was, however, a tendency for more rapid disaggregation after aggregation induced by these agents. Collagen-induced aggregation demonstrated a 41% decrease in initial slope, but the total extent of aggregation was unchanged. The bleeding time in this animal increased from 4.5 to 8 minutes. Whereas a small but significant percentage of animals do not form strong thrombi regardless of the provocation,10 it is not clear whether the response in this animal was merely coincidental with, or a result of, the infusion of the control antibody.
Antibodies 10E5 and 7E3

The responses of the 15 animals in phase 1 to infusion of the F(ab')2 fragments of 10E5 and 7E3 are reported in Table 1. Both 7E3 and 10E5 not only abolished the initial platelet thrombus formation within several minutes, but also prevented its return in response to the infusion of epinephrine and increasing the intimal damage with a hemostat. Microscopic analysis showed that the intimal injury induced by the hemostat was severe, with tears extending through the intima and into the media. The minimal dose needed to abolish the initial thrombi was 0.1–0.4 mg/kg with a mean value of 0.22 mg/kg. This value is likely to be an overestimation because in seven animals the lowest concentration of antibody tested abolished the thrombi, including one in which the lowest dose tested was 0.4 mg/kg. There was no significant difference between the potency of 7E3 and 10E5. In 12 of the 15 animals, the same dose of antibody that abolished the initial thrombus formation also prevented the return of thrombi when the animals were challenged with epinephrine infusion and increased intimal damage. In the other three animals, minor increases in dose were required to abolish the augmented thrombus formation.

Platelet aggregation to all of the agonists was inhibited by 10E5-F(ab')2 and 7E3-F(ab')2 in all of the animals in a dose-dependent manner. This correlated with increasing blockade of GPIIb/IIIa receptors and progressive prolongation of the bleeding time (Figure 2). Interestingly, in at least six of the 15 animals (10E5 nos. 1, 4, and 7; and 7E3 nos. 1, 5, and 6), abolition of spontaneous and augmented platelet thrombus formation could be achieved with antibody doses that did not abolish ex vivo platelet aggregation and had only modest effects on the bleeding time. Another five animals (10E5 nos. 2, 5, and 8; and 7E3 nos. 2 and 3) had both platelet aggregation and thrombus formation completely abolished at the lowest dose of antibody tested, raising the possibility that lower doses might have abolished platelet thrombus formation without abolishing platelet aggregation.

The data from this phase of the study suggested that vaso-occlusive platelet thrombus formation might be abolished in a substantial percentage of animals without either high-grade GPIIb/IIIa receptor blockade or abolition of platelet aggregation, and that only a minority of unblocked GPIIb/IIIa receptors might be required to maintain a relatively normal bleeding time. To test this hypothesis further, five additional monkeys were treated with a standardized protocol in which they received incremental amounts of 7E3-F(ab')2 to achieve cumulative doses of 0.1, 0.2, and 0.4 mg/kg (Figure 3). One of the five animals achieved complete abolition of augmented, in vivo thrombus formation at a dose of 0.1 mg/kg. Platelet aggregation in this animal was still present but inhibited by 54%, and 47% of the GPIIb/IIIa receptors were blocked. The other four animals achieved complete abolition of thrombus formation at 0.2 mg/kg. Platelet aggregation was not abolished in any of these animals at this dose. The bleeding times at the 0.2 mg/kg dose were prolonged compared with the control value, but the bleeding time exceeded 10 minutes in only one of the five animals. (9.1±1.4 minutes; mean±SD; range, 8–11.5 minutes). Increasing the dose to 0.4 mg/kg abolished platelet aggregation in all of the animals, produced nearly quantitative blockade of the GPIIb/IIIa receptors (95±1.3%) and induced a more significant prolongation of the bleeding time (14.2±1.5 minutes; range, 12–16 minutes; p<0.01, compared with 0.2 mg/kg dose value).

We calculated the fraction of injected antibody that became associated with the platelets in the phase 1 study from the percentage of GPIIb/IIIa receptors blocked, the total number of GPIIb/IIIa receptors present on the platelets, and the peripheral platelet count; the calculations were also based on data indicating that 15% of primate platelets reside in the spleen and the estimated blood volume of a monkey is 70 ml/kg of total body weight.20 At 0.2 mg/kg, the percentage of injected antibody that was recoverable on platelets was 54±18% (mean±SD; range, 29–83; n=7). There was no difference between 10E5-F(ab')2 (mean, 53%; n=4) and 7E3-F(ab')2 (mean, 56%; n=3). At higher doses, this percentage decreased because the platelet GPIIb/IIIa sites had already become nearly saturated; for example, at 0.6 mg/kg of 7E3-F(ab')2, the three values obtained were 21%, 26%, and 37%.

Discussion

We previously demonstrated that a dose of 0.8 mg/kg 7E3-F(ab')2 fragments could abolish in vivo platelet thrombus formation in this model in both...
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<th>Animal</th>
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<th>Inhibition of thrombus formation</th>
<th>Blockade of GPIIb/IIIa receptors (%)</th>
<th>Bleeding time (min)*</th>
<th>Platelet count (×10⁷/µl)</th>
<th>Total no. GPIIb/IIIa sites/platelet</th>
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*The control (preinfusion) bleeding time is given in parentheses.

P, partial; C, complete; NT, not tested; 0, no inhibition.

Antibodies were infused with incremental doses of the antibodies to achieve the indicated cumulative doses. Platelet aggregation and in vivo thrombus formation were characterized as either partially (P) or completely (C) inhibited. Thrombus formation augmented by epinephrine infusion and increased intimal damage was similarly analyzed. Percentage of GPIIb/IIIa receptors blocked by infusion of 7E3-F(ab')2 was determined by analyzing the amount of radiolabeled intact 7E3 that could bind to platelets ex vivo after 7E3-F(ab')2 was infused and comparing it with the amount of radiolabeled intact 7E3 that could bind to the same animal's platelets ex vivo before infusion. Bleeding times were performed in duplicate and results indicated are mean values; bleeding times of the animals before infusion are given in parentheses. Whole blood platelet count is indicated. Total number of GPIIb/IIIa sites per platelet was calculated from maximum amount of radiolabeled intact 7E3 that could bind to platelets ex vivo under equilibrium conditions, assuming monovalent binding.
FIGURE 3. Correlation between dose of antibody administered and epinephrine + ADP–induced platelet aggregation, bleeding time, percentage of GPIIb/IIIa receptors blocked and abolition of augmented, in vivo, platelet thrombus formation in five monkeys. After platelet thrombus formation was induced, animals were given control $F(ab')_2$ (OC-125) at 0.2 mg/kg (n=3). This had no significant effect on any measurements and data are shown to left of zero concentration mark. Animals were then given incremental doses of 7E3–F(ab')$_2$, achieving the cumulative doses noted on abscissa. Aggregation response, judged by initial slope of aggregation in response to epinephrine (11 μM)+ADP (5 μM), is expressed as percentage of control value, whereas abolition of thrombi and GPIIb/IIIa receptors blocked are expressed as percentage of maximum possible. Bleeding time is expressed in minutes. All error bars represent ±SEM. Note that at 0.2 mg/kg, thrombus formation was abolished in all five animals even though platelet aggregation was not abolished and only ~71% of GPIIb/IIIa receptors were blocked.

dogs and monkeys, and that this was accompanied by abolition of platelet aggregation in response to several agonists and by blockade of approximately 85% of the GPIIb/IIIa receptors. The present study was designed to extend these observations by defining the dose-response relation for each of these phenomena and correlating them with the template bleeding time. We chose to confine these studies to monkeys because it allowed us to also assess the effects of 10E5–F(ab')$_2$, another murine monoclonal antibody directed at GPIIb/IIIa that blocks platelet aggregation but differs from 7E3 in its epitope and in its more limited cross-reactivity (primates but not canines). In addition, we hoped that the primate model would more closely approximate the response of humans than the canine model, especially because the antibodies were made to human platelets. This prediction has been borne out by our recent preliminary study performed on a newly dead human in which platelet aggregation induced by ADP was abolished at 0.2 mg/kg.

We previously had difficulty in obtaining reproducible bleeding times in animals, but the present technique using a template device to make an incision on the tongue was easy to perform and reproducible. We modified the template so the depth of incision was approximately 1.5 mm rather than 1 mm, because this depth was required to make incisions that bled reproducibly. Our platelet aggregation studies included aggregation induced by a combination of both epinephrine and ADP because epinephrine has been shown to augment ex vivo platelet aggregation induced by a variety of agonists and to overcome the inhibition induced by several agents, including PG12. Thus, this is a more stringent test of platelet inhibition. It also permitted an ex vivo simulation of the in vivo use of epinephrine to augment platelet thrombus formation. We believe that the abolition of augmented thrombus formation provoked by the combination of epinephrine infusion and increased intimal damage is a much better test of the potency of an antiplatelet agent than abolition of the initial thrombus formation because many relatively weak inhibitors of platelet function abolish the initial thrombus formation, but only the strongest inhibitors abolish the augmented thrombus formation.

Our results showed that the minimum effective dose to abolish augmented in vivo thrombus formation was 0.1–0.4 mg/kg, with most of the animals responding to 0.2 mg/kg or less. 10E5–F(ab')$_2$ and 7E3–F(ab')$_2$ were equally effective in inhibiting platelet function in vivo, reflecting the similarity in their affinities for platelets. Data from the first phase of the studies suggested that abolition of augmented thrombus formation could occur at doses of antibody that did not completely abolish platelet aggregation, and this was confirmed in the five animals in the second phase.

The correlation between in vivo blockade of GPIIb/IIIa receptors and inhibition of ex vivo platelet aggregation was very similar to the correlation we previously observed in vitro with 10E5, with approximately 40–60% inhibition of platelet aggregation when approximately 50% of the sites are blocked, and abolition of aggregation when more than 80% of the sites are blocked. Abolition of platelet thrombus formation appeared to require more than 50% blockade of GPIIb/IIIa receptors.

The most striking finding in our study was that the minimum doses of antibody that completely abolished platelet thrombus formation rarely prolonged the bleeding time to more than 10 minutes.
even though higher doses were capable of producing more dramatic prolongations. This indicates that retention of a minority of unblocked GPIIb/IIIa receptors is adequate for preserving much of the primary phase of hemostasis but inadequate to support vaso-occlusive thrombus formation in this model. In contrast to these results, we previously found that in a dog model of coronary artery reocclusion after thrombolysis with recombinant tissue plasminogen activator, nearly complete or complete inhibition of platelet aggregation was required to prevent reocclusion.26 This difference probably reflects both the more severe thrombogenic stimulus present after the lysis of a fully formed clot and the tighter, fixed stenosis (>90%) used in that study.

Hanson et al27 studied two different murine monoclonal antibodies to GPIIb/IIIa in a baboon model of thrombus formation in Dacron vascular grafts. Antibody AP-2 produced dose-dependent inhibition of platelet aggregation and inhibition of platelet thrombus formation on the graft. As in the present study, the bleeding time was only minimally prolonged at antibody concentrations that significantly impaired platelet aggregation. In contrast to our results with antibodies 10E5 and 7E3, however, even at a dose of 8 mg/kg, ADP–induced aggregation was not abolished. Antibody LJ-CP8, which was tested only at 10 mg/kg, abolished platelet aggregation, markedly prolonged the bleeding time, and inhibited platelet deposition in the graft. In another model, however, this antibody failed to protect against thrombotic occlusion of a small-diameter polytetrafluoroethylene femoral artery graft in baboons.28 Thus, these results are qualitatively similar to ours, but it appears that these antibodies have lower affinities for primate platelets than do ours. Most recently, preliminary data reported by Takami et al,29 using antibodies to porcine platelet GPIIb/IIIa, showed only a modest effect on the bleeding time with antibody doses that abolished platelet aggregation.

The complex effects of anti–GPIIb/IIIa antibodies on platelet aggregation and adhesion make it possible to propose several different mechanisms to explain the observation that the bleeding time is relatively well preserved despite blockade of a significant percentage of GPIIb/IIIa receptors. One interpretation is that the residual, unblocked GPIIb/IIIa receptors permit the formation of small platelet aggregates near the vessel wall that are able to arrest hemorrhage despite their weakness; when these aggregates begin to grow into the lumen, however, their frailty becomes compounded with each layer until they cannot withstand the forces produced by the flowing blood, and therefore, they cannot cause vaso-occlusion. Alternatively, because in ex vivo models of platelet-vessel wall interaction, 10E5 at near-saturating concentrations (~5–12 μg/ml) inhibited platelet adhesion, in addition to inhibiting platelet thrombus formation at high shear rates30,31 but not at low shear rates,30 it is possible that the prolongation of the bleeding time is reflecting an effect on platelet adhesion in the microcirculation where the shear rate is high. If the inhibition of adhesion requires high doses of antibody, this might explain our observation that doses of antibody in excess of those required to abolish in vivo platelet aggregation caused progressive prolongation of the bleeding time. Although platelet adhesion to subendothelial surfaces is generally thought to reflect the interaction of receptors other than GPIIb/IIIa with collagen, von Willebrand factor, and perhaps vitronectin, fibronectin, and laminin,32 there are several potential mechanisms by which GPIIb/IIIa blockade may lead to decreased adhesion. Thus, fluid-phase von Willebrand factor and fibronectin bind to GPIIb/IIIa on platelets activated with thrombin, and von Willebrand factor binds to GPIIb/IIIa on ADP–activated platelets33 and platelets subjected to high shear rates,34 making it possible for platelets activated in vivo by these mechanisms to adhere to the immobilized von Willebrand factor and fibronectin present on the subendothelium by GPIIb/IIIa receptors. Moreover, if fibrinogen becomes immobilized on the subendothelium by interactions with fibronectin or other elements, platelet GPIIb/IIIa might support adhesion to this fibrinogen. Finally, even if GPIIb/IIIa receptors are not responsible for the initial adhesion event, it is possible that they stabilize the adherent platelets against rapid embolization by furnishing additional interactive sites between the platelet and the adhesive glycoproteins in the subendothelium. This might be reflected in the morphological criterion of “spreading,” and in fact, 10E5 has been shown to have a pronounced effect on platelet spreading in these ex vivo models.30,31 To what extent the ex vivo models of platelet-vessel wall interaction measure the very initial event in adhesion compared with the subsequent events that stabilize adhesion remains unknown.

The ability of the antibodies to inhibit vaso-occlusive thrombus formation raises the possibility that appropriate GPIIb/IIIa blockade could protect humans with vascular disease against thrombotic ischemic damage. It is important, however, to note that such an interpretation rests on the assumption that the model used in this study accurately reflects the pathogenesis of human vascular disease. Considerable circumstantial evidence supports the similarity between the model and certain forms of thrombotic vascular disease in humans; for example, coronary angiography has demonstrated that most patients with unstable angina have active intracoronary thrombus formation despite the absence of fully occlusive disease,4 and cyclical coronary artery occlusions much like those produced by this model have been found to have an ominous predictive value for the development of permanent reocclusion during and immediately after thrombolytic therapy of myocardial infarction.8,9,35
Whereas the doses of the antibody that were effective in preventing thrombus formation did not produce markedly prolonged bleeding times, there is a temptation to conclude that such doses might, therefore, not produce excessive hemorrhage. However, the correlation between bleeding time and hemorrhagic diathesis is imperfect at best, and thus, judgment regarding this important point must be reserved. Although the bleeding time is prolonged, for example, in many disorders associated with hemorrhage, there are dissociations between the bleeding time and hemorrhagic risk such as the apparently low risk of bleeding accompanying therapy with ticlopidine despite the considerable bleeding-time prolongation.36,37

It has been reported that 7E3 interacts with a GPIIb/IIIa-like receptor on cultured human umbilical vein endothelial cells.38 Recent evidence indicates that this receptor is probably not authentic GPIIb/IIIa but rather the vitronectin receptor, which is composed of a protein homologous to but clearly different from GPIb and a protein probably identical in primary structure to GPIIIa.39 A small number of vitronectin receptors have also been recently identified on platelets.40,41 Thus, it is possible that some of the inhibition of thrombus formation we observed was due to an interaction of 7E3 with the vitronectin receptor on platelets and endothelial cells. We consider this unlikely, however, because 10E5 does not bind to the vitronectin receptor on platelets or endothelial cells (our unpublished data; IF Charo and MA Gimbrone, personal communications), and yet it was equally effective in inhibiting platelet thrombus formation.

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References


19. Folts JD, Coller BS: Role of von Willebrand in acute platelet thrombus formation in stenosed coronary arteries (abstract). Blood 1987;70(suppl 1):402a


Abolition of in vivo platelet thrombus formation in primates with monoclonal antibodies to the platelet GPIIb/IIIa receptor. Correlation with bleeding time, platelet aggregation, and blockade of GPIIb/IIIa receptors.

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