Platelet Thrombus Formation on Collagen at High Shear Rates Is Mediated by von Willebrand Factor–Glycoprotein Ib Interaction and Inhibited by von Willebrand Factor–Glycoprotein IIb/IIIa Interaction

Ya-Ping Wu, Tom Vink, Marion Schiphorst, G. Henrita van Zanten, Martin J.W. IJsseldijk, Philip G. de Groot, Jan J. Sixma

Abstract—We studied the role of von Willebrand Factor (vWF) in platelet thrombus formation in flowing blood by using a perfusion system and mutant forms of vWF lacking either interaction with glycoprotein Ib (GpIb) or with glycoprotein IIb/IIIa (αIIb-β3). These mutants were added to the blood of patients with severe von Willebrand’s disease (vWD) or to normal blood reconstituted with a human albumin solution instead of plasma. This blood was then perfused over collagen type III spray-coated on a glass surface and preincubated for 2 hours with 20 μg/mL plasma vWF. In this way, the adhesion step was mediated by the preincubated plasma vWF bound to collagen type III, whereas thrombus formation was mediated by mutant vWF added to the perfusate. Thrombus formation was absent at all 3 shear rates studied (300, 800, and 2600 s⁻¹) when ΔA1-vWF, lacking interaction with GpIb, was added to the perfusate, indicating the importance of GpIb-vWF interaction for thrombus formation. The interaction of vWF and GpIb is currently thought to be possible under physiological conditions in which the conformation of vWF has been changed by adsorption to a surface. Our results regarding the role of GpIb-vWF interaction in thrombus formation suggest that a second mechanism may operate by which a change may occur in GpIb on the surface of adhered platelets either by activation of the molecule or as a consequence of shear stress. Increased thrombus formation was observed when the Arg-Gly-Gly-Ser–vWF, which does not interact with αIIb-β3, was added to vWD blood and perfused at 2600 s⁻¹. This increase was not observed in vWD blood at lower shear rates or after addition of Arg-Gly-Gly-Ser–vWF to reconstituted normal blood. Thrombus formation at a high shear rate was largest when either vWF or fibrinogen was present as a single ligand for αIIb-β3 at a high shear rate. When both were present, thrombus formation was decreased. We postulate that thrombus formation is less efficient because of incomplete bridge formation when vWF and fibrinogen are both present as ligands for αIIb-β3. (Arterioscler Thromb Vasc Biol. 2000;20:1661-1667.)

Key Words: von Willebrand factor • thrombus formation • GpIb • collagen

Platelet adhesion to collagen in flow is followed by thrombus formation, which is, in many respects, similar to the aggregate formation in plasma that occurs when platelets are activated and stirred in a cuvette. This aggregation is dependent on the integrin glycoprotein IIb/IIIa (αIIb-β3). Fibrinogen (and perhaps also fibronectin) serves as a bridge between adjacent platelets.¹ A role for von Willebrand factor (vWF) has not been shown for aggregation in a cuvette; in this respect, aggregation differs from thrombus formation in flow, which is dependent on vWF at high shear.²,³

Exposure of platelets to high shear stress in a cone/plate viscometer causes platelet aggregation⁴,⁵ as consequence of interaction between vWF and glycoprotein Ib (GpIb). This interaction leads to platelet activation and subsequent exposure of αIIb-β3⁶–⁸; this is then followed by binding to αIIb-β3 of ligands, which form a firm bridge between aggregating platelets. At the highest shear rates, vWF was found to be this ligand.⁹

We investigated whether platelet thrombus formation on collagen under flow (at shear rates that occur in the circulation) is dependent on GpIb-vWF interaction as well; we also investigated whether αIIb-β3-vWF interaction occurs at all in whole blood. To study this, we used vWF mutants defective in interaction with either GpIb or αIIb-β3.

Methods

Human collagen type III from placental origin was obtained from Sigma Chemical Co. Low molecular weight heparin (Fragmin) was from Kabi Pharmacia. Plasma-vWF was purified from cryoprecipi-
vWF in the void volume was stored at $-20^\circ$C. Human fibrinogen (98% clottable, American Diagnostica) was further purified by size exclusion chromatography on Sepharose 4B. Peak fractions were lyophilized, dissolved in Tyrode’s buffer (18 mg/mL), and stored at $-20^\circ$C. LJ-P5 (courtesy of Dr Z.M. Ruggeri, Scripps Clinic & Research Foundation, La Jolla, Calif) is a monoclonal antibody against αIIb-β3, inhibiting the binding of vWF but not of fibrinogen or fibronectin.11 Fab fragments of this antibody were prepared by using standard techniques.

Coating of the Coverslips
Glass coverslips (18×18 mm, Menzel Gläser) were cleaned overnight by a chromosulfuric acid (2% chromium trioxide) solution and rinsed with distilled water before spraying. Monomeric collagen type III was solubilized in 50 mmol/L acetic acid and sprayed with a density of 30 μg/cm² on glass coverslips with a retouching airbrush (Badger model 100, Badger Brush Co).12,13 After the spraying procedure, the collagen surface was blocked for 1 hour with 1% human albumin in PBS (10 mmol/L phosphate buffer, pH 7.4, and 0.15 mol/L NaCl) to prevent nonspecific protein binding during the subsequent perfusion and then incubated with 20 μg/mL vWF in PBS for 2 hours.

Perfusion Studies
Perfusion studies over collagen type III were carried out in a specially devised small parallel-plate perfusion chamber with well-defined rheological characteristics accommodating a glass coverslip as described previously.14 Whole blood was obtained by venipuncture from 3 patients with no vWF in either platelets or plasma (2 patients with severe von Willebrand’s Disease [vWD] and 1 patient with type-1 platelet-low vWD).15 The blood was anticoagulated with 1/10 vol of 200 U/mL low molecular weight heparin. In a separate series of experiments, reconstituted blood, prepared as described previously,18 was used.

Mutant vWF
The role of the platelet receptors GpIb and αIIb-β3 was studied by using mutant vWF. ΔA1-vWF lacks the AI repeat of vWF and is defective for the interaction with GpIb, under static and flow conditions.17 Arg-Gly-Gly-Ser (RGGS)-vWF, in which Asp (D)-1746 has been changed to Gly (G), has virtually no binding to thrombin-stimulated platelets, indicating that the interaction of this vWF with αIIb-β3 is defective. RGGS-vWF does not support platelet adhesion in flow when coated to a surface, indicating that this defect is also present under flow conditions. The construction of these mutants has been described.16,17 For the present study, we used wild-type and mutant vWF without propeptide. This was achieved by transfection of a stable BHK cell line overexpressing furin, as published previously.18 Recombinant vWF was purified by immunoaffinity purification as described.18 Plasma vWF (pvWF), wild-type recombinant vWF (rvWF), ΔA1-vWF, and RGGS-vWF were added to the blood of the patients with vWD to a concentration of 10 μg/mL plasma. As a control, we also used ΔA3-vWF, a mutant vWF in which the A3 repeat of vWF has been deleted and which lacks the ability to bind to collagen type I and III.19 Experiments with this mutant indicate that the preincubated vWF was sufficient to support the adhesion step.

Evaluation of Platelet Deposition and Thrombus Formation
Platelet deposition was evaluated with a light microscope equipped with a JAI-CCD camera coupled to a Matrox frame grabber (Matrox Electronic Systems Ltd) with the use of Optimas 6.0 software (DVS) for image analysis. Platelet deposition was expressed as percentage of the surface covered with platelets. Thrombus size en face was evaluated by using the Watershed program provided by the Optimas software package. The Watershed technique separates confluent or overlapping objects on the basis of color intensity levels. This technique was reproducible with a variation coefficient of 9.8%.

The height of thrombi was determined by confocal laser scanning microscopy (CLSM) in 1 series of experiments. For this determination, coverslips were fixed for 30 minutes in 3% paraformaldehyde/0.025% glutaraldehyde in PBS, blocked by 0.15 mol/L glycine and 3% BSA in PBS (30 minutes), and permeabilized with 0.5% Triton X-100 in PBS (5 minutes). The coverslips were then incubated with 100 μL Bodipy FL-phallacidin (0.13 μmol/L, Molecular Probes Inc) in PBS with 3% BSA. After each step, the coverslips were washed 3 times with PBS. The coverslips were mounted in Mowiol 40-88 (Aldrich-Chemie) and 2.5% diazobicyclo-octane (Sigma) on concave slides with a depth of 0.79 mm. The thrombus height was measured by CLSM (Leica TCS 4D). For each slide, 10 fields of 0.25×0.25 mm² were selected to measure the maximal height of each thrombus. Calibration was performed by using 5.5- and 7.4-μm FITC-conjugated standard beads (FCS Corp).

Scanning Electron Microscopy
For scanning electron microscopy, coverslips with platelets fixed for 1 hour with 2% glutaraldehyde/PBS at room temperature were postfixed in 1% osmium tetroxide for 1 hour, dehydrated in a graded series of ethanol, and dried by the critical-point procedure with use of CO₂ as a transitional fluid. The samples were sputter-coated with a thin layer of gold and viewed in a scanning electron microscope (Philips XL30).

Statistics
All perfusions were performed in triplicate for each condition. The results are presented as the mean±SEM from experiments with blood from 3 different patients or 3 separate series of experiments with reconstituted blood, unless indicated otherwise. The difference between the various conditions was tested by a general linear model for repeated measurements as described in the SPSS package. The data on thrombus size obtained with the Watershed technique were tested by the χ² test.

Results
Studies in vWD Blood
To study the role of vWF in thrombus formation independent of its role in platelet adhesion, we first created a good adhesive surface by precoating collagen type III with pvWF and subsequently varied the perfusate by adding different mutant forms of vWF to the whole blood of patients with severe vWD. These patients have no vWF in either their plasma or platelets. The morphology of the platelet deposition at shear rate of 2600 s⁻¹ under different conditions as shown by scanning electron microscopy is presented in Figure 1; the surface coverage was quantified by image analysis after May-Grünwald-Giemsa staining and is summarized in Table 1.

The following results were obtained. With no vWF in the system (no preincubation of collagen with vWF and no addition of vWF to the blood), deposition of only a few single platelets (surface coverage 1%) was observed. When collagen type III was preincubated with vWF but no vWF added to the blood, platelet deposition was still small (7.1±0.9% [mean±SEM]) and consisted of single platelets. After preincubation with and the addition of vWF, platelet deposition increased to a surface coverage of 30%. The actual values were similar for pvWF (31.4±4.3%) and rvWF (29.5±3%). Platelet deposition with RGGS-vWF was higher (39.2±1.9%). Platelet deposition after the addition of ΔA1-vWF was low (3.3±0.2%), lower even than that without added vWF.

Figure 1 shows that platelet deposition after the addition of pvWF, rvWF, and RGGS-vWF consisted of thrombi. The size of these thrombi en face was evaluated by the Watershed technique. The results are presented in Table 2. Platelet deposition with vWF
thrombi were largest after the addition of RGGS-vWF. The addition of pvWF yielded larger thrombi than those found after the addition of rvWF. Platelet deposition after the addition of ΔA1-vWF consisted completely of single platelets.

Data on thrombus height as measured by CLSM, with the use of fluorescent phallacidin as a platelet label, are presented in Table 1. Mean thrombus height was higher with RGGS-vWF than with rvWF and pvWF.

Platelet deposition was also studied at shear rates of 300 and 800 s⁻¹ in a single triplicate experiment with vWD blood. The results are summarized in Table 3. Single platelets were found after the addition of ΔA1-vWF. Addition of other forms of vWF yielded thrombi. Platelet deposition with RGGS-vWF was not increased, in contrast to deposition found at 2600 s⁻¹.

**Studies With Reconstituted Blood**

The role of the interaction of vWF with GpIb and αIIb-β3 was further studied in reconstituted blood. The results are summarized in Table 4 for shear rates of 2600 s⁻¹ and 300 s⁻¹. Thrombi were absent when no vWF or ΔA1-vWF was added. The deposition was slightly, but not significantly, lower after the addition of RGGS-vWF than after rvWF. Analysis of

<table>
<thead>
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<th>Perfusion</th>
<th>Surface Coverage, %</th>
<th>Thrombus Height, μm</th>
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<tr>
<td></td>
<td>Mean ± SEM</td>
<td>P</td>
</tr>
<tr>
<td>A. Without vWF</td>
<td>1 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>B. Preincubated</td>
<td>7.1 ± 0.9</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>C. Preincubated + pvWF</td>
<td>31.4 ± 4.3</td>
<td>0.03 (BvsA)</td>
</tr>
<tr>
<td>D. Preincubated + rvWF</td>
<td>29.5 ± 3</td>
<td>0.012 (BvsA)</td>
</tr>
<tr>
<td>E. Preincubated + ΔA1-vWF</td>
<td>3.3 ± 0.2</td>
<td>&lt;0.005 (EvsD)</td>
</tr>
<tr>
<td>F. Preincubated + RGGS-vWF</td>
<td>39.2 ± 1.9</td>
<td>&lt;0.02 (FvsD)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 separate experiments. Each perfusion was performed in triplicate. Perfusion with vWD blood were performed for 5 minutes at 2600 s⁻¹. Blood was perfused over collagen type III coated onto glass coverslips. For preincubated values, collagen type III was preincubated with vWF 20 μg/mL plasma. The perfusate was vWD blood to which various mutants of vWF had been added to a final concentration of 10 μg/mL plasma.

**Figure 1.** Scanning electron micrograph of platelet adhesion to collagen type III preincubated with 20 μg/mL vWF. Perfusion was for 5 minutes at 2600 s⁻¹. The perfusate was vWD blood to which various mutants of vWF had been added to a final concentration of 10 μg/mL plasma. A, No vWF addition. B, Plasma-vWF. C, rvWF and vWF. D, RGGS-vWF. E, ΔA1-vWF.
thrombus size with the Watershed technique showed smaller thrombi with RGGS-vWF than with rvWF or pvWF. This difference was more pronounced at 2600 s⁻¹ than at 300 s⁻¹ (Table 5).

Role of αIIb-β3

The experiments in which RGGS-vWF was present as a single ligand in reconstituted blood (Table 4) may suggest that αIIb-β3 is not involved in thrombus formation. RGGS-vWF was unable to interact with αIIb-3, and there was no other ligand present. Previous studies involving Glanzmann’s disease have demonstrated the complete absence of thrombus formation in flow.20,21 We confirmed the importance of αIIb-β3 by adding an antibody to β3 to the perfusate (C17, courtesy of Dr A.E.G.K. von dem Borne, Central Laboratory of Blood Transfusion Service, Amsterdam, the Netherlands). This blocked thrombus formation completely (results not shown).

Ligands secreted from platelet α granules (fibrinogen, fibronectin, and vWF) have to be responsible for thrombus formation. We investigated the role of vWF by using Fab fragments of LJ-P5, an antibody directed against αIIb-β3, which blocks the interaction with vWF but not with fibrinogen or fibronectin. We first tested the specificity of the Fab fragments. They completely blocked platelet adhesion in flow to surface-coated vWF and had no effect on adhesion in flow to surface-coated fibrinogen (results not shown). The Fab fragments of LJ-P5 reduced platelet deposition to vWF-

Table 3. Perfusion Studies With Blood From a vWD Patient: Platelet Deposition at 300 and 800 s⁻¹

<table>
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<tr>
<th>Perfusion</th>
<th>Surface Coverage, %</th>
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<tr>
<td></td>
<td>300 s⁻¹</td>
</tr>
<tr>
<td>Without vWF</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td>Preincubated</td>
<td>11.4±1.7</td>
</tr>
<tr>
<td>Preincubated + pvWF</td>
<td>17.3±4.4</td>
</tr>
<tr>
<td>Preincubated + rvWF</td>
<td>16.9±1.8</td>
</tr>
<tr>
<td>Preincubated + ΔA1-vWF</td>
<td>10.4±0.4</td>
</tr>
<tr>
<td>Preincubated + RGGS-vWF</td>
<td>18.8±2.1</td>
</tr>
</tbody>
</table>

Values are mean±SD. Experiment was performed in triplicate under conditions described in Table 1.
be that fibrinogen and vWF are both present in vWD blood, whereas vWF is the single initial ligand in reconstituted blood. To investigate this further, we added fibrinogen to reconstituted blood (Table 6). This caused inhibition in the case of rvWF but no inhibition in the case of RGGS-vWF. The maximal effect was at fibrinogen concentrations of ≥1 mg/mL (dose response not shown).

Discussion

Platelet adhesion and platelet thrombus formation are generally viewed as completely different processes with their own special ligands and adhesion receptors. Adhesion concerns nonactivated platelets and involves vessel wall molecules and some plasma proteins binding to it as ligands and GpIb and integrins of the β1 family as receptors. Adhesion to fibrinogen/fibrin is an exception: the β3 integrin αIIb-β3 is the adhesion receptor for this ligand. Thrombus formation, ie, aggregation, starts from a first layer of adhered and activated platelets. It has been thought to be mediated exclusively by the β3 integrin αIIb-β3, with fibrinogen, fibronectin (vitronectin), and vWF used as ligands. Previous studies involving the binding of vWF to αIIb-β3 of activated platelets showed strong competition by fibrinogen: this competition was so powerful that no vWF binding occurred at plasma concentrations. Thus, it was unexpected that perfusion studies in which the blood of patients with vWD was circulated over rabbit subendothelium showed a deficit in thrombus formation at a shear rate of 2600 s⁻¹. The same authors suggested in a later study that thrombus formation at this relatively high shear rate was mediated by the vWF–αIIb-β3 interaction. A role for GpIb-vWF interaction in platelet aggregate formation was found in experiments in a cone-plate viscometer. This role is difficult to investigate under flow conditions, because inhibition of GpIb affects adhesion and the subsequent thrombus formation. Goto et al. approached this by introducing a 2-step procedure in which adhesion was achieved in a short perfusion of 1 minute. Thrombus formation was then studied in the presence or absence of relevant antibodies by perfusing for another 4 minutes. This procedure has the drawback that the antibodies may still have an effect on the platelets that had adhered during the first minute.

We approached this issue by using vWF mutants. Adhesion was supported by pvWF that had been preadsorbed to collagen type III by preincubation. The role of vWF in thrombus formation was then studied by adding various mutant forms of vWF to the vWF-free blood.

The experiments showed unequivocally that the interaction of vWF with GpIb is absolutely required for thrombus formation. This is based on the observation that no thrombus formation occurred at all when ΔA1-vWF was added to whole blood (Figure 1 and Tables 1 through 3), whereas thrombus formation occurred when either pvWF or rvWF was added.

Earlier studies involving Glanzmann’s thrombasthenia have demonstrated without any doubt that αIIb-β3 is also absolutely required for thrombus formation.20,21 Our data suggest that adhesion and thrombus formation are analogous processes. In adhesion, platelet GpIb interacts with vWF in a rolling interaction.22 Firm attachment is then caused by interaction with an integrin receptor, in this case α2β1. Direct studies of platelet thrombus formation in real time (Y.-P.W., unpublished data, 1999) show that platelets also roll on top of the thrombi that form on collagen fibers and that this rolling is then followed by firm attachment. We postulate that the rolling is mediated by GpIb-vWF and that the firm attachment is mediated by an integrin (in this case αIIb-β3).

An interesting consequence of our observation is the different mechanism that may lead to vWF-GpIb interaction in the adhesive situation versus the thrombotic situation. According to our present insights, the GpIb-vWF interaction in adhesion is mediated by a conformational change in vWF caused by binding to a surface.23 In the case of the thrombotic situation, one has to postulate that a change in conformation occurs in GpIb present on an adhered platelet. This conformational change may be due to an activation process caused by adhesion or may be due to the effect of shear stress of the passing plasma on GpIb. The spontaneous binding of vWF to GpIb in platelet-type vWD, caused by a point mutation in GpIb, indicates that conformational changes in GpIb may indeed cause binding of vWF.

The studies performed at low shear rates indicate that in contrast to the original idea, GpIb-vWF interaction is of importance for thrombus buildup at all the shear conditions existing in arteries. This is of interest for the potential use of the GpIb-vWF interaction as a target in drug development. In contrast to the use of αIIb-β3 inhibitors, GpIb inhibitors may be expected to inhibit thrombus formation only under flow conditions and not under static conditions. It would be interesting if one could separate the effect of inhibitors on platelet adhesion from the effect of inhibitors on platelet thrombus formation. In principle, this may be possible. The difference in the protein undergoing the conformational change, in vWF in adhesion or in GpIb in thrombus formation, may be used to generate inhibitors that would block one interaction and not the other.

The preferred ligand for αIIb-β3 remains to be determined. The data on RGGS-vWF addition in vWD blood as presented in Tables 1 and 2 show increased thrombus formation with this ligand, and this may mean that vWF/αIIb-β3 may be less effective than fibrinogen–αIIb-β3 interaction. On the other hand, we find in Table 6 that the addition of fibrinogen to reconstituted blood containing rvWF yields a decrease in thrombus formation. This would indicate that vWF–αIIb-β3 interaction is more effective than fibrinogen–αIIb-β3 interaction. This discrepancy becomes better understandable when

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<th>Perfusion</th>
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<tr>
<td></td>
<td>rwWF</td>
</tr>
<tr>
<td>Series A</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>45.8±4.7</td>
</tr>
<tr>
<td>LJ-P5 F(ab)₂ (20 μg/mL)</td>
<td>29.0±1.4*</td>
</tr>
<tr>
<td>Series B</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>42.0±2.4</td>
</tr>
<tr>
<td>Fibrinogen (1 mg/mL)</td>
<td>28.9±1.1*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 3 separate experiments performed in triplicate. Perfusions were performed at 2600 s⁻¹ for 5 minutes. Series A was performed independently from series B.

*0.02>P>0.01; †P=NS.
we see that fibrinogen does not inhibit thrombus formation when RGGS-vWF is added to reconstituted blood. RGGS-vWF does not interact with αIIb-β3, and ligands secreted from platelet α granules have to act as a bridge between platelets. LJ-P5 Fab fragments did not inhibit thrombus formation in this case, which indicates that it is fibrinogen rather than vWF from α granules that is responsible for thrombus formation. We now begin to see that whenever a single ligand is present, thrombus formation is higher than when vWF and fibrinogen are both present. This leads us to the hypothesis shown in Figure 2. When a single ligand for αIIb-β3 is present (Figure 2A and 2B), bridge formation will be efficient and complete. When 2 ligands are present (Figure 2C), part of the bridges will be incomplete. The ligand bound to αIIb-β3 on one of the platelets cannot interact with the adjacent αIIb-β3 on the other platelet because this is occupied by the other ligand. Whether it can displace this will depend on the affinity and local concentration of the 2 ligands. In the case of homotypic interaction of fibrinogen or vWF with the αIIb-β3-molecules on adjacent platelets, bridge formation will be easier, because the affinity for the bound versus the free ligand will at least be equal, which will lead to easier displacement.

In developing this hypothesis, we have only considered fibrinogen and vWF as ligands. We believe that this is justified. Fibrinogen is present at a 10-fold higher concentration than fibronectin, and vWF is already present on the platelet surface, interacting with GPⅢb. The negative effect of the presence of 2 ligands was observed only at 2600 s⁻¹ but not at 800 and 300 s⁻¹. This may be caused by the higher shear stress.

Recently, Ruggeri et al²⁴ published a study in which they also focused on the role of GPⅢb versus αIIb-β3 and vWF versus fibrinogen in thrombus formation in collagen in flow. There are a number of methodological differences, which may have an effect on the results, but the overall conclusion was similar. GPⅢb was important for thrombus formation at shear rates between 300 and 1500 s⁻¹. They also found that vWF may act as single ligand in thrombus formation at 1500 s⁻¹. Addition of fibrinogen caused lower initial thrombus formation but stabilized thrombus formation at later times. They could investigate a situation in which fibrinogen was the only ligand for αIIb-β3 because they did not use mutant vWF, and they needed vWF for the GPⅢb. Their observation of reversible thrombus size with vWF at later times and of the stabilizing effect of fibrinogen will require further study.

The different roles of vWF and fibrinogen as ligands for αIIb-β3 may have clinical consequences. Preliminary data in the blood of a patient with afibrinogenemia showed that the large thrombi that formed on collagen became smaller after the addition of fibrinogen. Further studies will be required to look more precisely at shear rate and time effects. Real time perfusion studies as performed by Ruggeri et al²⁴ and use of mutants as performed by us may be required to get the full picture.

Figure 2. Drawing of the hypothetical interactions involved in platelet thrombus formation. Platelets are always linked by GPⅢb-vWF interactions. In panels A and B, either fibrinogen (Fg) or vWF was present as a ligand for αIIb-β3. Because these ligands only need to displace themselves, effective bridge formation will occur. In panel C, Fg and vWF were both present as ligands. Displacement of a ligand from αIIb-β3 on an adjacent platelet may be more difficult. We call this phenomenon incomplete bridge formation.

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