Platelet–Collagen Interactions: Increase in Rate of Adhesion of Fixed Washed Platelets by Factor VIII-Related Antigen

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A simple technique using an aggregometer and fixed washed human platelets (FWP) and fibrillar collagen has been used to evaluate the contribution of the two components of the factor VIII (FVIII) complex to platelet–collagen interactions. FWP bound individually to collagen fibrils in suspension, and both the total number of FWP bound and the rate of adhesion increased with increasing collagen concentration. Von Willebrand's disease (vWD) type I or normal plasma immunoadsorbed with anti-factor VIII-related antigen (anti-FVIII:Ag) antiserum gave 20% and vWD type II, gave 50% of the rate of adhesion obtained with normal, hemophilia A, or hemophilia A with inhibitor plasma, but the same percent adhesion was found with all plasmas. The rate of adhesion of both vWD type I and type II, was corrected by the addition of purified FVIII complex. These results indicated that the FVIII:Ag and not the factor VIII coagulant activity (FVIII:C) in normal plasma or purified FVIII complex caused an accelerating effect on the rate at which FWP bound to collagen. Collagen fibrils not only bound FWP, but also adsorbed the FVIII complex with preferential adsorption of the forms of FVIII:Ag with the greatest ristocetin cofactor (FVIII-RCoF) activity. Saturation of collagen with FWP did not change the adsorption pattern of the FVIII complex. Also anti-FVIII:Ag blocked the accelerating effect of the FVIII complex but not the adhesion of FWP. Thus, FWP and FVIII:Ag appeared to bind to separate sites on collagen.

Von Willebrand factor (vWF) is required to initiate the formation of a platelet plug at the site of exposed subendothelium. Use of a perfusion chamber containing everted vessels denuded of endothelium has shown that vWF is also required in the adhesion of platelets to areas of exposed subendothelium and collagen. The role of vWF in platelet–collagen interactions is unclear, but it is thought to be different from the role it plays in the aggregation of platelets in in vitro systems as a cofactor with ristocetin (FVIII-RCoF) and certain snake venoms. Activity of vWF is associated with a series of high molecular weight multimers that compose the factor VIII-related antigen (FVIII:Ag) component of the factor VIII (FVIII) complex. The largest forms of FVIII:Ag have the highest activity toward platelets, and possibly, more than size is responsible for the specificity of aggregation.

None of the current methods allows measurement of the rate at which platelets bind to collagen. We have used a technique, modified from that described by Spaet and Lejnieks, to measure both the amount and rate of adhesion of formaldehyde-fixed washed platelets (FWP) to suspended fibrillar collagen and to show that FVIII:Ag affects the rate, but not the amount, of adhesion.
Collagen was bovine Achilles tendon collagen dispersed in dilute acetic acid (Ethicon Inc., Somerville, NJ). It is mostly type I collagen and was shown to be polymeric by electron microscopic (EM) studies. A weighed sample of collagen suspension was dried under vacuum to constant weight and the concentration was determined to be 16 mg/g of original suspension. The fibrillar collagen reagent used in the following experiments was prepared fresh daily by suspending 0.5 g of the collagen suspension in 16 ml of distilled water. The suspension was rocked for 2 hr at 4°C and then mixed with an equal part of cold 0.3 M NaCl and stored in an ice bath until used. The final reagent contained 250 μg/ml of fibrillar collagen in 0.15 M NaCl. Denatured collagen was prepared by heating at a final concentration of 400 μg/ml in 0.15 M NaCl for 10-15 min in a boiling water bath. Cytodex 3 beads (Pharmacia Fine Chemicals AB, Uppsala, Sweden) consist of denatured collagen coupled to a crosslinked dextran matrix.

Purified human FVIII was prepared from fresh human plasma by gel filtration on Sepharose CL-4B. Void volume fractions were assayed, pooled, and dialyzed against 0.05 M cacodylate saline buffer overnight at 25°C. The dialyzed material was divided into aliquots and stored at −20°C.

Immunabsorbed normal human plasma was prepared as follows: 20 μl of the anti-human FVIII:Ag rabbit antiserum was mixed with 1 ml of normal human plasma and the mixture incubated for 2 hr at 37°C and then overnight at 5°C. Supernatant plasma (ristocetin cofactor (FVIIIR:RCoF) <0.06 U/ml, factor VIII coagulant (FVIII:C) 0.37 U/ml, FVIIIR:Ag <0.06 U/ml) was obtained by centrifugation of the mixture at 1,500 g for 20 min at 25°C. Nonimmune rabbit serum (20 μl) was mixed with 1 ml of normal plasma and processed similarly for the control. In some experiments, the FVIII:C activity in the normal plasma was neutralized by incubation with an inhibitor plasma (614 Bethesda Unit/ml) from a patient with severe hemophilia A (FVIII:C ≤ 0.01 U/ml).

Bioassays of the Activities of the FVIII Complex

Ristocetin cofactor (FVIIIR:RCoF) activity was assayed using a Payton dual-channel aggregometer, as previously described. Factor VIII-related antigen (FVIII:Ag) was assayed by Laurell immunoelectrophoresis, using 1% Seakem ME agarose and 1:1,000 dilution of a rabbit antiserum prepared against the purified human FVIII complex. Factor VIII coagulant activity (FVIII:C) was measured by a modification of the one-stage method. One unit of these activities (FVIIIIR:RCoF, FVIIIIR:Ag, and FVIII:C) is, by convention, that amount found in 1 ml of normal plasma.

Adherence of FWP to Collagen

Adherence of FWP to collagen was evaluated in a Payton dual-channel aggregometer. The buffer used was 0.05 M cacodylate saline (pH 7.3, F/2 = 0.15). In the test system, 200 μl of FWP (7.5 × 10⁶/ag/ml) in buffer was mixed with 200 μl of buffer or test material in a glass cuvette and set for 0% transmission; 100% transmission was set with 200 μl buffer plus 200 μl test sample or buffer. After 3-5 min of stirring at 37°C, 100 μl of collagen was added to the cuvette and the change in transmission was recorded on a Beckman recorder (5 mV for 100% light transmission). Some of the preliminary experiments were done at different bar speeds. In the control, no change in light transmission was observed when collagen was added to platelet-poor plasma or buffer. However, when collagen was added to FWP in buffer or plasma, the light transmission increased, approaching a plateau within 2-3 min. As no platelet-platelet cohesion was observed in these mixtures, the initial slope and the total change in percent transmission at 5 min after the addition of collagen were interpreted as measuring the rate of adhesion and percent of adhesion of FWP to collagen, respectively. The calculated percent adhesion agreed well with percent adhesion determined by direct count (Coulter counter) of the filtrate obtained after filtering the collagen-platelet mixtures with a 5-μM Unipore filter (BioRad Laboratories, Richmond, CA).

**RESULTS**

FWP adhered to fibrillar collagen along each collagen fiber (Fig. 1). Platelets not adhering to collagen were seen as single discrete platelets. Stability of the fibrillar collagen suspension for long periods of time was difficult to achieve. It was found that dispersion of the stock collagen preparation in unbuffered 0.15 M NaCl resulted in a suspension that was stable for at least 8 hr when kept at 4°C. Adequate stability was defined as the ability of the suspension to maintain a fine fibrillar structure when examined by phase-contrast microscopy, with zero to minimal aggregation of the fibrils.

Both the rate at which the platelets adhered as well as the percent of platelets that adhered to a constant concentration of collagen increased with decreasing platelet concentration. This would be expected, as the data also establish that 1 μg of collagen can only bind an average of 2.6 × 10⁶ FWP (Table 1). Bovine serum albumin or plasma decreased the rate at which FWP...
adhered to collagen, but had no effect on the final number of FWP adhering.18 However, the decrease in rate was consistently less in normal plasma than in bovine serum albumin, suggesting that some component of normal plasma can overcome the inhibitor effect of protein concentration on the rate of platelet adhesion. For this reason, protein concentration was carefully controlled in all experiments in the present report.

Experiments to Standardize the Assay for Platelet Adherence to Collagen

The effects of varied concentrations of collagen were tested. The initial slope or rate of adhesion, as well as the total number of platelets binding (percent), increased with an increasing concentration of collagen (Fig. 2). When the same experiment was run at a setting of 300 rpm, total platelet adhesion remained the same, but the slope of adhesion was less than one-half that obtained at a setting of 600 rpm.18 Therefore, this experiment cannot be compared with other experiments in this report that were run at a setting of 300 rpm.

![Figure 2](image-url)

Fig. 2. Rate of platelet adhesion to collagen and percent adhesion as a function of collagen concentration. A quantity of 0.1 ml collagen was added to 0.2 ml FWP in normal plasma and 0.2 ml buffer. Stirring setting was 600 rpm, 37°C. Final platelet concentration 3 x 10^7/μl; collagen shown as final concentrations. Percent adhesion was measured after it had reached a plateau at 3 mm. Standard deviation, where calculated, is shown by (§). Number of determinations for 25 μg collagen was 25, for 50 μg, 29, for 100 μg, 6, for 500 μg, 4.

Binding of FWP to Denatured Collagen

This was tested using heat-denatured collagen and Cytodex 3 beads. There was no significant deviation from baseline when 100 μg/ml of heated collagen was added to FWP (3 x 10^7/μl) in the aggregometer cuvette. In addition, phase microscopy after incubation of 0.2 ml of beads (20 mg/ml) with 0.2 ml of FWP (8, 4, 2, 1 x 10^7/μl) for 5 min at 37°C revealed no adherence of FWP at any concentration used to the surface of Cytodex 3 beads.

Effects of Plasmas on Rate and Degree of FWP Adhesion to Collagen

The effects of various plasmas (normal and deficient) on the rate and degree of FWP adhesion to collagen were studied. The FVIII-related properties of the test plasmas are shown in Table 2. Only the slopes of FWP adhesion were different with the various plasmas (Table 3). The slope in the presence of hemophilic plasma and hemophilic inhibitor plasma was not significantly different from that of normal plasma. The slope of normal plasma was not changed by neutralization of FVIII:C with a naturally occurring human inhibitor to FVIII:C. The slopes, and thus the rates, of FWP adhesion to collagen in the presence of homozygous vWD type I plasma and vWD type I inhibitor plasma were only about 20% of that in the presence of normal plasma.

The rate of adhesion (slope) of FVIII immunoadsorbed normal plasma (not shown) when tested at twice the concentration of collagen (50 μg/ml) was similar to that obtained for vWD type I plasma; 1.1 ±

Table 2. FVIII-Associated Properties in Units per Milliliter for Test Plasmas

<table>
<thead>
<tr>
<th>Plasma</th>
<th>FVIII:C</th>
<th>FVIII:Ag</th>
<th>FVIII:RCoF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pool</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>vWD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>≤0.01</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>Type I and inhibitor</td>
<td>≤0.01</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>Type II</td>
<td>0.50</td>
<td>2.2</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>≤0.01</td>
<td>1.98</td>
<td>0.76</td>
</tr>
<tr>
<td>Hemophilic with inhibitor</td>
<td>≤0.01</td>
<td>1.78</td>
<td>1.60</td>
</tr>
</tbody>
</table>

*Lacks high molecular weight multimers by two-dimensional immunoelectrophoresis.
0.2 (FVIII absorbed normal plasma), 0.8 ± 0.2 (homozygous vWD type I plasma). Both of these values were 20%-30% of the slope found at this concentration of collagen for normal plasma; 3.7 ± 0.3. In addition, the increased rate of adhesion of FWP in normal plasma could be eliminated by the addition of anti-FVIIIR:Ag antiserum (1/40 volume of normal plasma, 1 mm, 37°C) directly to the incubation mixture. This amount of antiserum was sufficient to inhibit totally the FVIIIR:RCoF activity in 1 ml of normal plasma in 1 min at 37°C. The effect on rate of adhesion could only be observed when the antisera were added to plasma and platelets prior to the introduction of collagen. Anti-FVIIIR:Ag antiserum alone had no effect in the absence of normal plasma or if it was added to the system after the adhesion pattern reached a plateau. In a separate experiment, FWP were incubated for 30 min with the anti-FVIIIR:Ag antiserum. The mixture was nonreactive with ristocetin plus normal plasma. After the platelets were washed three times, the response to ristocetin and normal plasma was identical to control normal FWP that had been treated with nonimmune serum and then washed. The washed antibody-treated platelets also adhered to collagen with the same rate and degree of adhesion as the control platelets.

**Effect of Purified FVIII Complex on the Adhesion of FWP to Collagen**

The purified FVIII complex, expressed as FVIIIIR:RCoF, increased the adhesion rate of FWP to collagen (Fig. 3). Above 0.1 U/ml FVIIIIR:RCoF, the rate of adhesion increased with increasing concentration, while the total number of platelets binding to collagen (percent adhesion) remained relatively unchanged.

Added purified FVIII complex increased the adhesion rate in normal and vWD type I and IIa plasmas, but not in vWD type I plasma with inhibitor (Table 4).

**The Binding of the FVIII Complex to Collagen**

FVIII:C, FVIIIIR:Ag, and FVIIIIR:RCoF activities all bound to collagen (Fig. 4). Preliminary results (not shown) revealed that maximum adsorption of FVIIIIR:Ag occurred within the first 5 min of incubation. No further antigen was removed, even after 20 min. The adsorption of FVIIIIR:RCoF after 10 min was greater at all collagen concentrations than that of FVIIIIR:C or FVIIIIR:Ag. Eighteen percent of the FVIIIIR:C remained in the supernatant after incubation of normal plasma with 800 µg/ml collagen, whereas all the measurable FVIIIIR:Ag and FVIIIIR:RCoF was adsorbed. Previous reports found no effect of temperature on the binding of FVIIIIR:Ag to collagen. In our studies, however, a linear effect on the binding of

**Table 3. Effect of Various Plasmas on the Rate of Adhesion and Percent Adhesion of FWP to Collagen**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Adhesion Rate (Slope)</th>
<th>Platelet Adhesion (%</th>
<th>Cone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.9 ± 0.3</td>
<td>29 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>vWD</td>
<td>0.4 ± 0.2</td>
<td>27 ± 5</td>
<td>0.05</td>
</tr>
<tr>
<td>Type I</td>
<td>0.4 ± 0.2</td>
<td>25</td>
<td>0.02</td>
</tr>
<tr>
<td>Type I with inhibitor</td>
<td>0.85</td>
<td>27</td>
<td>0.02</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>1.5 ± 0.2</td>
<td>22 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>Hemophilic-inhibitor</td>
<td>1.8 ± 0.2</td>
<td>23</td>
<td>0.02</td>
</tr>
<tr>
<td>Normal + hemophilic*</td>
<td>1.5 ± 0.1</td>
<td>23 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Normal + hemophilic-inhibitor*</td>
<td>2.0 ± 0.4</td>
<td>23 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>Inhibitor (Neat)</td>
<td>1.6 ± 0.2</td>
<td>30 ± 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Inhibitor (1:60)</td>
<td>1.6 ± 0.1</td>
<td>31 ± 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Normal + buffer*</td>
<td>6.5% BSA in CBS</td>
<td>0.2</td>
<td>24</td>
</tr>
<tr>
<td>Buffer alone</td>
<td>2.4 ± 0.3</td>
<td>30 ± 2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*These mixtures were preincubated in a cuvette at 37°C for 30-60 min, and 0.2 ml added to 0.2 ml FWP in CBS buffer, followed by 0.1 ml collagen, to give a final concentration of 25 µg/ml. Stirring speed 300 rpm, 37°C. Final platelet concentration, 3 x 10^9/µl.

**Table 4. Rate of FWP Adhesion to Collagen Before and After Addition of FVIIIIR:RCoF**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Adhesion Rate (Slope) Before</th>
<th>Adhesion Rate (Slope) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.2</td>
<td>5.4</td>
</tr>
<tr>
<td>vWD</td>
<td>0.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Type I</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Type I with inhibitor</td>
<td>0.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

*Final concentration of added FVIIIIR:RCoF was 1.6 U/ml.
FVIII:Ag to collagen was found between 6° and 37°C. At 200 μg/ml collagen, for example, 40% FVIII:Ag bound at 6°C, increasing to 56% bound at 37°C.

When the plasma of a patient with vWD type II, was studied, the amount of residual FVIII:Ag at the same concentrations of collagen differed from those shown in Fig. 4 for normal plasma. For example, at a concentration of 200 μg/ml of collagen, 60%-70% of the FVIII:Ag in the variant vWD type II, plasma remained in the supernatant after 10 min at 37°C. Such functionally abnormal FVIII:Ag, however, could be completely adsorbed by the higher concentration of collagen, i.e., 800 μg/ml, but at 400 μg/ml of collagen, only 50% was adsorbed. No evidence was found for activation of FVIII:C by collagen.

FVIII:RCoF activity in the purified FVIII complex also bound to collagen (Fig. 5) and was completely removed by as little as 50 μg/ml collagen, whereas quantitative removal of the FVIII:RCoF from normal plasma required almost 200 μg/ml (Fig. 4). Adsorption was also tested by using denatured collagen. FVIII:Ag in normal plasma was not adsorbed by 200 μg/ml of heated collagen. Similarly, when Cytodex 3 beads (20–80 mg/ml) were incubated with 0.2 ml normal plasma for 10 min at 37°C, there was no evidence of binding of FVIII:Ag or FVIII:RCoF to the beads.

**Effect of FWP on Binding of FVIII:Ag to Collagen**

The effect of the presence of FWP on the binding of FVIII:Ag to collagen was tested by mixing 0.2 ml of normal plasma, 0.1 ml of Tris buffer or FWP in Tris (final concentration 2 x 10⁷/μl), and 0.2 ml of collagen (final concentration 125, 250, 375, and 750 μg/ml). The collagen in Tris buffer binds about 1.6 x 10⁶ platelets/μg, determined from data similar to those shown in Table 1 for cacodylate buffer. At a final concentration of 125 μg/ml, the collagen in this experiment would be completely saturated with FWP. The residual FVIII:Ag determined for each of the concentrations of collagen used gave values in the presence of FWP that were 54%, 40%, 32%, and 0%, and in the absence of FWP, the values were 54%, 40%, 29%, 0%.

**DISCUSSION**

Adherence of fresh platelets is not cation dependent and is not affected by a 50% depletion of glycogen stores. This suggested that FWP could be used to advantage in a collagen system. Although FWP appeared to adhere individually to collagen fibrils (Fig. 1), and nonadherent platelets appeared singly in the supernatant, it is not certain that there were no platelets superimposed upon an adherent platelet.

The aggregometer method used is simple and allows the evaluation of both the rate of adhesion and the number of platelets adhering to various concentrations of collagen. Other platelet functions, such as release and aggregation, do not obscure the result. The degree and the rate of adhesion could be increased either by lowering the platelet number or increasing the collagen concentration (Table 1). When FWP were incubated with collagen (Fig. 2), up to 91% bound. Our data with FWP are not consistent with suggestions that there are two populations of platelets, adherent and nonadherent, with the latter group comprising 25%-50% of the platelets in plasma.

As factor VIII complex is known to participate in
platelet–collagen interactions, the effects of various plasmas were compared in the aggregometer system. The rate of adhesion with homozygous vWD type I plasma was usually only 20% of the rate obtained for normal plasma (Table 3). Despite this difference in rate, the degree or total number of platelets adhering with both plasmas was essentially the same. The increased rate of adhesion elicited by normal plasma could be eliminated by prior neutralization of FVIII:RCoF with rabbit anti-FVIII:Ag antiserum. These results indicate that ristocetin cofactor activity is at least one factor in normal plasma that can increase the rate of FWP adhesion to fibrillar collagen, but has no effect on the number of FWP that adhere. Plasma from a patient with vWD type IIa, containing elevated levels of the lower molecular weight multimers of FVIII:Ag but low ristocetin cofactor activity, resulted in a rate of adhesion that was slower than normal plasma but higher than vWD type I plasma, indicating that low molecular weight multimers have some effect.

Only the FVIIIIR:Ag component of the FVIII complex appeared to participate in the increased rate of adhesion. Plasma from an individual with classic hemophilia A and normal plasma in which the FVIII:C component of the FVIII complex had been neutralized by an inhibitor gave results similar to those obtained for normal plasma (Table 3).

Experiments using purified FVIII complex also confirmed the effect of FVIIIIR:RCoF on FWP adhesion to collagen. The rate of adhesion of FWP to collagen was increased by concentrations of FVIIIIR:RCoF above 0.1 U/ml (Fig. 3). The rate of adhesion of FWP to collagen in the presence of vWD (types I, II) plasma was increased 4–10-fold by the addition of purified FVIII complex. The same concentrations of the purified preparation, however, did not correct the defect in adhesion rate when vWD type I inhibitor plasma was used (Table 4). Presumably, in these experiments, not enough FVIII complex was added to completely overcome the strong inhibitor. There may be other plasma proteins, such as fibrinogen and fibronectin, also involved in the interaction of platelets with collagen.

When collagen was incubated with normal citrated plasma, FVIIIIR:Ag, FVIIIIR:C, and FVIIIIR:RCoF were bound to the collagen fibrils (Fig. 4). The binding of the FVIIIIR:RCoF required one-third as much collagen as the value reported earlier by Santoro. The preferential adsorption of FVIIIIR:RCoF suggests a greater affinity of collagen for the higher molecular weight multimers of FVIII. Such a hypothesis is also supported by experiments using vWD type II, plasma, where little adsorption of FVIIIIR:Ag was observed at concentrations of collagen between 25 and 200 μg/ml. Collagen binds other proteins besides those of the factor VIII complex, so that there is probably some competition for binding sites. As one would expect, removal of VIIIIR:RCoF from purified FVIII preparations required less collagen than for removal of FVIIIIR:RCoF from plasma (Fig. 5).

The presence of FVIIIIR:C in the absence of other activities associated with the FVIII complex after incubation with collagen confirms the nature of FVIIIIR as a complex of more than one entity. It also suggests that there may be a conformational change in the FVIIIIR:Ag multimer upon binding to collagen. This structural alteration could decrease noncovalent interactions within the complex, resulting in the dissociation of some of the FVIIIIR:C. It is also possible that FVIIIIR:C unassociated with FVIIIIR:Ag, or associated with some other macromolecular complex (such as phospholipid), exists in normal plasma. This aspect of the binding of the FVIII complex to collagen will require additional studies. Activation of FVIIIIR:C by collagen, as described by Nymann, was not observed. FVIIIIR:Ag in normal plasma could not be adsorbed by denatured collagen, either as heated collagen or as Cytodex 3 beads. No inhibition of FVIIIIR:Ag binding to fibrillar collagen by denatured collagen was observed, confirming the report by Santoro et al.

FWP and FVIIIIR:Ag appear to bind independently to collagen. When collagen was saturated by FWP and the mixture used to adsorb FVIIIIR:Ag from plasma, the same results were obtained as found with collagen alone in Fig. 4. The effect of a commercial antiserum to human FVIIIIR:Ag on platelet–collagen interactions has been reported. In these studies, preincubation of antiserum with fresh washed platelets in EDTA inhibited platelet adhesion to collagen when no FVIIIIR:Ag was added. Using a fluorescent technique, the antibody was shown to bind to the platelet surface. On the other hand, the rabbit antiserum to human FVIIIIR:Ag used in our studies did not decrease the percent adhesion or rate of fixed washed platelet adhesion to collagen when tested in the absence of plasma, but our antiserum did inhibit the ability of added FVIIIIR:Ag to increase the rate of FWP adhesion. This effect was lost when the antibody-treated FWP were washed. Thus, our data with FWP do not support the earlier suggestion that “vWF” already bound to fresh washed platelets mediates platelet adhesion to collagen.

The effect of FVIIIIR:Ag on the rate of platelet adhesion to collagen may be significant, because platelets approaching an arteriolar injury 100 μm long are exposed to the vascular subendothelial structure only for about 2 msec. Even when platelets are subjected to pathologic flow patterns, the total exposure time to the site of injury can still be measured in tenths of a second.
REFERENCES

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M Aihara, HA Cooper and RH Wagner