CONCISE REPORT

Investigation of the Role of von Willebrand Factor in Thrombotic Thrombocytopenic Purpura

By Eric C-Y. Lian and Farooq A. Siddiqui

Von Willebrand factor (vWF) has been implicated as a cofactor in platelet aggregation induced by thrombotic thrombocytopenic purpura (TTP) plasma. To investigate further this role of vWF, we have used rabbit monoclonal anti-FvIII/vWF antibodies and a monoclonal antibody to platelet glycoprotein Ib (GP Ib) that blocks the ristocetin-induced platelet aggregation. The monoclonal anti-platelet GP Ib antibody inhibited the platelet aggregation induced by ristocetin in the presence of normal plasma, but not that by any of the five TTP plasma samples. The TTP plasma samples from five patients were incubated with the monospecific antibodies to FvIII/vWF. In all of the samples, the FvIII/vWF:Ag was drastically reduced; however, there was almost no effect on the platelet-aggregating activity. Therefore, it is concluded that vWF is unlikely to play a major role in platelet aggregation induced by majority of TTP plasmas and that the site of platelet GP Ib, to which vWF binds in the presence of ristocetin, is not involved in TTP plasma-induced aggregation.

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Study of the effect of monospecific anti-FVII/VWF IgG on the TTP plasma-induced platelet aggregation. A 0.4-mL sample of TTP plasma in a plastic tube was mixed with varying amounts of anti-FVII/VWF IgG to give a final concentration of 0, 20, or 100 μg of IgG per milliliter. The tubes were incubated for two hours at 37°C and one hour at 4°C. The mixture was centrifuged at 48,000 g for one hour at 4°C to remove antigen-antibody complexes. The supernatant was used for the determination of platelet-aggregating activity and FVIII:Ag as described previously.

RESULTS

Effect of monoclonal anti-platelet GP I b antibody on the TTP plasma-induced platelet aggregation. After incubation of anti-platelet GP I b antibody at the concentration of 6.6 μg/mL at 37°C for 30 minutes, the platelet aggregation induced by ristocetin (at the final concentration of 1.2 mg/mL) in the presence of normal plasma was completely inhibited. In contrast, at the same concentration of anti-platelet GP I b antibody, the platelet aggregation induced by any of the five TTP plasma samples was not inhibited (Fig 1).

Effect of anti-FVII/VWF IgG on the TTP plasma-induced platelet aggregation. As shown in Table 1, the FVIII/VWF:Ag was significantly depleted from all of the TTP plasma samples after incubation with rabbit anti-FVIII/VWF IgG; however, there was very little change in the platelet-aggregating activity.

DISCUSSION

TTP is a syndrome manifested by microangiopathic hemolytic anemia, thrombocytopenia, changing neurologic signs, renal abnormalities, and fever. The syndrome is caused by diverse etiologies. The development of TTP is likely caused by the unbalanced interaction between environmental inciting agents and intrinsic host factors. Our laboratory has discovered that plasmas from some TTP patients induced the aggregation of autologous as well as homologous platelets. The factors responsible for the platelet aggregation are heterogeneous. Other than primary intravascular platelet aggregation or agglutination, endothelial cell injury induced by antienzyme B cell antibody, circulating immune complexes, or other agents has also been proposed as the cause of platelet thrombi in the microvessels in TTP. The platelet aggregation or agglutination could be influenced by host factors, such as defective immune response, to produce specific inhibitory immunoglobulin, prostacyclin synthesis, or stabilization, decreased fibrinolysis, hyperactive platelets, and other plasma proteins.

Recently, it has been shown that unusually large vWF multimers are increased in amounts in patients with chronic relapsing TTP during remission. These multimers disappear during relapse of chronic TTP and during active disease of acute TTP. Kelton et al reported that large vWF multimers prepared from normal cryoprecipitate enhanced the agglutination of platelets. These workers suggested that large vWF multimers are possibly consumed in platelet aggregation and patients may be more susceptible to TTP because of congenital or acquired abnormality in processing unusually large multimers of vWF. However, patients with type IIb or pseudo-van Willebrand's disease do not have clinical manifestations of TTP, even if vWF is consumed as a result of platelet aggregation. Furthermore, most of the patients with TTP experience improved conditions by plasma infusion despite the presence of vWF multimers in the normal plasma, from which Kelton et al prepared the cryoprecipitate and large vWF multimers for the in vitro platelet agglutination test. These clinical observations raised some doubts that vWF multimers are important in the development of TTP.

Here we demonstrated that the platelet aggregability of

Fig 1. Effect of monoclonal anti-platelet GP I b antibody on the TTP plasma-induced platelet aggregation. Buffer or monoclonal anti-platelet GP I b antibody (1.5 μL) containing 0.67 μg protein were admixed with 0.1 mL of washed platelet suspension (750 × 10^6 per liter) and incubated for ten minutes. To this, (A) 0.3 mL of normal plasma, 0.074 mL of Tris-saline buffer, and 0.025 mL of ristocetin (24 mg/mL) were sequentially added; (B through F) Four-tenths milliliter of each TTP plasma, undiluted or diluted with Tris-saline buffer, pH 7.4, was added. The platelet aggregation reflecting the change of optical density was recorded.
Table 1. Effects of Anti-FVIII/vWF lgG on the TTP Plasma-Induced Platelet Aggregation

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Anti-FVIII/vWF lgG</th>
<th>Platelet Aggregation (%)</th>
<th>Platelet Aggregation (%)</th>
<th>Platelet Aggregation (%)</th>
<th>Platelet Aggregation (%)</th>
<th>Platelet Aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma 1</td>
<td>0 µg/mL</td>
<td>3.40</td>
<td>100</td>
<td>0.56</td>
<td>100</td>
<td>1.72</td>
</tr>
<tr>
<td>Plasma 2</td>
<td>20 µg/mL</td>
<td>3.00</td>
<td>100</td>
<td>&lt;0.08</td>
<td>100</td>
<td>1.12</td>
</tr>
<tr>
<td>Plasma 3</td>
<td>100 µg/mL</td>
<td>&lt;0.08</td>
<td>86</td>
<td>—</td>
<td>—</td>
<td>&lt;0.08</td>
</tr>
</tbody>
</table>

Platelet aggregation induced by TTP plasma without anti-FVIII/vWF lgG is regarded as 100%.

The majority of TTP plasmas was not affected after marked reduction of vWF, and the monoclonal anti-platelet GP Ib antibody that specifically blocks ristocetin-induced platelet aggregation did not inhibit the platelet-aggregating activity of all TTP plasma samples tested. The difference between our results and those of Kelton et al may be due to different platelet-aggregating or -agglutinating factor present in the TTP plasmas or other unknown factors involved in the tests. From our studies, vWF appears unlikely to play a major role in aggregation by the majority of TTP plasmas, and the site on platelet GP Ib, to which vWF binds in the presence of ristocetin, is not involved in TTP plasma-induced aggregation.

ACKNOWLEDGMENT

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REFERENCES

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