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) in humans is known to cause the platelets to adhere to the subendothelium and to aggregate in the presence of ristocetin. Recently, it has been reported that large vWF multimers may be responsible for the development of platelet microthrombi in chronic relapsing thrombotic thrombocytopenic purpura (TTP) and may function as a cofactor in the platelet aggregation induced by TTP plasma. To investigate further the latter role of vWF, we have used rabbit monospecific anti-FVIII/vWF antibodies and a murine monoclonal antibody to platelet glycoprotein Ib (GP Ib) that blocks 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.

Materials and Methods

Preparation of plasma and platelets. Human plasma and platelets were prepared as described previously. The washed platelets were suspended in the Tris–saline buffer, pH 7.4, containing 0.133 mol/L NaCl, 0.015 mol/L Tris–Cl, 0.005 mol/L KCl, and 0.001 mol/L MgCl₂, and adjusted to 750 × 10⁵ per liter for the platelet aggregation studies. The TTP plasma samples were obtained from five patients with classic manifestation of TTP during active disease. All five patients initially responded to either plasma infusion or exchange plasmapheresis. Four of these patients recovered completely; the other succumbed to the disease after recurrent relapses. All five TTP plasma samples were shown to cause the aggregation of washed platelets, which was inhibited by preincubation with normal plasma, but not with hirudin or heparin in the presence of antithrombin III, using the method described previously.

Study of the effect of monoclonal anti-platelet GP Ib antibody on the TTP plasma-induced platelet aggregation. A 1.5-μL of buffer or monoclonal anti-platelet GP Ib antibody solutions (which was kindly supplied by Dr Barry Coller, SUNY, Stony Brook, NY, and designated 6D1) containing 0.67 μg protein was mixed with 0.1 mL of washed platelet suspension (750 × 10⁵ per liter) and incubated at 37°C for ten minutes. To this, 0.3 mL of normal plasma, 0.074 mL of Tris–saline buffer, and 0.074 mL of ristocetin were added. The mixture was incubated at 37°C for ten minutes. After this, 0.4 mL of TTP plasma undiluted or diluted with Tris–saline buffer, pH 7.4, was added. The platelet aggregation reflected by the change in optical density was recorded.

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-FVIII/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-FVIII/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\(^1\) and FVIII:Ag\(^6\) as described previously.

RESULTS

Effect of monoclonal anti-platelet GP Ib antibody on the TTP plasma-induced platelet aggregation. After incubation of anti-platelet GP Ib 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 Ib antibody, the platelet aggregation induced by any of the five TTP plasma samples was not inhibited (Fig 1).

Effect of anti-FVIII/vWF IgG on the TTP plasma-induced platelet aggregation. As shown in Table I, 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.\(^7\) The syndrome is caused by diverse etiologies.\(^8\)-\(^10\) 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.\(^5\) The factors responsible for the platelet aggregation are heterogeneous.\(^9\)-\(^10\) Other than primary intravascular platelet aggregation or agglutination, endothelial cell injury induced by antiendothelial cell antibody,\(^11\) circulating immune complexes,\(^12\) 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,\(^9\) prostacyclin synthesis,\(^11\) or stabilization,\(^14\) decreased fibrinolysis,\(^15\) 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.\(^1\) These multimers disappear during relapse of chronic TTP\(^1\) and during active disease of acute TTP. Kelton et al\(^4\) 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-von Willebrand's disease do not have clinical manifestations of TTP, even if vWF is consumed as a result of platelet aggregation.\(^16\) Furthermore, most of the patients with TTP experience improved conditions by plasma infusion despite the presence of vWF multimers in the normal plasma,\(^17\) from which Kelton et al\(^4\) 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
Table 1. Effects of Anti-FVIII/vWF IgG on the TTP Plasma-Induced Platelet Aggregation

<table>
<thead>
<tr>
<th>Anti-FVIII/vWF IgG</th>
<th>Plasma 1</th>
<th>Plasma 2</th>
<th>Plasma 3</th>
<th>Plasma 4</th>
<th>Plasma 5</th>
</tr>
</thead>
<tbody>
<tr>
<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>20 μg/mL</td>
<td>3.00</td>
<td>&lt;0.08</td>
<td>100</td>
<td>&lt;0.08</td>
<td>1.12</td>
</tr>
<tr>
<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 IgG 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.

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REFERENCES

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