Plasma $\beta$-Thromboglobulin: Differentiation Between Intravascular and Extravascular Platelet Destruction

By Ping Han, Alexander G. G. Turpie, and Edward Genton

To elucidate the usefulness of $\beta$-thromboglobulin ($\beta$TG) in the differentiation of the mechanism of thrombocytopenia, plasma $\beta$TG concentration was measured in one patient with amegakaryocytic thrombocytopenia, four patients with autoimmune thrombocytopenia (ATP), two patients with thrombotic thrombocytopenia (TTP), and one patient with thrombocytopenia secondary to disseminated intravascular coagulation (DIC). Plasma $\beta$TG was not measurable in amegakaryocytic thrombocytopenia, was normal in ATP, and was increased in TTP and DIC. These data indicate that in thrombocytopenic patients, increased plasma $\beta$TG concentration may result from intravascular platelet consumption with release of platelet constituents in contrast to extravascular platelet destruction by the macrophage-monocyte system.

THERE ARE three main mechanisms that result in thrombocytopenia: (1) increased platelet destruction, (2) decreased platelet production, and (3) increased platelet sequestration. Increased platelet destruction can occur extravascularly in the macrophage-monocyte system in autoimmune thrombocytopenia (ATP) or by intravascular aggregation and consumption in thrombotic thrombocytopenic purpura (TTP), in some types of septicemia, and in large cavernous hemangioma.

Chromium-51-labeled platelet survival time has been used to differentiate between thrombocytopenia caused by decreased production from that due to increased destruction, but does not differentiate between intravascular and extravascular platelet destruction. Several platelet proteins, including platelet factor 4 and $\beta$-thromboglobulin ($\beta$TG), which are released from platelets when they are activated, have been isolated and characterized. Radioimmunoassays have been developed to assay the concentration of these proteins in plasma, which may prove useful in differentiating intravascular from extravascular platelet consumption. To test this hypothesis, we have compared the plasma $\beta$TG concentration in patients with autoimmune thrombocytopenia (ATP), with thrombotic thrombocytopenia (TTP), and disseminated intravascular coagulation (DIC), which are characterized by intravascular platelet consumption resulting in microcirculatory thrombosis. In addition, to determine the platelet specificity of $\beta$TG, we measured the concentration of $\beta$TG in the plasma of one patient with amegakaryocytic thrombocytopenia.
MATERIALS AND METHODS

Venous blood (2.7 ml) was collected without stasis, using 21-gauge needles, into 3-ml plastic syringes and was mixed immediately with 0.3 ml anticoagulant mixture containing EDTA, 0.219 M, theophylline, 30 mM, and PGE1, 30 tM, at 4°C. Platelet-poor plasma (PPP) was prepared by centrifugation at 2000 g for 60 min at 4°C, and the top 0.5 ml of PPP was collected and stored at -70°C for βTG assay.

Plasma βTG concentration was measured using an optimized radioimmunoassay with a sensitivity to 3 ng/ml, and platelet βTG was measured after lysis with sodium lauryl sulphate.

In patients with ATP, platelet survival time was determined using 3tCr-labeled homologous ABO-Rh-matched platelets. The mean platelet survival time was calculated using the multiple-hit model by least-square regression analysis with computer assistance.

Patients

Group A—Autoimmune Thrombocytopenic Purpura (ATP)

Case 1. A 22-yr-old male had a platelet count of 17 × 10⁹/liter on routine blood count with no history of bruising or bleeding. Hemoglobin (Hb) was 15.8 g/dl, and WBC was 14.7 × 10⁹/liter with normal differential count. Evaluation of the thrombocytopenia revealed no splenomegaly, Antinuclear Factor (ANF) negative, increased numbers of megakaryocytes of the marrow, shortened platelet survival, and increased platelet antibody (PAIgG). He was taking no drugs.

Case 2. A 48-yr-old female with systemic lupus erythematosus was found to be thrombocytopenic (20 × 10⁹/liter) on routine follow-up. She gave a history of easy and spontaneous bruising for 6 mo and a 1-yr history of arthralgia. At the time of presentation, she was taking quinidine. Evaluation of thrombocytopenia revealed Hb 12.3 g/dl, WBC 4.2 × 10⁹/liter with normal differential count, ANF 1:512, positive Lupus Erythematosus (LE) cells, and Erythrocyte Sedimentation Rate (ESR) 102. She had a raised PAIgG, short platelet survival time, and no antibodies to quinidine. Bone marrow aspirate contained normal numbers of megakaryocytes.

Case 3. A 52-yr-old female had a history of chronic ATP and a platelet count of 16 × 10⁹/liter. Evaluation revealed increased marrow megakaryocytes, short platelet survival time, and raised PAIgG. Hb was 13.0 g/dl, WBC 5 × 10⁹/liter with normal differential count. There was no evidence of systemic lupus erythematosus.

Case 4. A 27-yr-old female presented with a history of chronic thrombocytopenia; platelet count was 82 × 10⁹/liter. Evaluation revealed no evidence of systemic lupus erythematosus; splenomegaly was present but no hepatomegaly nor lymphadenopathy. Hb was 13.8 g/dl, WBC 3.6 × 10⁹/liter with normal differential count. She had a raised PAIgG and a short platelet survival time.

Group B—Thrombotic Thrombocytopenic Purpura (TTP)

Case 5. A 50-yr-old female with TTP presented with a 6-mo history of spontaneous bruising and 1-wk history of fluctuating neurologic symptoms; she experienced transient aphasia, peripheral numbness, and dysphagia. Evaluation revealed no evidence of extracranial arterial disease or valvular heart disease. CT scan was normal, but EEG showed multiple, discrete small localized lesions consistent with cerebral emboli. Hb was 9.6 g/dl, WBC 13.3 × 10⁹/liter with normal differential count, and platelet count of 20 × 10⁹/liter. Peripheral blood film showed a microangiopathic picture. There was no laboratory evidence of disseminated intravascular coagulation. Patient recovered completely after treatment with steroids, platelet suppressant drugs, heparin, and plasmapheresis.

Case 6. A 27-yr-old female with TTP presented with a 3-wk history of sore throat and upper respiratory tract infection and easy bruising, petechiae, menorrhagia, and hematuria. Evaluation revealed no neurologic symptoms or signs, splenomegaly but no lymphadenopathy or hepatomegaly. Hb was 10.0 g/dl, WBC 3.8 × 10⁹/liter with normal differential count, and platelet count 5 × 10⁹/liter. Blood film showed a microangiopathic picture with no laboratory evidence of disseminated intravascular coagulation. Patient recovered completely after plasmapheresis, with return of hematologic indices to normal.

Group C—Thrombocytopenia Secondary to Disseminated Intravascular Coagulation (DIC)

Case 7. A 69-yr-old female with staphylococcal septicemia developed DIC. Hb was 11.4 g/dl, WBC 14.2 × 10⁹/liter, platelet count 90 × 10⁹/liter, partial thromboplastin time (PTT) 47 (30) sec, PT 19
Table 1. Platelet Count, Platelet Survival, and Plasma βTG in ATP:
Platelet Count and Plasma βTG in TTP and DIC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patient No.</th>
<th>Platelet Count x 10^9/Liter</th>
<th>Platelet Survival (hr)</th>
<th>Plasma βTG (ng/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>1</td>
<td>17</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20</td>
<td>0.5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>82</td>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>TTP</td>
<td>5</td>
<td>20</td>
<td>ND*</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40</td>
<td>ND</td>
<td>72</td>
</tr>
<tr>
<td>DIC</td>
<td>7</td>
<td>90</td>
<td>ND</td>
<td>244</td>
</tr>
</tbody>
</table>

*Normal: 28 ± 16 ng/ml.
†ND, not done.

(11) sec, thrombin clotting time, 2 U, 37 (26) sec, 10 U 13 (10) sec, Fibrin Degradation Products (FDP) 192 μg/ml. At autopsy, multiple disseminated microthrombi were found.

Group D—Amegakaryocytic Thrombocytopenia

Case 8. A 52-yr-old male with pure red cell aplasia associated with a thymoma developed thrombocytopenia: Hb 7.7 g/dl, WCC 10.4 x 10^9/liter with normal differential, and platelet count < 10 x 10^9/liter by automatic counter. Platelets were not seen in peripheral blood film, and there were no megakaryocytes in the bone marrow. The patient was treated initially with a transfusion of autologous platelets, and recovered following cytotoxic therapy with a platelet count of 146 x 10^9/liter.

RESULTS

The mean plasma βTG level in 70 healthy subjects was 28 ± 8 (SD) ng/ml, (range 14–38 ng/ml), and there was no correlation between plasma βTG and platelet count in normals. Plasma βTG concentration was normal in 4 ATP
patients. In 3 of the patients, platelet survival time was less than 24 hr, and in 1 patient, was 150 hr (normal > 168 hr). In contrast, the plasma βTG level in the patients with TTP and DIC was elevated. The results are shown in Table 1. In one patient with TTP, plasma βTG was obtained serially during treatment with plasmapheresis, and plasma βTG remained elevated even when the platelet count was recovering, but when fragmented red cells were still present on the blood film. After 4 wk, when the patient was fully recovered and with the return of all hematologic parameters to normal, plasma βTG was normal (Fig. 1).

Plasma βTG was not measurable in the patient with amegakaryocytic thrombocytopenia (Table 2). After treatment with autologous platelet transfusion, plasma βTG was 4 ng/ml 1 hr after 16 U of platelets with a platelet count of 47 × 10⁶/liter; it was unmeasurable 24 hr after 8 U (platelet count 83 × 10⁹/liter) and was 5 ng/ml 24 hr after a further 8 U of platelets, when the platelet count was 145 × 10⁹/liter. Platelet βTG content was 8 ng/10⁶ platelets (normal 18–30 ng/10⁶ platelets), and stimulation with collagen released only 3% of the total platelet βTG. After bone marrow recovery with a native platelet count of 120 × 10⁹/liter, plasma βTG was 22 ng/ml.

DISCUSSION

Platelets in ATP are destroyed within the macrophage-monocyte (reticuloendothelial) system of spleen or liver, whereas in TTP and DIC, there is evidence that intravascular platelet consumption occurs with microvascular platelet aggregation and thrombus formation. In this study, βTG—a platelet-specific protein that is released from platelets during activation—was normal in four patients with ATP and raised in two patients with TTP and one patient with DIC. This observation suggests that plasma βTG may be an important indicator of platelet activation or destruction in the circulation, analogous to plasma hemoglobin as an indicator of intravascular hemolysis.

Plasma βTG has an in vivo half-life of 100 min. Hence, the observation that plasma βTG remained high even when platelet count was recovering in one TTP patient suggests that the pathogenic stimuli in TTP were still operative after institution of therapy, or that although therapy inhibited the active consumptive process, the continued presence of platelet/fibrin thrombi in the microcirculation during recovery remained as a stimulus for continued activation of platelet release. Plasma βTG returned to normal levels in both the TTP patients when their hematologic indices returned to normal. These observations suggest that plasma βTG may serve as a useful monitor of therapy in patients with intravascular destruction of platelets.

Table 2. Response of Platelet Count and Plasma βTG to Platelet Transfusion in Patient With Amegakaryocytic Thrombocytopenia: Plasma βTG After Recovery

<table>
<thead>
<tr>
<th>Platelet Transfusion</th>
<th>Time After Transfusion (hr)</th>
<th>Platelet Count (× 10⁹/Liter)</th>
<th>Plasma βTG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>—</td>
<td>&lt;10</td>
<td>0</td>
</tr>
<tr>
<td>8 U</td>
<td>24</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>16 U</td>
<td>48</td>
<td>145</td>
<td>5*</td>
</tr>
<tr>
<td>After marrow recovery</td>
<td>—</td>
<td>130†</td>
<td>22</td>
</tr>
</tbody>
</table>

*Platelet βTG content 8 ng/10⁶ platelets (normal: 18–30 ng/10⁶ platelets).
†Native platelets.
In one patient with marrow aplasia and without detectable megakaryocytes or platelets (i.e., $< 10 \times 10^9$/liter platelets on instrument count), there was no measurable plasma $\beta$TG by the sensitive radioimmunoassay. After platelet transfusion, the plasma $\beta$TG rose slightly to 4 ng/ml. Although the platelet count obtained by transfusion at the time of sampling was $145 \times 10^9$/liter, the platelet $\beta$TG content was low (8 ng/10$^8$ platelets) compared to normal platelets (mean 24.5 ng/10$^8$ platelets, range 18–30 ng/10$^8$ platelets), and stimulation by collagen released only 3% of platelet $\beta$TG, indicating reduced amounts and defective release of $\beta$TG from transfused platelets. The patients with thrombocytopenia due to extravascular destruction (ATP) had plasma $\beta$TG levels similar to subjects with normal platelet counts. Plasma $\beta$TG does not correlate with the platelet count in normal individuals. These data suggest that plasma $\beta$TG normally measured in plasma reflects minimal platelet activation in vivo.

The data indicate that plasma $\beta$TG is a useful index of intravascular consumption of platelets and may be useful in differentiating the mechanism of thrombocytopenia in ATP and TTP. Also, plasma $\beta$TG concentration may be a useful measurement for monitoring therapy of TTP and DIC.

REFERENCES

Plasma beta-thromboglobulin: differentiation between intravascular and extravascular platelet destruction

P Han, AG Turpie and E Genton