Monocyte–Platelet Interaction in Immune and Nonimmune Thrombocytopenia

By Mansoor N. Saleh, Daniel L. Moore, Jeannette V. Lee, and Albert F. LoBuglio

Platelets from 24 patients with immune thrombocytopenia resistant to standard therapy (refractory ITP), 35 patients with nonimmune thrombocytopenia (non-ITP), and 32 normal donors were studied in regard to platelet surface-bound IgG (PBlgG) and the ability of these platelets to be bound by human monocytes in vitro (monocyte-platelet rosette assay). Fourteen (58%) of the platelet samples from refractory ITP patients but none (0%) from the non-ITP or control donors had PBlgG >800 molecules IgG/platelet. Seventeen of 24 (71%) of the ITP patients had platelets which demonstrated increased monocyte-platelet rosette formation [rosette index (RI) >2], whereas only four (11%) of the non-ITP patients had such platelets. There was a direct correlation between PBlgG and rosette index for the platelets from resistant ITP patients. There was no correlation of severity of thrombocytopenia with PBlgG or rosette index. Monocyte–platelet interaction in the presence of elevated PBlgG is mediated through the monocyte Fc-receptor. Platelets from five of ten refractory ITP patients with PBlgG <800 molecules IgG/platelet had increased rosette formation. Monocyte–platelet interaction in the absence of increased PBlgG may be due to small amounts of platelet surface IgG which are still able to mediate monocyte Fc-receptor interaction or to alternate membrane receptor interaction through the monocyte C3 receptor. Our data underscore the pathophysiologic relevance of monocyte/macrophage-mediated interaction in immune platelet destruction syndromes.

Immune thrombocytopenic purpura (ITP) is a clinical disorder generally characterized by a shortened platelet survival as a result of reticuloendothelial sequestration. The humoral-mediated nature of this disease was recognized very early by Harrington et al. In the meantime, some researchers have demonstrated that this syndrome of accelerated platelet destruction is primarily mediated by IgG antibodies directed against as yet ill-defined platelet surface antigens. A monoclonal anti-IgG assay that quantitates the amount of IgG bound on the platelet surface is capable of differentiating very accurately between immune and non-ITP based on the amount of IgG on the platelet surface. By this assay, 90% of newly diagnosed ITP patients are shown to have >800 molecules IgG/platelet on their surface. In contrast only 67% of the treatment-resistant ITP patients showed a similar finding. This finding suggested the involvement of non-IgG-mediated processes in the pathogenesis of refractory ITP.

Previous studies have suggested that destruction of opsonized platelets is due to a macrophage-receptor-mediated phenomenon involving the organs of the reticuloendothelial system. One defined mechanism has been the macrophage Fc-receptor-mediated recognition and binding of IgG-coated platelets. Additionally, binding of IgM antibodies to platelet surface antigens may also result in complement activation. This process could lead to direct platelet lysis or deposition of complement components on the platelet surface with subsequent phagocytosis via macrophage complement receptors. Other investigators have reported that platelet-associated IgM as well as complement (C3) may be elevated in ITP patients. We previously described an assay for quantitative study of Fc-receptor-mediated interaction between antibody-coated platelets and Fc-bearing effector cells in vitro. In the present study, we examined the interaction between human monocytes and platelets from patients with treatment-resistant ITP as well as non-ITP. Data are provided to support the pathophysiologic relevance of receptor-mediated platelet destruction in refractory ITP.

Patient characteristics. Platelets from 60 thrombocytopenic patients and 32 normal subjects were studied and form the basis for this study. Twenty-four of the patients had a diagnosis of ITP based on accepted clinical criteria. All these patients had been previously treated extensively and were referred to us for evaluation because of persistent treatment-resistant thrombocytopenia. All patients (100%) had received corticosteroids, 30% had undergone splenectomy, and 5% had received additional immunosuppressive regimes. Thirty-six patients had thrombocytopenia considered nonimmune in origin. These included patients with aplastic anemia, chemotherapy-induced myelosuppression, septicemia, or disseminated intravascular coagulation (DIC).

Materials and Methods

Platelets were isolated from platelet-rich plasma (PRP) derived from the respective patients or healthy controls. An aliquot was used to quantitate the amount of IgG on the platelet surface using 125I-labeled monoclonal antibody (MoAb) to IgG. A second aliquot was used for the monocyte-platelet rosette assay. Monocytes were isolated from peripheral blood of healthy donors as previously described.

Monocyte-platelet rosette assay. Monocyte binding of platelets was determined by a modification of the classic monocyte-RBC rosette assay as previously described. One million monocytes were added to tubes containing 10 x 10^6 patient or control FITC-labeled platelets, and cell contact was initiated by centrifugation at 675 g for ten minutes at 4°C. The supernatant was aspirated and discarded. The cell pellet was resuspended in 50 μL phosphate buffer, and 50 μL fixative solution (5% acetic acid in ethanol cooled to –40°C) was

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Table 1. Monocyte-Platelet Interaction Between Normal Human Monocytes and FITC-Labeled Platelets Sensitized With Anti-PLA<sub>4</sub> Antiserum

<table>
<thead>
<tr>
<th>Subject</th>
<th>PBIG&lt;sup&gt;*&lt;/sup&gt; (Molecules IgG/Plt)</th>
<th>RI</th>
<th>Total</th>
<th>RI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>203 ± 5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>430 ± 5</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>650 ± 6</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>895 ± 14</td>
<td>5</td>
<td>0</td>
<td>19</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>1,460 ± 9</td>
<td>1</td>
<td>4</td>
<td>28</td>
<td>5.6</td>
</tr>
<tr>
<td>5</td>
<td>3,266 ± 20</td>
<td>18</td>
<td>8</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>20,084 ± 27</td>
<td>26</td>
<td>38</td>
<td>91</td>
<td>18.2</td>
</tr>
</tbody>
</table>

*PBIG in molecules IgG per platelet (Plt).

Results

Table 1 shows the results of an experiment to examine the effect of antibody density on both the RI and the number of platelets bound per monocyte using normal platelets sensitized in vitro with increasing amounts of anti-PLA<sub>4</sub> antiserum. Control platelets sensitized with autologous plasma yielded 5% rosettes with all rosettes composed of two platelets bound per monocyte. With increasing amounts of IgG on the platelet surface, rosette formation as well as the number of platelets bound per monocyte increased. With this antibody, an RI >2 was observed when platelets were sensitized with >800 molecules IgG per platelet.

As summarized in Table 2, platelets from the 32 healthy donors (control) had PBIG of 60 ± 6 (mean ± SEM) molecules IgG per platelet. Patients with refractory ITP had PBIG of 2,477 ± 822 molecules IgG per platelet and an RI of 4.5 ± 0.69 (mean ± SEM). Patients with non-ITP had a PBIG of 219 ± 23.5 molecules IgG per platelet and an RI of 1.3 ± 0.14 (mean ± SEM). The mean platelet count of the patient with immune ITP and non-ITP was comparable (46 ± 10<sup>9</sup>/μL vs 57 ± 10<sup>9</sup>/μL; P = .2).

The quantitative breakdown of PBIG and RI for platelets from both patient groups is summarized in Table 3. Platelets from 14 refractory ITP patients had >800 molecules IgG per platelet and 12 of them (86%) had an RI >2. Platelets from four ITP patients had 400 to 800 molecules IgG per platelet, and all (100%) had an RI >2. The remaining six ITP patients had <400 molecules IgG per platelet, and only one (17%) had an RI >2.

Fourteen of 24 (58%) refractory ITP patients had >800 molecules IgG per platelet. None of the 36 non-ITP patients had elevated PBIG (P < .001). Platelets from 17 of 24 (71%) refractory ITP patients yielded an RI >2, whereas platelets from four of 35 (11%) non-ITP patients demonstrated increased rosette formation (P < .001) (Table 3).

There was a direct correlation between PBIG and the RI for the ITP population (data not shown). The relationship between PBIG and the RI was best expressed by the log-linear equation (log PBIG = 2.56 + 0.10 x RI) (P = .002, r<sup>2</sup> = .35).

Discussion

The pathophysiology of acute ITP apparently involves binding of autoantibody (IgG) to platelet surface antigens and the subsequent Fc-receptor-mediated sequestration of opsonized platelets by the reticuloendothelial organs. Quantitation of IgG on the platelet surface can be very accurately determined by the monoclonal anti-IgG assay. With this assay, a majority (90%) of patients with ITP are shown to have PBIG levels >800 molecules per platelet. A lesser proportion of refractory ITP patients have PBIG >800 molecules IgG per platelet.

Table 2. Platelet Count, PBIG, and RI of Study Population

<table>
<thead>
<tr>
<th>Platelet Source</th>
<th>n</th>
<th>Plt Count (x 10&lt;sup&gt;9&lt;/sup&gt;/L) (Mean ± SEM)</th>
<th>Mean PBIG (Molecules IgG/Plt) (± SEM)</th>
<th>RI (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donor</td>
<td>32</td>
<td>&gt;200</td>
<td>60 ± 6</td>
<td>1</td>
</tr>
<tr>
<td>ITP (treatment resistant)</td>
<td>24</td>
<td>46 ± 5.53</td>
<td>2,477 ± 822</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>Non-ITP</td>
<td>36</td>
<td>57 ± 4.45</td>
<td>219 ± 24</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3. Quantitative Breakdown of PBIG and RI for Platelets From Refractory ITP and Non-ITP Patients

<table>
<thead>
<tr>
<th>PBIG Status</th>
<th>PBIG (Molecules IgG/Plt)</th>
<th>n</th>
<th>RI &gt; 2</th>
<th>RI &lt; 2</th>
<th>RI (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITP &gt;800</td>
<td>14</td>
<td>12</td>
<td>2</td>
<td>6.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>400-800</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>3.7 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>&lt;400</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>1.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Non-ITP &gt;800</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>400-800</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>&lt;400</td>
<td>35</td>
<td>4</td>
<td>31</td>
<td>1.3 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: Plt, platelet.
All 24 patients we reported had ITP resistant to standard medical treatment (refractory ITP). Our data regarding PBlgG in this larger study population of patients with refractory ITP are comparable to the results of our previous report. In addition, our present findings underscore the results of the previous report in that none of the patients with non-ITP had >800 molecules IgG per platelet.

The interaction between opsonized platelets and human effector cells plays a key role in the proposed pathophysiology of immune platelet destruction. We previously described a sensitive monocyte-platelet rosette assay to study in vitro the interaction between human monocytes and IgG-coated platelets. In this study, we present data examining the interaction in vitro between normal human monocytes and platelets from patients with clinical thrombocytopenia (in vivo sensitized).

The cutoff values for PBlgG (<400, 400 to 800, and >800 molecules IgG per platelet) we used are based on our previously reported finding in patients with new-onset ITP. Ninety percent of patients with new-onset ITP had platelets with >800 molecule IgG per platelet and >99% of normal donors had <400 molecules IgG per platelet. Supportive of the pathophysiologic relevance of platelet IgG, the in vitro sensitization of platelets with PLAl antisera requires >800 molecules antibody per platelet to demonstrate increased binding to human monocytes (Table 1).

At the onset of our current study, an RI >2 was arbitrarily selected to represent increased monocyte-platelet binding. The cumulative distribution of RI for the refractory ITP and non-ITP populations (data not shown) reveals that this cutoff value segregates the two groups at a statistically significant level of $P = <.001$.

Seventeen of 24 (71%) patients with refractory ITP demonstrated increased monocyte-platelet binding in vitro (RI >2). This included four patients with 400 to 800 molecules IgG per platelet and one patient with <400 molecules IgG per platelet. The ability of platelets with intermediate levels of PBlgG (400 to 800 molecules IgG per platelet) or normal levels of PBlgG (<400 molecules IgG per platelet) to bind to monocytes and demonstrate rosette formation can occur by two possible mechanisms. Monocyte-platelet interaction at low levels of PBlgG may be possible and would presumably depend on the subclass composition and distribution of PBlgG. This phenomenon is akin to immune hemolytic anemia observed concomitantly with low levels of RBC antibody and a negative Coombs reaction. On the other hand, mechanisms other than, or in addition to, IgG-Fc-receptor interaction may be operational and lead to rosette formation. Increased amounts of platelet-bound complement have been documented in ITP. Platelets from patients with surface-bound complement can potentially interact with effector cells through the monocyte/macrophage complement receptor. Monocyte-platelet binding in such a setting could either be solely mediated by surface complement–complement receptor interaction or in conjunction with IgG–Fc-receptor interaction (in the case of platelets with low levels of surface IgG). In selected cases (unpublished observation), we have been able to detect elevated levels of platelet-bound IgM and/or complement in patients with clinical ITP whose platelets demonstrated <800 molecules IgG per platelet yet yielded increased rosette formation by our in vitro assay.

Five patients with ITP had <800 molecules IgG per platelet and an RI <2. This observation suggests that a subset of patients with treatment-resistant ITP may have thrombocytopenia due to a nonimmune mechanism. None of the patients with thrombocytopenia due to nonimmune causes had PBlgG >400. Four of these patients (11%) had an RI >2. All four patients had severe systemic infections, and two had acute respiratory distress syndrome (ARDS) (Table 4). Activation of complement through the alternate pathway with C3 deposition on the platelet surface might be an explanation but additional studies to detect platelet surface C3 will be necessary to explore this mechanism.

Despite the pathophysiologic relevance of platelet surface IgG in most ITP cases, our data demonstrated no correlation between the amount of IgG on the platelet surface and the degree of clinical thrombocytopenia. This observation is consistent with previous findings. This phenomenon can be explained on the basis of the variable affinity of the human monocyte/macrophage Fc-receptor for the different IgG subclasses. The binding affinity for IgG1 and IgG3 is reportedly higher than for IgG2 and IgG4. Thus, platelets coated predominantly with IgG1 or IgG3 antibody would be more likely to undergo sequestration as opposed to platelets sensitized with comparable amounts of IgG2 or IgG4 antibody. This observation is supported by in vitro studies using RBCs sensitized with different subclass-specific RBC antibodies.

We did not detect an inverse relationship between the RI and the platelet count. This observation would indicate that factors other than receptor-mediated binding and sequestration of opsonized platelets may be important in determining the degree of thrombocytopenia in a given clinical setting (e.g., marrow megakaryocyte content, rate of megakaryocyte maturation, and platelet production). Various investigators have shown that autoantibodies in ITP not only react with antigens on the platelet surface but may also cross-react with bone marrow megakaryocytes and thereby influence the production of young platelets.

Our study underscores the multifactorial pathophysiology of ITP. The in vitro monocyte-platelet rosette assay supports the role of macrophage/monocyte–platelet interaction in the pathophysiology of ITP. The severity of thrombocytopenia, however, appears to be governed not only by factors relevant to the amount of antibody on the platelet surface and its interaction with the macrophage/monocyte receptors but presumably also by factors that govern the production of platelets by bone marrow megakaryocytes.
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REFERENCES

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