Severe Platelet Dysfunction in Hairy Cell Leukemia
With Improvement After Splenectomy
By Michael H. Rosove, Faramarz Naeim, Sylvia Harwig, and Jacob Zighelboim

A patient with hairy cell leukemia developed purpura not attributable to thrombocytopenia. We found markedly reduced platelet aggregation responses and malondialdehyde production, decreased serotonin uptake, and depleted dense granule contents. Ultrastructural studies showed that most platelets had few or no granules. All of the clinical and laboratory studies of platelet function and morphology improved after splenectomy. These findings indicate that qualitative defects in platelet function occurring in hairy cell leukemia may cause clinically important bleeding and that the bleeding diathesis may be ameliorated by splenectomy.

Hairy Cell Leukemia is a well-defined clinical syndrome characterized by circulating mononuclear cells with prominent cytoplasmic projections, splenomegaly, which may be massive, absent or minimal lymphadenopathy, pancytopenia, and a chronic course. The diagnosis is supported by the cytochemical demonstration of tartrate-resistant acid phosphatase in the abnormal cells and by the splenic histology, which shows mononuclear cellular infiltration of the red pulp and sinus engorgement. There is considerable controversy regarding the nature and cytogenesis of the hairy cell; it seems to be a cell that has lymphocytic characteristics. Splenectomy appears to be the preferred initial therapy.

Clinical studies of hairy cell leukemia note the high frequency of thrombocytopenia and 5%-20% mortality from hemorrhage; increasing attention has been given to qualitative platelet abnormalities that may occur and either cause or contribute to clinical bleeding. We describe a patient with hairy cell leukemia who developed a hemorrhagic diathesis associated with prominent abnormalities of platelet morphology and function. Following splenectomy, clinical bleeding regressed, and all studies of platelet morphology and function improved.

CASE REPORT

A 65-yr-old white female was found to have “lymphocytosis” in 1957 at age 43. In the most recent year, she had progressive fatigue, occasional sweats, weight loss, early satiety, and the sensation of weightiness in the left side of the abdomen. She began to bruise easily, often without recognized antecedent trauma, and had a constant petechial rash in the lower extremities. She had one episode of epistaxis, and bled excessively after a dental extraction. The Ivy constant petechial rash in the lower extremities. She had one episode of hemorrhage associated with child births in 1944 and 1947, or with hysterectomy in 1955. There was no history of menorrhagia. She took no medications containing aspirin, nonsteroidal antiinflammatory drugs, and antidepressants, or reserpine.

Physical examination showed a massively enlarged spleen, which extended to the iliac crest and to the right side of the abdomen, mild hepatomegaly, and evidence of numerous old and recent bruises with petechiae on the legs, trunk, and arms. There was no lymphadenopathy.

Before splenectomy, the Hb was 10.5 g/dl and WBC 49,000/µl (89% abnormal mononuclear cells, 2% small lymphocytes, 9% neutrophils). On the Wright-stained peripheral blood smear, many of the platelets were bluish-gray. The bone marrow aspirate and biopsy were hypercellular with 70% replacement by the abnormal cells. Megakaryocytes appeared normal in number and morphology. Normal values were obtained for the prothrombin and activated partial thromboplastin times, thrombin time, plasma levels of fibrinogen (thrombin clotting time), factor VIII-AHF, VIII-antigen, VIII-ristocetin cofactor, and platelet-associated IgG. A protamine paraclotting test and test for serum fibrin degradation products (Burroughs Wellcome) were negative.

Results of special studies were compatible with the diagnosis of hairy cell leukemia. The majority of the peripheral blood lymphoid cells stained strongly for tartrate-resistant acid phosphatase. They had typical hairy cell features by light and electron microscopy, including abundant cytoplasm, ill-defined cytoplasmic borders, and elongated cytoplasmic projections. No paraprotein was detectable by serum immunoelectrophoresis.

The patient received a transfusion of 2 x 1011 platelets (5 donors) immediately before the initial surgical incision. There was no excessive bleeding during splenectomy, and the postoperative course was uncomplicated. The spleen weighed 4500 g, and the cut surface had a homogeneous appearance. The histology showed red pulp and sinus infiltration by lymphoid cells. After operation, the WBC was 65,000/µl, the platelet count ranged from 220,000 to 300,000/µl, and the bleeding time was 4 min. Small numbers of petechiae continued to develop, but only in the feet. There was no further bruising.

Special Platelet Studies

Special studies of platelet morphology and function were performed on the patient before and 1 mo after splenectomy, and on the patient’s sister and two children. To perform platelet studies, venous blood was mixed with 3.8% sodium citrate (9:1 v/v) and centrifuged at 120 g at 25°C for 15 min to prepare platelet-rich
plasma (PRP). Platelet-poor plasma (PPP) was obtained by further centrifugation at 1800 g for 20 min. Platelet aggregometry was performed on a Sienco aggregometer using adenosine diphosphate (ADP) (Sigma Chemicals), epinephrine (Parke-Davis), collagen (Bio/Data Corp., Willow Grove, Pa.), and sodium arachidonate (prepared from arachidonic acid, Nu Chek Prep, Inc., Elysian, Minn.) as aggregating agents. Forty microliters of an aggregating agent in appropriate concentration was added to 360 µl PRP at 37°C with magnetic stirring, and the change in optical density was recorded. Citrated blood, PRP, and PPP contacted only plastic or siliconized surfaces.

Uptake of 14C-serotonin (Amersham, 58 mCi/mmol) by platelets was performed as described. Platelet adenosine triphosphate (ATP) and ADP were determined by the firefly luminescence method. Content of 5-hydroxyindoles as an estimate of platelet serotonin content was determined fluorimetrically. Platelet calcium content was measured by atomic absorption spectroscopy. Measurement of malondialdehyde (MDA) production during platelet aggregation was used as an indicator of the biotransformation of endogenous or added arachidonate to prostaglandin intermediates. The reaction was terminated 8 min after addition of epinephrine, collagen, or saline (5 min after addition of sodium arachidonate) by transferring the reaction mixture to 40% trichloroacetic acid in 1N HCl (8:3 v/v). MDA was measured colorimetrically after reaction with 2-thiobarbituric acid. The platelet count in PRP was adjusted down to 200,000/µl with autologous PPP for studies of platelet aggregation. 14C-serotonin uptake, and MDA production. Platelets were studied by electron microscopy as described. Studies were performed on blood samples taken on duplicate occasions both before (whole blood platelet counts 140,000 and 158,000/µl) and after splenectomy, and mean values are reported.

Before splenectomy, platelet aggregation responses were markedly abnormal. Only primary aggregation responses were observed with epinephrine, 10 µM, and ADP, 16 µM, and no optical density change was observed with collagen or arachidonate. After addition of ristocetin (1.5 to 1.2 mg/ml), maximal aggregation slope was normal. Levels of platelet ADP, 5-hydroxyindoles, calcium, and uptake of 14C-serotonin were markedly decreased (Table 1 and Fig. 1). When PRP was stirred with collagen or arachidonate, MDA was generated in detectable but markedly reduced amounts (Table 2). Ultrastructural features of the platelets are shown in Fig. 2. There was marked polymorphism with great variation in internal structure. Most platelets had few or no granules. Dense granules were rare. Scattered platelets demonstrated dilated canalicular systems or segments of demarcation membranes or mitochondrial granules. Other platelets showed centripetal localization of organelles.

Table 1. Platelet Content of Adenosine Triphosphate (ATP), Adenosine Diphosphate (ADP), 5-Hydroxyindoles, and Calcium From Normal Subjects (n = 12) and in the Patient Before and After Splectomy (splx)

<table>
<thead>
<tr>
<th>Aggregating Agent</th>
<th>Normal Subjects Mean ± SEM</th>
<th>Patient Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before splx</td>
<td>After splx</td>
</tr>
<tr>
<td>ATP (µmole/10^11 platelets)</td>
<td>5.32 ± 0.36</td>
<td>3.4</td>
</tr>
<tr>
<td>ADP (µmole/10^11 platelets)</td>
<td>2.72 ± 0.24</td>
<td>0.8</td>
</tr>
<tr>
<td>ATP/ADP ratio</td>
<td>2.01 ± 0.08</td>
<td>4.2</td>
</tr>
<tr>
<td>5-hydroxyindoles (µmole/10^11 platelets)</td>
<td>250 ± 16</td>
<td>&lt;40*</td>
</tr>
<tr>
<td>Calcium (µmole/10^11 platelets)</td>
<td>14.4 ± 1.1</td>
<td>6.2</td>
</tr>
</tbody>
</table>

*Undetectable.

Table 2. Malondialdehyde (MDA) Production by Platelets From Normal Subjects (n = 11) and From the Patient Before and After Splectomy (splx)

<table>
<thead>
<tr>
<th>Aggregating Agent</th>
<th>Normal Subjects Mean ± SEM</th>
<th>Patient Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before splx</td>
<td>After splx</td>
</tr>
<tr>
<td>Epinephrine (10 µM)</td>
<td>—</td>
<td>&lt;10*</td>
</tr>
<tr>
<td>Collagen (0.2 mg/ml)</td>
<td>106 ± 7</td>
<td>30</td>
</tr>
<tr>
<td>Collagen (0.13 mg/ml)</td>
<td>56 ± 6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Sodium arachidonate (1.2 mM)</td>
<td>1,210 ± 60</td>
<td>230</td>
</tr>
<tr>
<td>Sodium arachidonate (0.6 mM)</td>
<td>739 ± 49</td>
<td>18</td>
</tr>
<tr>
<td>Saline</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*Undetectable.
Fig. 2. The patient's platelets prior to the splenectomy are markedly pleomorphic, and most are variably degranulated. Scattered platelets demonstrate centripetal organelle localization. Dense granules are rare.

DISCUSSION

Defects in platelet function have been previously observed in vitro in association with hairy cell leukemia. Levine and Katayama,6 Golomb et al.,8 and Sweet et al.,9 and Zuzel et al.,10 studied 10, 11, and 8 patients, respectively, with hairy cell leukemia. The bleeding time was prolonged in 5/16 patients. A poor response to epinephrine, often a lack of the second wave,6 was the most frequently observed abnormality in platelet aggregation studies, and was found in 19/28 patients. Abnormal aggregation responses to ADP and collagen, decreased platelet factor 3 availability,6 and decreased serotonin uptake were also observed. Ultrastructure studies showed some platelets with granule abnormalities.6,10

Thus, abnormalities of platelet function are more common in hairy cell leukemia than has been generally recognized. However, it appears that in only a portion of patients with hairy cell leukemia are the abnormalities severe enough to result in clinical bleeding. Despite the fact that 8 of 10 patients studied by Levine and Katayama6 had one or more tests indicating abnormal platelet function, only 2 patients were considered to have abnormal bleeding. Unexpected bleeding was encountered during splenectomy in one patient and during dental extractions in another. None of the patients studied by Golomb and Sweet8 and Zuzel et al.,10 in whom platelet dysfunction was demonstrated had clinical bleeding.

Our patient did have a hemorrhagic tendency; it is reasonable to assume that the disorder was acquired and related to hairy cell leukemia, because of the recent onset in association with advancing lymphocytic proliferation, and because family members lacked any similar findings. The platelets had features of dense granule storage pool disease, including abnormal aggregation responses, subnormal MDA production, near absence of dense granules, and depletion of ADP, 5-hydroxyindoles, and calcium, with decreased serotonin uptake. Additional features were the polymorphism, alpha granule depletion, presence of "empty" platelets, and the centripetal localization of organelles.

It now seems evident that the spleen has a role in the genesis of the platelet defects in hairy cell leukemia. Feiner et al.7 observed a patient in whom the abnormal bleeding time and aggregation responses became normal after splenectomy. Sweet and Golomb9 observed a similar postsplenectomy correction of platelet aggregation abnormalities in three patients, as did Zuzel et al.,10 in one patient. The nearly complete reversal of platelet abnormalities following splenectomy in the present case contributes further evidence that the hairy cell spleen can provide the proper milieu for platelet alteration. However, splenectomy did not result in complete correction of all abnormalities in our patient; two patients initially studied by Golomb and Sweet8 after splenectomy had abnormal epinephrine responses. Accordingly, the spleen appears to contribute to platelet dysfunction in some patients with hairy cell leukemia, but it may not be the only determinant.

The precise mechanism by which the platelets might have been altered is not clear from this study, but some conjecture is possible. It appears that the platelets might have undergone the release reaction. The morphological alterations in particular are

Table 3. Platelet Granule Content as Determined by Electron Microscopy in the Patient Before and After Splenectomy (splx) and in Normal Subjects

<table>
<thead>
<tr>
<th>Dense Granules</th>
<th>Percentage of Platelets Showing</th>
<th>Alpha Granules</th>
<th>Percentage of Platelets Showing</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>None</td>
<td>1 - 4</td>
</tr>
<tr>
<td>Before splx</td>
<td>89</td>
<td>55</td>
<td>24</td>
</tr>
<tr>
<td>After splx</td>
<td>41</td>
<td>59</td>
<td>2</td>
</tr>
<tr>
<td>Normal (n - 4)</td>
<td>39</td>
<td>61</td>
<td>1</td>
</tr>
</tbody>
</table>

A quantity of 75-150 platelets were examined per specimen.
compatible with this hypothesis.\textsuperscript{23,24} It has been suggested that intravascular coagulation\textsuperscript{25} or antiplatelet antibodies\textsuperscript{26} can result in platelet alteration; however, there was no evidence of either in the present case. Perhaps hairy cells can interact directly with platelets to effect the release reaction. Evidence exists to support that platelet interaction and aggregation with other neoplastic cell types occurs.\textsuperscript{27} Although the spleen might provide the most favorable milieu for interaction to occur, hairy-cell-platelet interaction and varying degrees of platelet release could conceivably occur anywhere in the circulation, bone marrow, or parenchymal organs. This might explain the persistence of platelet abnormalities after splenectomy. We cannot exclude the possibilities that either a humoral factor might facilitate platelet alteration, or that platelets may be intrinsically abnormal.

The platelet abnormalities observed in hairy cell leukemia are not unique to this form of hematologic neoplasia, and abnormal platelet aggregation responses and deficient storage pool ADP have been described with other acute and chronic leukemias, lymphomas, and myeloproliferative disorders.\textsuperscript{22,28,29} Although intrinsic platelet defects probably exist in some of these conditions, perhaps interactions of platelets with various cell types contributes significantly to the genesis of platelet abnormalities in a variety of disease states.

**ACKNOWLEDGMENT**

The authors thank Dr. Jerry Waisman for the electron microscopy studies, and Dr. Stephen Chess (who performed the splenectomy) and Dr. K. Parsa for their care of the patient.

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Severe platelet dysfunction in hairy cell leukemia with improvement after splenectomy

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