Immune Thrombocytopenia in Lymphoproliferative Diseases

By Bruce R. Kaden, Wendell F. Rosse, and Thomas W. Hauch

We have studied the thrombocytopenia of lymphoproliferative disorders using a measurement of membrane-bound IgG by an antiglobulin consumption assay. Nine patients with chronic lymphocytic leukemia (CLL) and thrombocytopenia had increased membrane-bound IgG. Two patients with non-Hodgkin's lymphoma and 1 patient with Hodgkin's disease also had thrombocytopenia and increased membrane-bound IgG. Five of the patients with CLL had positive direct antiglobulin (Coombs) tests on red cells; of these, 3 had hemolytic anemia. In eight of the 9 patients with CLL, thrombocytopenia, and increased platelet-bound IgG, the platelet count increased with the administration of prednisone or an alkylating agent, with splenectomy, or with a combination of these.

Thrombocytopenia is a common complication of lymphoproliferative disorders, particularly chronic lymphocytic leukemia (CLL). In the latter disease it is often presumed to be due to marrow infiltration, hypersplenism, or chemotherapy, and it has been used as a criterion of poor prognosis, since in one series the median survival of patients who became thrombocytopenic was 12 mo, as compared with survival of more than 70 mo for nonthrombocytopenic patients. In a few cases the thrombocytopenia appears to be like that seen in idiopathic (immune) thrombocytopenic purpura (ITP), since megakaryocytes were abundant in the bone marrow and the spleen was not greatly enlarged. The platelet survival time in some of these patients, as measured by 51Cr labeling, was shortened. Antibody against platelets detected by platelet factor 3 release, 14C-serotonin release, and phagocytosis of antibody-coated platelets by human granulocytes has been found in the serum of some patients.

We have shown that the antiglobulin consumption assay is useful in the evaluation of thrombocytopenia in that it detects evidence of immune destruction of platelets. The assay quantitates the amount of membrane-bound IgG on the surface of the platelet. In patients with ITP the amount of membrane-bound IgG is increased, and it varies inversely with platelet count.

We have used this assay to evaluate the thrombocytopenia in patients with CLL and other lymphoproliferative disorders. We have found 9 patients with CLL and 3 patients with lymphoma who have thrombocytopenia that appears to be caused by immunologic destruction. The syndrome of CLL with immune thrombocytopenia is
often associated with immune hemolytic anemia and is not necessarily associated with a poor prognosis.

MATERIALS AND METHODS

Patients

Platelets from 36 patients with CLL were studied. Eighteen patients had platelet counts greater than 150 \times 10^9/liter, and 18 patients had platelet counts less than 150 \times 10^9/liter. In each of the 9 patients with increased surface-bound immunoglobulin the platelet count was less than 100 \times 10^9/liter and the bone marrow had increased or normal numbers of megakaryocytes and less than 50% lymphocytes; other illness associated with thrombocytopenia (collagen vascular disease, disseminated intravascular coagulation, or sepsis) was absent.

The platelets of 25 normal adult donors were evaluated. All these donors had platelet counts greater than 150 \times 10^9/liter, were not taking any medication, and had no known clinical illness.

The platelets of patients who did not have CLL and whose platelet counts were less than 130 \times 10^9/liter were also studied. These patients had acute leukemia, multiple myeloma, aplastic anemia, metastatic solid tumors, and disseminated intravascular coagulation. None of these patients had received platelet transfusions.

Buffers and Reagents

Veronal buffered saline (VBS) and buffered 0.015-M ethylenediamine-tetraacetic acid disodium salt (EDTA-VBS) were prepared as previously described.6

Platelet Preparation

Platelet-rich plasma was obtained by differential centrifugation of fresh blood that had been drawn by sterile venipuncture and anticoagulated by a 10% EDTA solution. The platelets were concentrated by centrifugation and were washed twice in EDTA-VBS and once in isotonic sodium chloride, using a total volume of six times the original platelet-rich plasma volume. Final dilution of platelet suspensions were made in isotonic saline. The platelet count of the initial concentration was done by the phase method. Great care was exercised to avoid contamination with white blood cells; at platelet counts of less than 10 \times 10^9/liter, white blood cell contamination was less than 2%.

Assay for Membrane-Bound IgG

Membrane-bound IgG was measured by a quantitative antiglobulin consumption assay as described by Dixon et al.,4 with some modifications. This assay quantitatively measures membrane-bound IgG by its ability to remove anti-IgG from solution. The amount of anti-IgG in solution is measured by lysis of IgG-coated sheep red blood cells by complement.

Human IgG and rabbit antihuman IgG were prepared as previously described.6 Sheep red blood cells coated with IgG (ElgG) were prepared by incubation of 0.14 ml of 0.8% glutaraldehyde in water with 0.5 ml of washed packed sheep red blood cells and 0.25 ml of purified human IgG (10 mg/ml) for 1 hr at room temperature. The cells were washed in isotonic saline and suspended at a concentration of 4.4 \times 10^6 cells/ml. These IgG-coated red cells (ElgG) were stable for 5 days.

The assay required three 30-min incubation steps at 37°C: (1) 0.1 ml of rabbit antihuman IgG in a dilution that would effect 50% maximal lysis was combined with dilutions of known concentrations of fluid-phase human IgG or serial dilutions of known amounts of washed platelets; (2) 0.1 ml of ElgG (4.4 \times 10^6 cells/ml) was then added; (3) 0.2 ml of guinea pig complement diluted 1 part in 20 was then added. The reaction was stopped by addition of 5 ml of VBS. After the platelets and ElgG were removed by centrifugation, the amount of free hemoglobin in the supernatant fluid was measured by the optical density (OD) at 412 nm.

The inhibition of lysis (I) was calculated as follows:

\[
I = 1 - \frac{(OD - CB)}{(OD_m - CB)}
\]

where CB, the cell blank, was the optical density of the supernatant in a tube where anti-IgG was
IMMUNE THROMBOCYTOPENIA

Fig. 1. Relationships between membrane-bound IgG and platelet counts in patients with CLL and ITP (◇), lymphoma and ITP (○), CLL and thrombocytopenia secondary to chemotherapy or progressive disease (▲), CLL associated with elevated membrane-bound IgG and a normal platelet count (□), and nonthrombocytopenic CLL controls (△). For comparison, patients with ITP without lymphoproliferative disorders, thrombocytopenic controls (see text), and normal controls are included; they are represented by the shaded and crosshatched areas.

omitted and ODₘₐₜ was the optical density of the supernatant fluid in a tube where no IgG or platelets were added.

A curve was then drawn relating the percentage inhibition of lysis to the concentration of fluid-phase IgG. With this curve the degree of inhibition of lysis by platelets could be related to the amounts of IgG necessary for an equivalent degree of inhibition. For a known amount of inhibition of lysis and a known number of platelets, the amount of platelet-bound IgG was calculated.

RESULTS

The platelets of the 25 normal donors (Fig. 1) had 6.36 ± 4.16 fg IgG/platelet (mean ± 1 SD). The thrombocytopenic controls had 6.50 ± 4.35 fg IgG/platelet. The maximum normal value at the time of this study was 15 fg IgG/platelet (2 SD from the mean value of the controls).

Of the 36 patients with CLL (Fig. 1), 16 patients had normal platelet counts and normal membrane-bound IgG. Seven patients had reduced platelet counts but normal membrane-bound IgG.

The platelets of 11 patients had elevated membrane-bound IgG; in each patient the platelet count was decreased (Fig. 1 and Table 1). In 8 of the 11 patients the platelet counts increased in response to administration of corticosteroids or an alkylating agent, in response to splenectomy, or in response to a combination of these. In 1 patient the platelet count did not respond to prednisone, vincristine, or splenectomy. Two patients were thrombocytopenic, with elevated membrane-bound IgG; in each patient both values returned to normal without therapy.

Two patients had elevated membrane-bound IgG and normal platelet counts (Fig. 1). The elevation in each case was small.

There was no consistent relationship between the disease activity of the CLL and the presence of thrombocytopenia with elevated membrane-bound IgG. Patients developed immune thrombocytopenia at the following times: at presentation (3 patients); prior to diagnosis of CLL (1 patient); during exacerbation of CLL (2 patients); during otherwise well-controlled CLL (3 patients) (Table 1). The latter 5 patients had received or were receiving chemotherapy, usually chlorambucil.

Five of the 11 patients with thrombocytopenia and increased membrane-bound IgG had positive direct antiglobulin (Coombs) tests on red cells. Three of these 5
Table 1. Clinical and Immunologic Studies of Patients With Elevated Platelet-Bound IgG and Lymphoproliferative Disorders

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age and Sex</th>
<th>Diagnosis</th>
<th>Onset of ITP*</th>
<th>Platelet Count (X 10^5/μl)</th>
<th>Platelet-Bound IgG (μg/platelet)</th>
<th>Total WBC (X 10^9/μl)</th>
<th>Percentage Lymphocytes</th>
<th>Direct RBC Antiglobulin Test</th>
<th>Evidence of Hemolysis</th>
<th>Serum γ-globulin</th>
<th>Treatment of Immune Thrombocytopenia</th>
<th>Response</th>
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<tr>
<td>1</td>
<td>58 F</td>
<td>CLL</td>
<td>34</td>
<td>1</td>
<td>Not done</td>
<td>5.2</td>
<td>47</td>
<td>+ + + +</td>
<td>Yes, Normal</td>
<td>Prednisone</td>
<td>Splenectomy, complete</td>
<td>Complete</td>
</tr>
<tr>
<td>2</td>
<td>70 M</td>
<td>CLL</td>
<td>2</td>
<td>10</td>
<td>760</td>
<td>22.3</td>
<td>87</td>
<td>+ + + +</td>
<td>Yes, Heterogeneous</td>
<td>Prednisone</td>
<td>Splenectomy, increase</td>
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<tr>
<td>3</td>
<td>58 M</td>
<td>CLL</td>
<td>24</td>
<td>16.3</td>
<td>Not done</td>
<td>24</td>
<td>92</td>
<td>+ +</td>
<td>No, Normal</td>
<td>Prednisone, splenectomy</td>
<td>Comple</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>48 M</td>
<td>CLL</td>
<td>22</td>
<td>2</td>
<td>861</td>
<td>13.1</td>
<td>54</td>
<td>-</td>
<td>No, Normal</td>
<td>Prednisone, chlorambucil</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>50 F</td>
<td>CLL</td>
<td>74</td>
<td>2</td>
<td>43</td>
<td>49</td>
<td>85</td>
<td>-</td>
<td>No, IGF spikes</td>
<td>Cytosar</td>
<td>Cyclophosphamide</td>
<td>Complete</td>
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<td>72</td>
<td>100</td>
<td>41</td>
<td>42.4</td>
<td>97</td>
<td>-</td>
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<td>Prednisone, chlorambucil</td>
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<tr>
<td>7</td>
<td>58 F</td>
<td>CLL</td>
<td>26</td>
<td>26</td>
<td>Not done</td>
<td>66</td>
<td>85</td>
<td>+ + +</td>
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<td>Prednisone</td>
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<td>Complete</td>
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<td>CLL</td>
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<td>88</td>
<td>84</td>
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<tr>
<td>9</td>
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<td>4</td>
<td>14</td>
<td>49</td>
<td>75</td>
<td>-</td>
<td>No, Prednisone</td>
<td>None</td>
<td>Splenectomy, vinristine</td>
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<td>10</td>
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<td>CLL</td>
<td>24</td>
<td>80</td>
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<td>CLL</td>
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<td>85</td>
<td>21</td>
<td>18</td>
<td>57</td>
<td>-</td>
<td>No, Normal</td>
<td>None</td>
<td>Splenectomy, complete</td>
<td>None</td>
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<tr>
<td>12</td>
<td>55 M</td>
<td>H.D., M.C.</td>
<td>9</td>
<td>29</td>
<td>98</td>
<td>6.1</td>
<td>25</td>
<td>-</td>
<td>No, Normal</td>
<td>MOPP††</td>
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<tr>
<td>13</td>
<td>54 M</td>
<td>NHL, DMLH‡</td>
<td>22</td>
<td>24</td>
<td>240</td>
<td>4.5</td>
<td>40</td>
<td>-</td>
<td>No, Normal</td>
<td>Prednisone</td>
<td>Splenectomy, vinristine</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>71 M</td>
<td>NHL, DPDL‡</td>
<td>46</td>
<td>1</td>
<td>Not done</td>
<td>15</td>
<td>6</td>
<td>-</td>
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<td>Prednisone</td>
<td>Cyclophosphamide, vinristine, Predn</td>
<td>Complete</td>
</tr>
<tr>
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</table>

*Onset of ITP: minus indicates months prior to diagnosis of CLL or lymphoma; plus indicates months after diagnosis of CLL or lymphoma; no sign indicates concurrent onset of ITP and CLL or lymphoma.

†Hodgkin’s disease, mixed cellularity.

‡Non-Hodgkin’s lymphoma, diffuse, mixed histiocytic-lymphocytic.

§Non-Hodgkin’s lymphoma, diffuse, poorly differentiated.

¶Test performed 1 wk after high-dose prednisone.

**Platelet count returned to normal without any specific therapy.

††Nitrogen mustard, vinristine, procarbazine, prednisone.
patients had immune hemolytic anemia of the warm antibody type. Two of these had immune hemolytic anemia and thrombocytopenia simultaneously.

None of the patients had hypogammaglobulinemia. Two patients had a heterogeneous increase in \( \gamma \)-globulin, and one had a monoclonal increase in IgM.

One patient had moderate elevation of membrane-bound IgG and a normal platelet count at presentation. He later developed a marked increase in his membrane-bound IgG and severe thrombocytopenia, with many megakaryocytes in the bone marrow. Treatment with cyclophosphamide and prednisone corrected both abnormalities (Fig. 2).

One patient with Hodgkin disease and 2 patients with non-Hodgkin lymphoma had thrombocytopenia with elevated membrane-bound IgG (Fig. 1 and Table 1). When first seen, the patient with Hodgkin's disease had both active Hodgkin's disease and thrombocytopenia. Treatment with corticosteroids, splenectomy, and multiagent chemotherapy was unsuccessful in controlling either the lymphoma or the thrombocytopenia. Both of the patients with non-Hodgkin lymphoma required successful treatment of their malignancy before platelet counts and membrane-bound IgG returned to normal levels.

**DISCUSSION**

We have described 9 patients with CLL and 3 patients with lymphoma who had thrombocytopenia with elevated membrane-bound IgG. These patients had megakaryocytes in the bone marrow, small spleens, and no other illnesses (i.e., SLE, infection, drugs) associated with immune thrombocytopenia. Immune destruction may be suspected when these findings are present. In these patients the demonstration of an elevated membrane-bound IgG is further evidence of this mechanism, since previous reports have established the relationship between membrane-bound IgG and immune destruction of platelets. Furthermore, the correlation between the amount of membrane-bound IgG and the platelet count, both at presentation and during therapy, strongly supports both in these patients and in patients with ITP that membrane-bound IgG is responsible for the destruction of the platelet.

The occurrence of apparently immune thrombocytopenia did not correlate with
onset of the CLL or with the level of the peripheral lymphocyte count, since it was seen at the time of diagnosis of CLL in 3 patients, 7 yr prior to onset of CLL in 1 patient, and 2–7 yr after diagnosis in the remainder of the patients. The total white blood cell count at the time of thrombocytopenia in these patients varied from 6450/cu mm to 89,000/cu mm.

Thrombocytopenia associated with antibody is similar to immune hemolytic anemia in patients with CLL,\textsuperscript{9,10} and immune destruction of red cells and of platelets at the same time (the Evans-Duane syndrome\textsuperscript{11}) occurs frequently in patients with CLL.\textsuperscript{2,3} Five of our patients had positive direct antiglobulin (Coombs) tests on red cells, and 3 patients had associated hemolysis. Two of the episodes of hemolytic anemia occurred with thrombocytopenia.

The clinical syndrome of apparent immune thrombocytopenia in other lymphoproliferative disorders, particularly Hodgkin disease, has been described.\textsuperscript{12-15} We have documented in 3 patients that the platelets in such patients may have elevated platelet-bound IgG. Since immune hemolytic anemia is also seen in these patients, the underlying immunologic abnormalities may be similar to those in CLL.

Eight of the 9 patients with ITP and CLL responded to therapy with consequent diminution of membrane-bound IgG; this is usually observed in patients with “primary” ITP.\textsuperscript{6,7} This may occur even though the peripheral lymphocyte counts and the bone marrow do not return to normal. In patients with Hodgkin’s disease or lymphoma, normal platelet count and normal membrane-bound IgG did not occur until the underlying lymphoma was successfully controlled with chemotherapy; a larger group of these patients must be examined to establish this difference.

Identification of the cause of thrombocytopenia in CLL has important implications for its therapy. As in ITP, high-dosage prednisone is the initial treatment of choice for immune thrombocytopenia complicating CLL, whereas thrombocytopenia secondary to disease progression requires treatment of the primary disease.

Thrombocytopenia has been used as an indicator of limited prognosis in patients with CLL. In the group studied by Rai et al.,\textsuperscript{1} patients with CLL who developed thrombocytopenia had a median survival of 12 mo. Only 1 of our patients with apparent immune thrombocytopenia has died (from the effects of sudden severe thrombocytopenia). The median time of follow-up for the group is 16 mo, with a range of 8 to 103 mo. From this we conclude that thrombocytopenia on an immune basis does not appear to have the same dire prognostic significance as that noted by Rai et al.

This study emphasizes the association between immune thrombocytopenia and lymphoproliferative disorders. Although our population of patients is somewhat selected in that patients with complications of CLL are more likely to be followed at a referral center, nine new cases of immune thrombocytopenia and CLL were evaluated over a 2-yr period. Immune thrombocytopenia may be a more common cause of thrombocytopenia in lymphoproliferative diseases than has been previously reported. Thrombocytopenia from this cause does not seem to have the adverse prognostic significance of thrombocytopenia caused by other effects of the disease or its treatment.

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