Platelet-Associated IgG in Patients With Lymphoma

By Aron W. Berkman, Thomas Kickler, and Hayden Braine

Levels of platelet-associated IgG (PA-IgG) were studied in 72 patients with Hodgkin’s (HD) and non-Hodgkin’s lymphoma (NHL). Thirty-nine percent of patients with HD and 20% of patients with NHL had elevated PA-IgG levels. There was a positive correlation between disease activity and the presence of PA-IgG in HD and NHL. In patients with HD, PA-IgG strongly correlated with extent of disease and may serve as a marker of disease activity. PA-IgG may have facilitated platelet destruction in 5 of 11 thrombocytopenic patients with HD and increased PA-IgG and in 2 patients with HD and increased PA-IgG who developed severe thrombocytopenia when treated with chemotherapy.

RESULTS

Patient Selection and Characteristics

Seventy-two consecutive patients who had a diagnosis of Hodgkin’s disease or non-Hodgkin’s lymphoma on the inpatient and outpatient services of the Johns Hopkins Oncology Center, between July 1, 1981, and February 1, 1982, were screened for the presence of PA-IgG. The data obtained in these patients were compared to data obtained previously on a group of hospitalized patients in the Johns Hopkins Medical Institutions. In the comparison group of patients from the Johns Hopkins Medical Institutions, 52 had idiopathic immune thrombocytopenia. These patients had been prospectively referred for study with a clinical diagnosis of ITP based on standard criteria. Twenty-nine patients had thrombocytopenia from other known causes. Diagnoses in this group were: aplastic anemia—8, solid tumors with chemotherapy-induced thrombocytopenia—9, Laennec’s cirrhosis and hypersplenism—6, Wiscott-Aldrich syndrome—1, absent radius syndrome—1, thrombotic thrombocytopenic purpura (TPP)—2, paroxysmal nocturnal hemoglobinuria (PNH)—1, and preeclampsia—1. Fifty patients were nonthrombocytopenic with a variety of general medical illnesses (not including chronic inflammatory states or active infection).

The characteristics of the lymphoma patients studied are described in Table 1. Twenty-eight patients had Hodgkin’s disease (HD) and 44 patients had non-Hodgkin’s lymphomas (NHL). Patients were subclassified according to disease activity. Those patients in clinical remission or with minimal residual disease responding to chemotherapy were said to have minimal disease, whereas all other patients were classified as having active disease. Seventeen of 28 patients with Hodgkin’s disease and 25 of 44 patients with non-Hodgkin’s lymphoma had active disease. In the HD group, 4 had lymphocyte predominance, 7 had mixed cellularity, 15 had nodular sclerosis, and 2 were indeterminate. Four had stage I, 7 stage II, 9 stage 3, and 8 stage IV disease. In the group of patients with non-Hodgkin’s lymphoma, 11 were nodular poorly differentiated, 8 nodular mixed, 1 nodular histiocytic, 5

MATERIALS AND METHODS

Platelet-Associated IgG Assay

Platelet-associated IgG was measured by a modification of the radiolabeled antiglobulin test described by Cines and Schreiber. Whole blood was collected in 5% EDTA, and platelets were separated according to standard technique within 1–2 hr. Leukocyte contamination was less than 5%, as measured by microscopy. Platelets (10⁶) were incubated for 30 min at 37°C with 125I-goat anti-human IgG, washed, and assayed for radioactivity. PA-IgG was expressed as an index of radioactivity in the test sample divided by the average of three negative controls, each run in duplicate. A positive PA-IgG index was defined as greater than 1.7 (3 standard deviations above the mean of the controls).

From the Johns Hopkins Oncology Center and the Johns Hopkins Hospital, Baltimore, MD.

Supported in part by Grant CA-06973 of the National Institutes of Health.

Submitted April 21, 1983; accepted November 1, 1983.

Address reprint requests to Dr. Aron W. Berkman, Johns Hopkins Oncology Center, 600 North Wolfe Street, Baltimore, MD 21205.

© 1984 by Grune & Stratton, Inc.

0006-4971/84/4/6006-4971(304-0031$03.00)0
Table 1. Characteristics of Patients Screened for Presence of Platelet-Associated IgG

<table>
<thead>
<tr>
<th></th>
<th>Hodgkin’s Disease</th>
<th>Non-Hodgkin’s Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>28</td>
<td>44</td>
</tr>
<tr>
<td>Age (range) (yr)</td>
<td>40.9 (20-76)</td>
<td>57.2 (29-85)</td>
</tr>
<tr>
<td>Male/female</td>
<td>14/14</td>
<td>24/20</td>
</tr>
<tr>
<td>White/black</td>
<td>26/2</td>
<td>38/6</td>
</tr>
<tr>
<td>Active disease</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Remission</td>
<td>11</td>
<td>19</td>
</tr>
</tbody>
</table>

Platelet-Associated IgG Levels in Various Groups of Hospitalized Patients

Figure 1 demonstrates the range of values for PA-IgG indices in control groups of hospitalized thrombocytopenic and nonthrombocytopenic patients, and in patients with idiopathic ITP. Results are expressed with a mean and standard deviation for each group. The group of patients with immune thrombocytopenia had the highest levels of PA-IgG, with a mean index of 5.8 compared to 1.02 and 1.1 for groups of hospitalized patients without thrombocytopenia and with thrombocytopenia of nonimmune etiology, respectively.

Platelet-Associated IgG Levels in Patients With Lymphoma

Figure 1 also demonstrates the range of PA-IgG indices in the group of 72 patients with lymphoma. The mean index for this group was 1.63 (σ = 1.38). Twenty of the 72 patients (28%) had elevated levels of PA-IgG. This included 11 of 28 patients with Hodgkin’s disease (39%) and 9 of 44 patients with non-Hodgkin’s lymphomas (20%).

Platelet-Associated IgG Levels in Patients With Hodgkin’s Disease

Figure 2 illustrates the relationship among disease activity, extent (stage) of disease, and PA-IgG levels in patients with Hodgkin’s disease. Of the 11 patients with Hodgkin’s disease and elevated levels of PA-IgG, all had evidence of active disease. Three of these patients were newly diagnosed, two had just relapsed, four had progressive disease, and two were early in the course of chemotherapy. There was a strong correlation between the presence of elevated levels of PA-IgG and advanced stage of disease. Ten of the 11 positive patients had stage III or IV disease, and all 7 patients with active stage IV disease were positive. Figure 3 illustrates the distribution of patients according to histologic subtype. All positive patients had either mixed cellularity or nodular sclerosing subtypes, although no patients with lymphocyte depletion subtype were studied.

In order to determine the relationship between disease activity and PA-IgG levels in individual patients, serial determinations were performed in six patients who were initially positive (Fig. 4). In five of six patients, PA-IgG index normalized during the course
HISTOLOGIC SUBTYPE

Fig. 3. Relationship between histologic subtype and PA-IgG index in patients with Hodgkin’s disease. (•) Active disease, (○) no active disease, (shaded region) normal PA-IgG levels.

of chemotherapy as clinical remission occurred. In the sixth patient, active disease persisted, as did abnormal levels of PA-IgG. In addition, two patients who were believed to be in remission were noted to have elevated PA-IgG levels and later found to have relapsed (one in spleen and one in liver).

Patients with elevated levels of PA-IgG tended to have lower platelet counts, although the overall difference in platelet counts between PA-IgG positive and negative patients was not statistically significant (Fig. 5). Five of 11 patients with Hodgkin’s disease and elevated PA-IgG levels were thrombocytopenic. One of these patients (D.M. in Fig. 4) had resolution of thrombocytopenia and normalization of PA-IgG levels after splenectomy and chemotherapy. Two other patients had resolution of thrombocytopenia with chemotherapy, but subsequent PA-IgG levels were not obtained. Two others died with refractory Hodgkin’s disease, thrombocytopenia, and elevated PA-IgG. It should be noted that two of the six patients who had elevated PA-IgG levels but were not initially thrombocytopenic became profoundly thrombocytopenic (platelet count <50,000/cu mm) after chemotherapy was administered.

Only 4 of 11 patients with abnormal levels of PA-IgG had undergone prior splenectomy, as opposed to 10 of 16 patients with normal levels (Fig. 5). Spleens were present in each of the 5 patients with positive levels, who were also thrombocytopenic. One of the two patients who had elevated levels of PA-IgG and who became thrombocytopenic with chemotherapy had a spleen.

Platelet-Associated IgG Levels in Patients With Non-Hodgkin’s Lymphoma

A similar analysis was performed on the group of 44 patients with non-Hodgkin’s lymphomas. Although the overall incidence of elevated PA-IgG levels was lower in this group of patients than in the group of patients with Hodgkin’s disease, there still appeared to be a correlation between elevated levels and disease activity (Fig. 6). Seven of the eight patients with positive values had evidence of active disease. Conversely, 7 of 25 (28%) patients with active non-Hodgkin’s lymphomas had abnormal levels of PA-IgG.
(as opposed to 65% of patients with Hodgkin's disease).

Figure 6 also demonstrates that there appeared to be no consistent relationship between extent of disease in the non-Hodgkin’s lymphomas and levels of PA-IgG. In addition, there was no significant difference in platelet counts or incidence of thrombocytopenia in these groups of patients.

When broken down into histologic subtypes, individual groups were too small to analyze statistically. Eight of nine patients who were positive had a large cell component. Conversely, 6 of 17 (35%) patients, who had a large cell component and active disease, had elevated platelet-associated IgG levels.

**Associated Medical Illnesses**

It should be noted that most patients included in this study were ambulatory outpatients. Only one patient, D.M., had elevated PA-IgG levels and significant fever. She had advanced Hodgkin’s disease, and fever was felt to be secondary to her underlying disease. No other patient had documented infections, significant liver function abnormalities, or associated chronic inflammatory states.

**Comparison of Gamma Globulin Levels in Platelet-Associated IgG Positive and Negative Patients**

In order to assess the possibility that elevated serum immunoglobulin levels were responsible for PA-IgG positivity, serum gamma globulin levels were compared in positive and negative patients. Eight PA-IgG positive and 22 PA-IgG negative patients were studied. Each group had an identical mean gamma globulin level of 1.1 mg/dl. One PA-IgG positive patient with Hodgkin’s disease had a broad-based hypergammaglobulinemia (1.9 mg/dl). No other patient was hypergammaglobulinemic. Of the 30 patients in whom gamma globulin levels were compared, 10 had Hodgkin’s disease and 20 had non-Hodgkin’s lymphoma. Those studied were evenly distributed according to stage of disease.

**DISCUSSION**

This study indicates that there is a significant incidence of elevated levels of PA-IgG in patients with lymphomas. In patients with Hodgkin’s disease, there was a strong correlation between levels of PA-IgG and extent and activity of disease. When patients with Hodgkin’s disease and elevated PA-IgG levels were treated for their disease, PA-IgG levels uniformly normalized as tumor load diminished. These data suggest that PA-IgG may be useful as a marker for disease activity in patients with advanced Hodgkin’s disease. If elevated upon presentation, these levels may provide one indication of response to therapy. In addition, these data suggest that PA-IgG may be an early indicator of relapse if measured periodically in patients who have completed therapy.

The significance of PA-IgG in patients with non-Hodgkin’s lymphomas is less clear. As in patients with Hodgkin’s disease, the incidence of positive levels was significantly increased in patients with active disease, especially if a large cell component was present. There was, however, no similar correlation between levels of PA-IgG and extent of disease in these patients. One explanation for this observation could be that patients with non-Hodgkin’s lymphomas tend to be less precisely staged than their counterparts with Hodgkin’s disease. More data are needed on the changes in levels of PA-IgG as non-Hodgkin’s lymphoma is treated. Usefulness as a disease marker at this point is unknown.

Of considerable interest was that, although patients with HD and PA-IgG tended to have lower platelet counts, PA-IgG was not uniformly related to the presence of thrombocytopenia. A similar observation has been made in other groups of patients who have autoimmune disorders. It is possible that, in some patients, platelet destruction is increased by the presence of PA-IgG but marrow function is able to compensate. This is suggested by the finding that two patients with Hodgkin’s disease and elevated levels of PA-IgG developed severe thrombocytopenia when given myelosuppressive chemotherapy. PA-IgG levels may therefore be helpful as a predictor of those patients who may develop untoward chemotherapy-induced thrombocytopenia. It is likely that PA-IgG contributed to platelet destruction in the five patients.
who presented with active Hodgkin's disease and thrombocytopenia. Although two of these patients had bone marrow involvement and three had splenic involvement, ITP was considered on a clinical basis.

The presence of a spleen correlated (although not invariably) with PA-IgG levels in patients with Hodgkin's disease. Whether normal splenic tissue is required for the synthesis of PA-IgG or whether PA-IgG is synthesized by tumor cells, present in spleen, is unknown. However, it does appear that patients who still possess a spleen and who have elevated levels of PA-IgG are at a very high risk of developing thrombocytopenia.

The identity and origin of immunoglobulins bound to platelets in patients with lymphoma is still unclear. The IgG may have idiotypic specificity for the platelet, bind via Fc receptors, or bind nonspecifically to platelet surfaces. Alternatively, PA-IgG may be present as immune complexes, which are known to be present in patients with lymphoproliferative disorders and other malignancies. Such immune complexes may be specifically associated with Fc receptors or nonspecifically associated with platelets, which may in turn serve as a reservoir for their disposition. It should be noted that PA-IgG has been observed in patients with other tumors, as well as in patients with autoimmune or other systemic illnesses. As a result, it is unknown whether PA-IgG is, in this case, specifically produced by the tumor (i.e., malignant lymphocyte) or is a nonspecific entity produced by the host in response to the insult created by the tumor.

Disturbances in immune function and immunoregulation are known to exist in patients with Hodgkin's disease and other lymphomas. These patients can also develop antibodies that bind to red cells and neutrophils. Platelet-associated IgG may represent another manifestation of this disordered immunoregulation. In any event, the relationship of these molecules to basic biologic phenomena and clinical manifestations of disease is of considerable interest. Studies are in progress to further establish the usefulness of platelet-associated IgG as tumor markers in patients with lymphoma and on effects of these molecules on platelet survival.

REFERENCES

Platelet-associated IgG in patients with lymphoma

AW Berkman, T Kickler and H Braine