Positive Coombs Test in Hodgkin’s Disease: Significance and Implications

By Alexandra M. Levine, Phyllis Thornton, Stephen J. Forman, Philip Van Hale, Diane Holdorf, Charles L. Rouault, Darlene Powars, Donald I. Feinstein, and Robert J. Lukes

To clarify the clinicopathologic characteristics of Coombs’ positivity in Hodgkin’s disease, the records of 71 cases were reviewed. The direct Coombs test was positive in seven. Mean age of the seven was 22 yr (range 11–33). All were males. All had extensive disease (pathologic stage III or IV) and six had systemic (B) symptoms. Four had mixed cellularity; three had nodular sclerosis. The positive Coombs test was detected at original diagnosis in three and at time of relapse in four. Although all were anemic, only three had evidence of overt hemolysis. The antibody responsible for Coombs positivity was characterized in three and fulfilled the criteria for IgG anti-I. The presence of a positive direct Coombs test in the patient with Hodgkin’s disease suggests active and advanced disease. The presence of IgG anti-I may represent a unique antibody in the Coombs-positive hemolytic anemia associated with Hodgkin’s disease.

Although the presence of a positive direct Coombs test in patients with Hodgkin’s disease has been noted in the past,1,4 its significance has not been appreciated. We have recently evaluated seven patients with active Hodgkin’s disease and a positive direct Coombs test. Three of these patients had overt hemolytic anemia. Red cell eluates from three patients, including two with hemolytic anemia, were found to contain IgG anti-I. This antibody has been described in the past,5 and although distinctly unusual, its presence has been found in isolated cases of Hodgkin’s and non-Hodgkin’s lymphoma.6,8

Previous reports of Coombs positivity in Hodgkin’s disease6,9 were written prior to the development of modern concepts of pathologic classification, staging, and therapy. We have therefore reviewed our recent experience, prospectively and retrospectively, in order to determine the significance of this finding. As suggested by Eisner,1 and recently discussed by Rosenthal,10 our data confirm the hypothesis that the presence of a positive Coombs test in a patient with Hodgkin disease implies active and extensive disease.

MATERIALS AND METHODS

Seventy-one cases of Hodgkin’s disease, staged and treated at the Los Angeles County–University of Southern California Medical Center in the 10-yr period from 1968 through December 1978 had Coombs tests performed. The test was positive in seven patients, who form the basis of this report. Three of these cases were studied prospectively, and four were found from retrospective analysis of patient charts and blood bank records.

Standard serologic techniques were employed for all antibody testing. Eluates from fresh patient cells, obtained prior to institution of chemotherapy, were prepared by an acid eluate method using Gamma Elu-Kit (Gamma Biologicals, Houston, Texas). Adult I adult i, adult i, and cord i cells were obtained from commercial panels (Panocel 16, Pfizer Diagnostics, Groton, Conn. and Panel and Ficin-Panel, Gamma Biologicals, Inc., Houston, Texas). Fresh Cynomolgus monkeys erythrocytes were obtained from the Vivarium of the University of Southern California School of Medicine. The antisera used were commercially prepared anti-IgG, anti-C3d, and anti-broad spectrum (Ortho). Cells and eluates were tested by standard controlled blood bank procedures in immediate spin, 37°C albumin, and indirect globulin phases.11 All tests were performed in duplicate, were read by two independent observers, and were graded 0–4+.

Biopsy material from each of the seven patients was reviewed by two of us (P.V.H, R.J.L.), and was interpreted according to the Rye modification12 of the Lukes-Butler classification.13 All tissue specimens were fixed in B-5, and sections were stained by standard hematoxylin and eosin (H & E), methyl green pyronin (MGP), and periodic acid Schiff (PAS) techniques. Bone marrow biopsies and marrow tissue particles collected by aspiration were fixed in Zenker’s solution and processed according to the technique described by Lukes and Tindle.14

RESULTS

The clinicopathologic characteristics of these patients are summarized in Table 1. All of the patients were young males with extensive disease (stage III or IV) at the time of Coombs positivity. Six of the seven patients had systemic (B) symptoms. A positive direct Coombs test was detected at the time of original diagnosis in three patients (patients 1,3,6), and at relapse in the remaining four.

Three of the patients had nodular sclerosis, and four had mixed cellular Hodgkin’s disease. Involved lymph nodes from three patients revealed extensive plasmacytosis. Two of these nodes were from patients in relapse. Lymph node sections at the time of original diagnosis in these patients did not demonstrate increased plasma cell infiltration. The temporal relationship between Coombs positivity and plasmacytosis.

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Table 1. Clinicopathologic Characteristics at Time of Coombs Positivity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Pathologic Stage</th>
<th>Pathologic Type</th>
<th>Onset Coombs ++</th>
<th>Survival From Original DX</th>
<th>Survival From Coombs ++</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>M</td>
<td>IVB</td>
<td>Nodular sclerosis</td>
<td>Presentation</td>
<td>4 + mo</td>
<td>4 + mo</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>IVB</td>
<td>Mixed cellular</td>
<td>Relapse†</td>
<td>56 + mo</td>
<td>10 + mo</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>M</td>
<td>IVB</td>
<td>Mixed cellular</td>
<td>Presentation†</td>
<td>9 + mo</td>
<td>9 + mo</td>
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</tbody>
</table>

Patients studied prospectively

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Pathologic Stage</th>
<th>Pathologic Type</th>
<th>Onset Coombs ++</th>
<th>Survival From Original DX</th>
<th>Survival From Coombs ++</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12</td>
<td>M</td>
<td>IIIBS</td>
<td>Nodular sclerosis</td>
<td>Relapse</td>
<td>78 + mo</td>
<td>46 + mo</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>M</td>
<td>IVB</td>
<td>Mixed cellular</td>
<td>Relapse</td>
<td>32 mo</td>
<td>2 mo</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>M</td>
<td>IIIA</td>
<td>Mixed cellular</td>
<td>Presentation</td>
<td>53 + mo</td>
<td>53 + mo</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>M</td>
<td>IIIB*</td>
<td>Nodular sclerosis</td>
<td>Relapse†</td>
<td>163 + mo</td>
<td>133 + mo</td>
</tr>
</tbody>
</table>

Patients studied retrospectively

Hematologic data on these patients are presented in Table 2. Bone marrow revealed evidence of Hodgkin's disease in two patients. Three patients had active hemolytic anemia at the time of Coombs positivity (patients 2,3 and 7), as evidenced by elevated reticulocyte counts and elevated indirect bilirubin levels. The remaining four patients had stable hematocrits with normal reticulocyte counts and did not seem to have overt hemolysis, although red cell survival studies were not done.

The patients were treated in a variable manner. Patient 2 was initially treated for hemolysis with prednisone and vincristine. This resulted in resolution of the hemolytic anemia, although the Coombs test remained strongly positive. Subsequent therapy with full-dose nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP) led to gradual decrease and eventual disappearance of the positive Coombs test. Patient 3, with active hemolysis and a 12% reticulocyte count, underwent splenectomy for staging purposes. This led to a marked diminution of the hemolytic process, with reticulocyte counts ranging from 3% to 6%, although the Coombs test remained strongly positive. The patient was then begun on full-dose MOPP chemotherapy with return of hemoglobin to normal levels, amelioration of the underlying Hodgkin's disease, and disappearance of Coombs positivity. The third patient with overt hemolysis, patient 7, was treated with MOPP and experienced complete remission without a precipitous fall in hemoglobin level. Three of the remaining patients, without overt hemolysis, were treated with either MOPP or cyclophosphamide, vincristine, procarbazine, prednisone (COPP), resulting in a complete remission of underlying disease and a reversion to a negative Coombs test. The remaining patient (5), received combination chemotherapy, but died of sepsis and extensive disease 1 mo later. The survival for each patient is noted in Table 1.

The antibody responsible for the positive direct Coombs test was characterized serologically in the three patients studied prospectively. These results are

Table 2. Hematologic Data at Time of Coombs Positivity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (g/dl)</th>
<th>Reticulocytes (%) Corrected</th>
<th>Indirect Bilirubin (mg/dl)</th>
<th>LDH Units</th>
<th>Marrow Involvement With Hodgkin's Disease</th>
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<tbody>
<tr>
<td>1</td>
<td>9.6</td>
<td>1.5</td>
<td>0.2</td>
<td>567</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>7.1</td>
<td>2.6</td>
<td>1025</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>8.3</td>
<td>12.0</td>
<td>1.7</td>
<td>629</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>10.2</td>
<td>1.8</td>
<td>0.1</td>
<td>870</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>12.0</td>
<td>0.5</td>
<td>0.3</td>
<td>ND*</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>11.2</td>
<td>1.8</td>
<td>0.3</td>
<td>310</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>7.9</td>
<td>8.7</td>
<td>0.4</td>
<td>ND*</td>
<td>-</td>
</tr>
</tbody>
</table>

*ND, not done.
COOMBS TEST IN HODGKIN’S DISEASE

Table 3. Characteristics of Eluted Antibody*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Direct Coombs</th>
<th>Red Cells</th>
<th>IS</th>
<th>37°C</th>
<th>IDC†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 10/78</td>
<td>(4+)</td>
<td>0, cord i 0, adult l 0, adult i Monkey cells</td>
<td>0 0 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 3/78</td>
<td>(4+)</td>
<td>0, cord i 0, adult l 0, adult i Monkey cells</td>
<td>0 0 4+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 3/78</td>
<td>(3+)</td>
<td>0, cord i 0, adult l 0, adult i Monkey cells</td>
<td>0 0 &lt;W</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 3 8/78  | negative      | 0, cord i 0, adult l 0, adult i Monkey cells | 0 0 0 | 0 0
|         | (post chemo- |              |    |      |      |
|         | therapy)      |              |    |      |      |

IS, immediate spin; IDC, indirect Coombs.
* = Grading was 0–4 +.
† Tested with both broad spectrum and anti-IgG Coombs reagent, and found to react with IgG Coombs and not with anti-C3d reagent.

summarized in Table 3. The antibody eluted from the patients’ red cells appeared to be IgG, as shown by its reactivity with broad spectrum and anti-IgG, but not with nongamma anti-human globulin. The eluates agglutinated cord i cells, adult l cells, and adult i cells, but did not react against red blood cells of the Cynomolgus monkey, which serve as negative control for anti-I.D. The pattern of reactivity in patient 1 was consistent with that described by Booth et al. for anti-I.D. In patients 2 and 3, the eluates reacted with the appropriate cells, although the pattern was not one of decreasing reactivity. This pattern variation has also been described by Haфleigh et al. for anti-I.D. The Coombs antibody could not be characterized in the four retrospectively studied patients.

DISCUSSION

Anemia of varying severity is not unusual in patients with Hodgkin’s disease. Mechanisms contributing to anemia include decreased red cell production secondary to chronic disease, shortened red cell survival, marrow involvement by Hodgkin’s disease, and the effects of radiation and/or chemotherapy on the marrow. In 1967, Eisner et al. drew attention to the occurrence of immune hemolytic anemia in patients with Hodgkin’s disease, reporting an overall incidence of 2.7% Coombs positivity in a group of 219 patients. With one exception, all the patients in Eisner’s series had clinical stage III disease at the time of detection of the positive Coombs test. However, these patients were diagnosed prior to the general use of modern pathologic subtyping, routine surgical staging, or routine use of MOPP chemotherapy. It is therefore difficult to assess the significance of Coombs positivity with respect to histologic type, extent of disease, therapeutic response, or survival. In addition, the significance of a positive Coombs test in relation to activity of the underlying Hodgkin’s disease has not been addressed.

All of our patients had active Hodgkin’s disease at the time a positive Coombs test was first noted. In three cases, Coombs positivity was found at initial presentation, whereas it was detected at the time of recurrence in four. Coombs-positive hemolytic anemia occurred in two successive relapses in one patient (patient 7). Thus, our data would suggest that the development of a positive Coombs test in a patient with a history of Hodgkin’s disease may be an indication of relapse and should be investigated accordingly. Further, the Coombs test should be used as a parameter of disease activity in those patients who are Coombs positive at initial diagnosis. This contrasts to immune thrombocytopenia occurring in patients with Hodgkin’s disease, which appears to be unrelated to the activity of the underlying disease.

At the time of Coombs positivity, all patients were found to have stage III or IV disease, and six of the seven patients had systemic symptoms. Although there have been isolated reports of Coombs-positive hemolytic anemia in patients with limited stage Hodgkin’s disease, most cases have occurred in patients with extensive and symptomatic disease. Our current study would confirm that Coombs positivity implies widespread involvement in the patient with Hodgkin’s disease. However, in spite of extensive disease, the finding of a positive Coombs test does not necessarily imply a poor prognosis, as only one of our patients has died, and the others remain alive at a median of 53+ mo from diagnosis, with an average follow-up interval of 56 mo.

Various forms of therapy have been used to treat hemolytic anemia occurring in the presence of Hodgkin’s disease, and include, principally, splenectomy and corticosteroids. Splenectomy was effective in slowing the hemolytic process in one of our patients, while prednisone and vincristine were equally effective in a second patient. Neither of these therapeutic modalities resulted in conversion to a negative Coombs test. However, definitive therapy with either MOPP or COPP was associated with a gradual decline in the Coombs titer, and eventual disappearance of the positive Coombs test in our patients, without a precipitous fall in hemoglobin levels. Therefore, definitive therapy for the underlying Hodgkin’s disease, including the use of marrow-suppressive agents, appears to be neces-
sary to successfully treat the immune hemolytic anemia that may be seen in this setting.

The antibody associated with the positive direct Coombs test in our three prospectively studied patients was identified as IgG anti-I'. The Ii antigenic system of erythrocytes undergoes a series of changes in the course of neonatal and postnatal development. At birth and during the first 4 mo of life, the “i” antigen is predominant on erythrocytes, while in later life, a transition occurs, leading to the “I” antigen expression. Thus, cord cells are predominately “i” positive, while adult cells are “I” positive. The existence of the I' antigen was first demonstrated serologically by Booth et al., who described an IgM autoantibody that reacted strongest with cord i cells, weaker with adult I cells, and weaker still with adult i cells. Additionally, the antibody had no reactivity against red cells from the Cynomolgus monkey, which express the i antigen strongly. The pattern of reactivity of this antibody suggested that it was directed against the developing I antigen during its transition from i to I. Hence, it was termed the I transition (I') antigen. Although the transitional nature of I' in relationship to the development of the Ii system is only theoretical, Garratty et al., using fetal erythrocytes, did demonstrate that I' is a fetal antigen.

In 1972, Garratty et al. described the presence of an autoantibody with anti-I' specificity in an American patient with Hodgkin’s disease. The antibody in this patient was of the IgG class, was transient in nature, and was not associated with overt hemolysis. Garratty and his group extended their observations in 1974, reporting three additional patients with autoimmune hemolytic anemia secondary to the presence of an IgG antibody with I' specificity. Anti-I' was not found in 50 patients with Hodgkin’s disease who had negative Coombs tests, nor was it found in a group of 70 patients with Coombs-positive hemolytic anemia associated with a variety of underlying diseases, including non-Hodgkin’s lymphoma. The authors noted that although their series of patients was small, a possible relationship existed between autoimmune hemolytic anemia in Hodgkin’s disease and the anti-I' antibody.

Hafleigh et al. have recently described the presence of anti-I' of the IgG type occurring in three patients who did not have Hodgkin’s disease, although two had non-Hodgkin's lymphoma. None of these patients had evidence of shortened red cell survival. We also found one patient with anti-I' in whom overt hemolytic anemia could not be demonstrated, although the remaining two cases with anti-I' did have active hemolysis. Thus, while the underlying diseases associated with anti-I' may be more diverse than previously believed, to date, every case of anti-I' associated with hemolytic anemia has occurred in patients with Hodgkin’s disease.

The reason for the presence of anti-I' in some patients with Hodgkin’s disease remains speculative. Similar to carcinoembryonic antigen (CEA) elevation in patients with various solid tumors, it is possible that I' may represent the derepression of a fetal antigen on the red blood cell. Alternatively, anti-I' may arise as a consequence of cross-reactivity with an infectious agent. Based on epidemiologic characteristics, some investigators have suggested a possible infectious etiology in some patients with Hodgkin’s disease. Although intriguing, this hypothesis has not been proven. Thus, at the present time, the etiologic significance of anti-I' in the patient with Hodgkin’s disease remains unknown.

REFERENCES

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AM Levine, P Thornton, SJ Forman, P Van Hale, D Holdorf, CL Rouault, D Powars, DI Feinstein and RJ Lukes