Immunologic Aberrations in the Di Guglielmo Syndrome

By Harvey E. Finkel, Mark J. Brauer, Robert N. Taub and William Dameshek

It is well known that immunologic disturbances, particularly autoimmunity, are frequently associated with the chronic lymphoproliferative diseases. Indeed, it is sometimes difficult in such cases to be certain where the immunologic disorder ends and the neoplastic process begins. Included in the long list of autoimmune phenomena found in this disease group have been autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura (ITP), rheumatoid arthritis, thyroiditis, systemic lupus erythematosus, and the Sjogren syndrome. Malignant disease of nonhemic origin has also been occasionally marked by the presence of altered immunologic reactivity—for example, autoimmune hemolytic anemia associated with ovarian tumors, the high incidence of dermatomyositis in patients with carcinoma of the breast, stomach, and lung, and the occurrence of anergy in some patients with disseminated carcinoma.

On the other hand, a close relationship between disturbed immunologic mechanisms and the myeloproliferative disorders has not been apparent. Indeed, we have often stressed the great frequency of immunologic aberrations, including autoimmunity, in lymphoproliferative disorders, as opposed to the low incidence of these findings in the myeloproliferative diseases. In the last few years, however, we have been struck by the rather frequent occurrence of autoimmune phenomena and abnormally high concentrations of immunoglobulins in a series of patients with one particular type of myeloproliferative
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The Di Guglielmo Syndrome

During the period 1961–1965, 49 patients seen in this laboratory conformed to the diagnostic criteria of the Di Guglielmo syndrome as they have evolved since the early reports of Di Guglielmo.13–15 This condition is viewed as an acquired proliferative disorder of the entire bone marrow. The earliest clinical feature is usually refractory anemia accompanied by striking erythroid hyperplasia in the marrow, which may show partial or well-defined megaloblastosis, and which is marked by immaturity and other features indicative of a neoplastic disturbance of erythroid tissue (large nucleoli, nuclear-cytoplasmic dissociation, abnormal mitoses, vacuolization, sheets of cells approaching a syncitium, and bizarre, giant, often multinucleated primitive red cells). Chromosomal aberrations have been observed,16,17 and the normoblasts frequently contain a mucopolysaccharide which stains with periodic acid-Schiff reagent.15 In this, the stage of erythremic myelosis, enzymes involved in heme synthesis may be deficient2 and hemoglobin production is impaired,17 resulting in the accumulation of nonheme iron in the red cell precursors and the formation of “ringed” sideroblasts. As shown by studies of pigment metabolism, ferrokinetics, and of red cell survival time, the anemia results in part from decreased production and in part from an intramedullary hemolysis of the type seen in pernicious anemia (ineffective erythropoiesis).19 The erythroid dysplasia is manifest in the circulating erythrocytes; they are often misshapen, and may be hypochromic and macrocytic. In time, abnormalities of the granulocytes and megakaryocytes appear, often of a subtle degree initially. Finally, the disease may progress through the stage of erythroleukemia, with a mixed proliferation of red cells and white cells, and eventually terminate as myeloblastic leukemia.21 The total course may vary from that of an acute fulminant disease complicated by severe anemia, sepsis, and hemorrhage, to that of a smoldering, chronic, mild anemia lasting many years. The acquired, self-perpetuating nature of this disease; the indications of a new “race” of red cell precursors; the gradual evolution in many cases to frank myeloblastic leukemia; and the lack of appreciable response to many forms of therapy—has led us to consider that it is basically neoplastic in character.22 We have further speculated that a fundamental defect in DNA or nucleoprotein metabolism might be present.23

It is important to recognize that the picture of refractory sideroblastic anemia may be associated with a number of other conditions, congenital and acquired, intrinsic to the bone marrow as well as secondary manifestations of a variety of pathologic processes. Thus, one must consider the possible presence of such diseases as thalassemia, hereditary pyridoxine-responsive anemia, lead
poisoning, chronic infection, uremia, and cancer, with the same effort that is applied to searching out potential causes of leukemoid reactions in leukemia suspects.

Report of Selected Cases

Of the 49 patients with the Di Guglielmo syndrome, six recently studied cases were selected for illustrative purposes, particularly with reference to the protein and immunologic abnormalities.

Case 1 (N.E.M.C.H. 25-9-60). B. F., a 57-year-old man, was admitted to New England Medical Center Hospitals on October 26, 1963, because of anemia. Three weeks earlier, weakness and angina prompted admission to another hospital; the Hb was 8 Gm./100 ml. and a bone marrow specimen was interpreted as erythroleukemia. He was given 3 units of blood. One week later anemia and angina recurred, and 2 attempts at transfusion resulted in severe reactions with hypotension and hemoglobinuria. At this time, direct and indirect Coombs tests were positive; the eluted antibody was found to have anti-Lewis activity. The patient was given prednisone; 20 mg. daily; for 4 days and transferred to this hospital for further evaluation.

Physical examination was within normal limits except for pallor. Hct was 29 per cent, WBC 2900/mm.³, with 44 per cent mature neutrophils, 6 per cent band forms, 40 per cent lymphocytes, and 6 per cent plasma cells. A bone marrow aspiration revealed erythroid hyperplasia and immaturity, and many myeloblasts. The serum iron was 202 μGm./100 mg., with a total iron binding capacity of 260. The direct Coombs test was positive; the eluted antibody showed anti-D specificity. Since the patient had previously been typed as D, it was felt that this represented a true autoantibody.

Five units of type-specific blood were administered. The hemoglobin rose and the angina was relieved. On the fourth hospital day, prednisone. 150 mg. daily, was begun. The patient was discharged on the twelfth hospital day and instructed to taper the prednisone dosage.

Three weeks later the WBC was 3750/mm.³, with 42 per cent neutrophils, 40 per cent lymphocytes, 2 per cent monocytes, 10 per cent myelocytes, and 6 per cent myeloblasts. The bone marrow now showed overwhelming myeloblastosis. The direct and indirect Coombs tests were negative; using undiluted reagent, dilute reagent, and enzyme-treated red cells. His condition rapidly deteriorated; the WBC increased to 30,000 (95 per cent myeloblasts) and was accompanied by thrombocytopenia and staphylococcal and fungal sepsis. The patient was treated with cyclophosphamide, 6-mercaptopurine, and multiple antibiotics. He expired 6 weeks later.

Comment: This patient, studied during the terminal phases of the Di Guglielmo syndrome, developed isoimmunity against transfused Lewis-A positive blood within one week of his first transfusions, with resultant severe hemolytic transfusion reactions. Soon thereafter, he developed an autoantibody which appeared to be suppressed by high doses of prednisone.

Case 2 (N.E.M.C.H. 269-618). H. G., a 68-year-old man, entered New England Medical Center Hospitals on February 15, 1965, because of increasing fatigue and anemia since 1960. He previously had been in good health. Treatment had included iron and various vitamins.

Physical examination revealed an active white male with increased pigmentation. There was no icterus, purpura, lymphadenopathy, or splenomegaly. The liver edge was palpable 4 cm. below the right costal margin. The remainder of the examination was within normal limits.

Hb was 9.6 Gm./100 ml., Hct 26 per cent, RBC 3.4 M/mm.³, WBC 4500/mm.³, with 33 per cent neutrophils. 59 per cent lymphocytes. 6 per cent monocytes, and 2 per cent
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myeloblasts. The reticulocyte count was 0.8 per cent, sedimentation rate 122 mm./hr., platelet count 70,000/mm.3. Peripheral blood smear showed anisocytosis, poikilocytosis, macrocytosis, enlarged and bizarre platelets, and immature granulocytes. Bone marrow was hypercellular with notable immaturity of the erythroid series. Numerous myeloblasts were present, as well as eosinophils with exceptionally large granules. Plasma cells were not increased, but there were relatively large focal collections of small lymphocytes. Megakaryocytes were diminished in number. Normoblasts contained PAS positive material, stainable iron was increased, and many ringed sideroblasts were noted. Serum iron was 211 μGm./100 ml. with full saturation. Liver function tests were normal. Serum albumin was 3.0 Gm./100 ml. and the globulins were elevated to 4.2 Gm./100 ml. Paper electrophoresis showed a broad-based elevation (34 per cent) of gamma globulin, which was confirmed by immunoelctrophoresis as IgG. Direct Coombs test was positive, the eluate reacting with E antigen. Rheumatoid factor and antithyroid antibodies were demonstrated in the serum. LE preparations were positive. Antinuclear antibody and leucocyte agglutinins could not be found. The Hinton test was positive in low titer (1:2); Reiter protein complement fixation test gave doubtful results. Cerebrospinal fluid was normal.

Therapeutic trials with pyridoxine, oxymetholone, prednisone, busulphan, and penicillin (for the possible latent syphilis) produced no change. Blood transfusion was the only effective means of correcting the anemia.

Comment: In addition to the Di Guglielmo syndrome, this patient showed several indications of abnormal immunity, including positive LE preparations, rheumatoid factor, antithyroid antibody, hypergammaglobulinemia, and an erythrocyte isoantibody. The possibility of latent syphilis must be considered; however, in view of the equivocal serologic findings and the other immunologic abnormalities, the Hinton test probably represented a biologic false positive reaction, and thus another indication of immunologic abnormality.

Case 3 (N.E.M.C.H. 171-091). F. K., a 71-year-old woman, was admitted to New England Medical Center Hospitals for evaluation of refractory anemia of 2 years duration. Previous treatment had included folic acid, vitamin B12, and iron. She had no symptoms suggestive of immunologic disease and had never received a blood transfusion. She had had two normal pregnancies, the last 40 years ago.

On physical examination the patient showed indications of moderate weight loss and was slightly pale. There was no jaundice, purpura, or lymphadenopathy. The liver was palpable 5 cm. below the right costal margin; the spleen extended 2 cm. below the left. The remainder of the physical examination was not notable.

Hb was 9.9 Gm./100 ml., Hct 35 per cent, RBC 3.5 M/mm.3, WBC 2900/mm.3, with 59 per cent mature neutrophils, 30 per cent lymphocytes, 8 per cent monocytes, 2 per cent eosinophils, and 1 per cent basophils. Reticulocyte count was 3.2 per cent. platelet count 137,000/mm.3, sedimentation rate 122 mm./hr. Peripheral blood smear showed macrocytosis, hypochromia, enlarged platelets, and rouleaux formation. Bone marrow was hypercellular with marked erythroid hyperplasia, immaturity, and partial megaloblastosis. Granulocytes were increased and there was a left shift. Moderate plasmacytosis was present. Red cell precursors contained PAS positive material. Bilirubin was 0.2 mg./100 ml., serum alkaline phosphatase 5.1 Bodalsky units, prothrombin time 18 seconds, BSP retention 10 per cent in 45 minutes. LDH and transaminases were normal. Total serum protein was 7.1 Gm./100 ml., 2.8 albumin and 4.3 globulin. On the electrophoretic pattern there was a marked increase in gamma globulin (32 per cent of the total protein). Immunoelectrophoresis showed increased IgG. Hinton test was positive, but only to a titer of 1:2. Reiter protein complement fixation test was negative. Indirect Coombs test was positive; the serum contained anti-Wright and anti-S antibodies, and a weak cold agglutinin. Direct Coombs test was negative, as were lupus preparations. Rheumatoid factor was present in the serum.

Further evaluation was unrevealing, and the patient's condition remained stable during the next 6 months.

Comment: This patient presented the hematologic picture of erythremic myelosis, per-
haps complicated by liver disease. From the immunologic standpoint there were several points of interest. These included plasmacytosis with hypergammaglobulinemia, a biologic false positive serologic test for syphilis, the positive latex fixation test, and the presence of rather unusual erythrocyte isoantibodies.

Case 4 (N.E.M.C.H. #170-521). W. D., a 59-year-old man, entered New England Medical Center Hospitals on March 27, 1965, because of anemia of 4 years duration. There was no history of exposure to marrow toxins. The anemia had not responded to oral iron, folic acid, pyridoxine, or to parenteral vitamin B₁₂. Progressive enlargement of the liver and spleen was observed between 1961 and 1965. The Hb dropped as low as 3.5 Gm./100 ml., and the patient had received 10 blood transfusions prior to the present admission.

Physical examination revealed marked pallor but no icterus, purpura, or lymphadenopathy. The liver was palpable 8 cm. below the right costal margin, the spleen 5 cm. below the left.

Hb was 8.0 Gm./100 ml., Hct 25 per cent, RBC 2.2 M/mm.³, WBC 9700/mm.³, with 61 per cent neutrophils, 7 per cent bands, 18 per cent lymphocytes, 9 per cent monocytes, 3 per cent eosinophils, and 2 per cent myeloblasts. Reticulocyte count was 2.6 per cent, sedimentation rate 20 mm./hr., platelet count 567,000 (indirect). Peripheral blood smear showed nucleated red cells, bizarre platelets, and misshapen and enlarged erythrocytes. Bone marrow aspirate was hypercellular, consisting largely of immature erythroid elements. Many nucleated red cells had megaloblastic features and contained large nucleoli. Evidence of panmyelosis was seen with a marked left shift of granulocytes and megakaryocytes. Some eosinophils contained huge granules. Lymphocytes and plasma cells appeared normal in number. The PAS stain of erythroid precursors was positive, stainable iron was abundant, and many ringed sideroblasts were noted. Leukocyte alkaline phosphatase score was 1. Serum iron was increased and serum proteins were normal. Liver biopsy disclosed extramedullary hematopoiesis with normal liver parenchyma. Rheumatoid factor was present in the serum, and LE preparations were positive. Other immunologic tests were within normal limits.

The patient's anemia remained refractory to therapy with prednisone and oxymetholone, and frequent transfusions were required.

Comment: This patient with chronic erythremic myelosis had positive LE and latex fixation tests without clinical counterparts.

Case 5 (N.E.M.C.H. #171-840). J. M., a 61-year-old man, was referred to New England Medical Center Hospitals for evaluation of anemia of 5 years duration. In 1959, pallor and weakness were noted and anemia was discovered. No cause for the anemia could be found; there was no response to iron, liver extract, folic acid, or vitamin B₁₂. The patient required several transfusions between 1959 and the present. Each of the last three was followed by the passage of dark, almost black urine and the occurrence of generalized myalgia. The most recent transfusion had been 2 months before admission.

Physical examination revealed only slight pallor. Hb was 10 Gm./100 ml., Hct 30 per cent. RBC 2.63 M/mm.³, reticulocyte count 3.8 per cent, platelet count 265,000/mm.³, sedimentation rate 70 mm./hr., WBC 7000/mm.³, with 59 per cent neutrophils, 2 per cent band forms, 30 per cent lymphocytes, 8 per cent monocytes, and 1 per cent eosinophils. Peripheral blood smear showed platelet clumping and red cell agglutination, macrocytosis and hypochromia, but no abnormal leukocytes. Bone marrow examination revealed hypercellularity with marked erythroid hyperplasia and immaturity, a tendency toward megaloblastosis, as well as scattered giant, bizarre, primitive red cells. Granulocytes were increased and generally immature. The eosinophils contained abnormally large granules. There was moderate plasmacytosis. Atypical megakaryocytes were present. Prussian blue stain revealed markedly increased iron and strikingly large numbers of ringed sideroblasts. Cytogenetic study of the marrow showed abnormally large chromosomes with numerous breaks and gaps. Serum iron was 180 μGm./100 ml., total iron binding capacity 242. Studies of vitamin B₁₂ and folate metabolism revealed no abnormality. Renal and hepatic function, plasma
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hemoglobin and serum bilirubin were normal. Crosby test was negative, and hemosiderin
was not found in the urine. Total serum protein was 7.6 Gm./100 ml., 3.2 albumin and 4.4
globulin. On the electrophoretic pattern, gamma globulin made up 34 per cent of the total
protein. Immunelectrophoresis showed the increase to be chiefly Ig G. Direct Coombs test
was positive; the eluate proved to be a panagglutinin with uniform potency. Indirect
antiglobulin test was also positive due to the presence of anti-C and anti-E antibodies, and
a cold agglutinin. Other immunologic tests were negative.

The patient was advised to take pyridoxine and folic acid. On follow-up examination, his
condition had not changed.

Comment: This patient with erythremic myelosis demonstrated hypergammaglobulinemia
and a positive direct Coombs test due to a nonspecific panagglutinin. In addition, abnormal
erthrocyte isoantibodies were found in the serum.

Case 6 (N.E.M.C.H. #120-312). C. G., a 65-year-old woman, was
admitted to New England Medical Center Hospitals in November 1965 because of anemia. Recurrent epi-
isodes of fever, polyarthritis, pleuritic chest pain, and a sun-sensitive malar eruption began
in 1956. Leukopenia, anemia, an elevated sedimentation rate, and positive LE preparations
were found. Peripheral blood smear showed anisocytosis and poikilocytosis. macrocytosis
and hypochromia, and nucleated red blood cells. Prednisone was administered in varying
doses during the next 9 years, depending upon the activity of the systemic lupus.

During this time, periodic transfusions were required for increasingly severe anemia.
Bleeding and hemolysis were ruled out by appropriate investigations. The patient became
progressively weaker and was admitted for evaluation and treatment.

Physical examination revealed a very pale, feeble woman with an erythematous rash on
the bridge of the nose and both malar areas. Multiple ecchymoses were present. There was
no jaundice, lymphadenopathy, or hepatosplenomegaly.

Hb was 3.7 Gm./100 ml., Hct 13 per cent. RBC 1.56 M/mm.³, platelets 75,000/mm.³.
reticulocytes 0.8 per cent, sedimentation rate 30 mm./hr. WBC was 1950/mm.³, with 16
per cent mature neutrophils, 8 per cent bands, 54 per cent lymphocytes, 8 per cent
monocytes, 2 per cent plasma cells, 6 per cent metamyelocytes, 4 per cent myelocytes, and
2 per cent promyelocytes. Several nucleated red blood cells were noted on the peripheral
smear. In the bone marrow there was marked erythroid hyperplasia with large numbers of
immature, megaloblastic, multinucleated cells containing prominent nucleoli and numerous
cytoplasmic vacuoles. Mitoses were abnormal, and the maturation of nuclei and cytoplasm
were asynchronous. Granulocytes were abundant and generally immature. Erythroid cells
stained with PAS. Bone marrow iron was greatly increased and there were many ringed
sideroblasts. Renal function and serum proteins were normal. Serum iron was 150
µGm./100 ml., total iron binding capacity 177. Lupus preparations and tests for antinuclear
antibody and rheumatoid factor were positive. Coombs test was negative.

Transfusions, pyridoxine, and anti-inflammatory agents were administered, but with no
more than temporary benefit.

Comment: Unlike the other patients in this series who demonstrated only in vitro
manifestations of autoimmunity, this patient with erythremic myelosis showed a clinically
important autoimmune disease—i.e., systemic lupus erythematosus. Definite signs of primary
bone marrow dysfunction had been present for at least 9 years. As the hematologic
disease progressed, indications of the lupus erythematosus lessened in severity.

RESULTS OF IMMUNOLOGIC STUDIES

Of the group of 49 patients with the Di Guglielmo syndrome, 17 manifested
at least one distinctly unusual immunologic feature, including the presence of
various antibodies and of hypergammaglobulinemia. The results are sum-
marized in Table 1. Eleven of the 37 patients who had adequate studies of
serum proteins showed hypergammaglobulinemia, defined by a concentration
of 1.6 Gm./100 ml. or higher. The distribution of the gamma globulin levels in
Table 1.—Summary of Immunologic Findings in 49 Cases of the Di Guglielmo Syndrome

<table>
<thead>
<tr>
<th>Immunologic Finding</th>
<th>Number of Patients Positive/Number Studied</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>17/49</td>
</tr>
<tr>
<td>Hypergammaglobulinemia</td>
<td>11/37</td>
</tr>
<tr>
<td>Erythrocyte autoantibodies</td>
<td>4/38</td>
</tr>
<tr>
<td>Erythrocyte isoantibodies</td>
<td>5/38</td>
</tr>
<tr>
<td>LE factor</td>
<td>4/15</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>8/22</td>
</tr>
<tr>
<td>Others: Antithyroid antibody</td>
<td>2</td>
</tr>
<tr>
<td>Biologic false positive STS</td>
<td>2</td>
</tr>
<tr>
<td>Leukoagglutinin</td>
<td>1</td>
</tr>
<tr>
<td>Hemoglobin H</td>
<td>2</td>
</tr>
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</table>

our patients is plotted in Figure 1. Immunelectrophoretic determinations were performed on 15 patients;* in those showing increased levels of gammaglobulin, the Ig G fraction was consistently elevated.

Erythrocyte autoantibodies were found in four patients (one nonspecific panagglutinin, two with anti-D specificity, and one with anti-E specificity), and isoantibodies were found in 5 of 38 patients appropriately tested. Lupus preparations were positive in 4 of 15 patients, and rheumatoid factor was present in the sera of 8 of the 22 patients in whom latex fixation tests were performed. Other signs of possible immune dysfunction were encountered in individual cases: these included antithyroid antibodies, leukoagglutinins, biologic false positive serologic tests for syphilis, and one case of preexisting pernicious anemia.

For purposes of comparison, similar immunologic abnormalities were searched for in 99 recently studied patients with other myeloproliferative diseases. The frequencies of their occurrence are listed in Table 2.

**DISCUSSION**

*Immunologic Abnormalities*

In general, the antibodies found were not associated with clinical counterparts. However, in several patients with positive direct Coombs tests, the hemolytic component present was probably on the basis of the erythrocyte autoantibody; and in Case 6, as noted above, systemic lupus erythematosus was an early manifestation. In addition, the presence of high concentrations of abnormal erythrocyte isoantibodies in some cases was important because of consequent transfusion reactions and difficulties in finding suitable blood donors.

It is notable that coexistent diseases that might conceivably stimulate immune responses were generally absent. There was, however, one case of liver disease, one of possible late latent syphilis, and (as described) one of systemic lupus.

*Performed through the courtesy of Dr. Robert S. Schwartz.*
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Fig. 1.—Distribution of serum gamma globulin levels.

Immunoglobins. Although dysproteinemia occurs in various neoplastic states, it has been reported to be an infrequent finding in myeloproliferative disorders. Waldenström states that chronic granulocytic leukemia is rarely a cause of gamma globulin disturbance;24 he cites reviews by Rundles25 and others to substantiate this. Polycythemia vera and myelofibrosis with myeloid metaplasia have also been considered normal in this regard.26

On the other hand, Riva,27 Wuhrmann and Wunderly,28 Miller,29 and Boggs and Fahey30 found gamma globulin elevations in many of their patients with granulocytic leukemia. These changes seemed to revert to normal during remission of the active disease. Andersen, studying the turnover of radioactively labelled gamma globulin in patients with chronic granulocytic leukemia, noted frequent elevations due to increased synthesis.31

Table 2.—Immunologic Findings in Other Myeloproliferative Diseases

<table>
<thead>
<tr>
<th></th>
<th>Myeloblastic leukemia</th>
<th>Chronic granulocytic leukemia</th>
<th>Myelofibrosis with myeloid metaplasia</th>
<th>Polycythemia vera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>1/24</td>
<td>7/23</td>
<td>8/24</td>
<td>2/28</td>
</tr>
<tr>
<td>Hypergammaglobulinemia</td>
<td>1/16</td>
<td>3/13</td>
<td>3/20</td>
<td>2/14</td>
</tr>
<tr>
<td>Erythrocyte autoantibodies</td>
<td>0/23</td>
<td>0/18</td>
<td>1/18</td>
<td>0/15</td>
</tr>
<tr>
<td>Erythrocyte isoantibodies</td>
<td>1/23</td>
<td>1/18</td>
<td>2/18</td>
<td>0/15</td>
</tr>
<tr>
<td>L.E. factor</td>
<td>0/4</td>
<td>0/1</td>
<td>0/5</td>
<td>0/3</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>1/7</td>
<td>4/7</td>
<td>3/11</td>
<td>0/4</td>
</tr>
<tr>
<td>Biologic false positive STS</td>
<td>0/24</td>
<td>0/23</td>
<td>0/24</td>
<td>0/28</td>
</tr>
</tbody>
</table>
The gamma globulin elevations were considerable in several of our cases: examples of three of these are shown in Figure 2. The almost identical electrophoretic patterns in these cases are of interest and may indicate similar immunologic reactions to similar abnormal antigens. That the gamma globulin abnormalities were not limited to the 7S constituents is indicated by the presence of the macroimmunoglobulins responsible for the positive serologic tests for syphilis and latex fixation tests.

**Erythrocyte Antibodies.** Of particular interest was the occurrence of positive direct Coombs tests in four of our patients, indicating that the red cells were
coated with globulin. The blood types of these patients had been determined before the Coombs tests became positive, as well as later in their courses. The antibody nature of these proteins was confirmed by demonstrating that after elution they would coat other red cells in a similar manner. Furthermore, the blood group antigens involved were identical with those of the patients; hence, we may view the coating proteins as autoantibodies, probably 7S gamma globulins.\textsuperscript{22} Autoantibodies of this type previously have been rarely observed in association with myeloproliferative disorders.\textsuperscript{33}

The erythrocyte isoantibodies were often present in high titer, and in two patients were associated with severe hemolytic transfusion reactions. On occasion, multiple antibodies were present in the serum of a single patient, and cross-matching was difficult. In three patients the isoantibodies appeared to be transfusion-induced, but in two instances their presence was difficult to explain. Thus, rather than being immunologically deficient, these patients were outstanding antibody producers.

On the other hand, the appearance of autoantibodies could not be equated with the presence of severe hemolysis. It is well-known that the coating of a red cell by antibody does not necessarily lead to its premature destruction. The "hemolytic effectiveness" or "avidity" of the antibody for its antigen is an important determinant of the degree of hemolysis.\textsuperscript{34}

\textbf{Lupus Factor.} In one of the four patients with positive lupus preparations (Case 6), clinical and laboratory evidence of both systemic lupus erythematosus and erythremic myelosis were well defined. Since evidence of similar immunologic abnormalities were found in several of our other patients, the coexistence of these two rare diseases in the same patient must be regarded as more than coincidental. In view of the long-standing refractory anemia not due to hemolysis or bleeding, and of the abnormalities initially found in the peripheral blood, it is likely that erythremic myelosis had been present at least as long as the overt lupus.

The lupus cell phenomenon is a diagnostic hallmark of systemic lupus erythematosus, a disease associated with a complex and poorly understood state of autoimmunity involving many tissues.\textsuperscript{35} Characteristic tissue injuries were not present in the other three patients in whom lupus cells were found. Although these patients certainly did not have systemic lupus in the usual sense, the occurrence of this factor in their sera indicates the presence of autoantibodies against DNA-histone nuclear complexes.\textsuperscript{36} These are 7S gamma globulins,\textsuperscript{37} produced either by an immune system with defective self-recognition, or as a response to exposure of "new" antigens. The speculative possibility that erythremic myelosis is based upon a neoplastic disturbance or alteration of DNA synthesis involving at least nucleated red cells may be of some relevance in this context. Changes in nuclear proteins undoubtedly are present in neoplastic cells (witness the frequent chromosomal changes); it is therefore conceivable that the entire cell may be altered antigenically as well as functionally, thus resulting in an autoimmune mechanism.

\textbf{Rheumatoid Factor.} The occurrence of so-called rheumatoid factor in the sera of several patients is of considerable interest and agrees with the observa-
tions of Caplan. This protein has been characterized as a heterogeneous 19S immunoglobulin capable of reacting with a variety of antigenic determinants on 7S gamma globulin molecules, be they heterologous, iso logous, or autologous, and is generally regarded as autoantibody. Alternative hypotheses have emphasized the protective role of this protein in its preferential reaction with altered gamma globulin, its interaction with potentially toxic immune complexes, and its anticomplementary activity.

The serologic similarity of human rheumatoid arthritis to syndromes produced by chronic immunization in animals suggests that rheumatoid factor may be an index of chronic antigenic stimulation. The frequent occurrence of this factor in our patients may be interpreted as a reflection of long-term antigenic stimulation by altered bone marrow tissue.

At this point, the findings in the other myeloproliferative groups (Table 2) should be considered. Virtually no immunologic abnormalities were detected in patients with myeloblastic leukemia, nor in those with polycythemia vera. The duration of disease in the former may be too short for the development of a recognizable immune response. In the case of polycythemia, the disease may be viewed as a benign neoplastic process not possessing sufficiently altered antigenicity, nor being sufficiently invasive to stimulate antibody production. On the other hand, a rather high incidence of rheumatoid factor, and a small but definite number showing elevated levels of gamma globulin, were found among patients with chronic granulocytic leukemia and myelofibrosis with myeloid metaplasia. These are both chronic diseases of intermediate malignancy and might be expected to behave immunologically in a manner somewhat similar to the Di Guglielmo syndrome. However, involvement of erythroid tissue is not prominent in these two states, and abnormalities related to neoplastic transformation of red cells—i.e., erythrocyte autoantibodies—would not be expected to occur with the same frequency, indeed, they do not.

Possible Immunopathogenetic Mechanisms

It is important to examine the abnormal antibodies in these cases with the intent to determine whether they represent an appropriate response of a normally functioning immune system to a markedly abnormal state involving nonimmunocytic tissue, or whether the immune system itself is pathologically involved in the Di Guglielmo syndrome. The apparent low frequency of histologic involvement of immunologically competent tissue, as manifested by lymphoid hypertrophy or plasmacytosis, does not necessarily exclude abnormal functioning. Although immunologic capacity was not tested prospectively in these cases, there is little to suggest any breakdown of immune function in the Di Guglielmo syndrome. In fact, many of these patients seem to be unusually good antibody producers. The clinical courses were not punctuated by frequent bacterial or viral infections, and abnormalities of delayed hypersensitivity were not encountered. Nevertheless, a fundamental, subtle immunologic deficiency predisposing to the development of bone marrow neoplasia cannot be excluded.

Since the Di Guglielmo state represents a generalized proliferative disorder of bone marrow, it is possible that bone marrow immunocytes also may be
involved in the pathologic process. We have observed apparently proliferating groups of abnormal reticulum cells (stem cells, immunoblasts) in several of our cases, and others have also speculated upon the relationship of the reticuloendothelial system to the Di Guglielmo syndrome. Thus, the immune system might respond autogenously, or in an exaggerated and perhaps nonspecific fashion, to usually ineffective antigenic material. Our own thinking regarding non-neoplastic myeloid and lymphoid reactions has been that the two are ordinarily not directly related and, in fact, may be reciprocal. For example, when myeloid tissue is unduly reactive, lymphoid activity may be reduced, as in acute "stress"; the reverse is often true as well.

A second possibility is that the proliferative activity of the neoplastic tissue may uncover previously "hidden" antigens. Similar in mechanism to antibodies formed against the lens of the eye and spermatozoa after trauma or autoinoculation, it is conceivable that hyperproduction and destruction of defective marrow cells serves to expose antigenic sites to the immune system for the first time. Thus, these antigens are not recognized as "self," and an antibody response ensues.

Finally, the immunologic abnormalities observed in our patients may be considered as perhaps induced by the altered antigenicity of malignant myeloid tissue. The appearance of hypergammaglobulinemia and autoantibodies might indicate that some element(s) of the neoplastic process had become antigenic, as if it were an allograft. The subject of tumor antigenicity, particularly with reference to the establishment and perpetuation of neoplasia, has been under study for a number of years. Specific tumor antigens have been demonstrated in a variety of ways, both in experimental animals and in naturally occurring human carcinomas and leukemia.

The mechanism by which neoplastic tissue becomes autoantigenic is presently controversial. Two possible mechanisms have been given considerable attention. The first, antigenic simplification or deletion as advocated by Potter suggests the loss of antigen from the transformed cell. The existence of such deletions has been confirmed in rodent tumors and human carcinomas and leukemias. Zilber has proposed that the change in antigenic material is the result of the action of certain carcinogens upon the genetic determinants of cells. Alternatively, antigenic "fortification" or "gain" may occur, with the addition of cellular protein causing a change in configuration, thus stimulating an immune response. Furthermore, as suggested by the work of Whitcutt and Elson, both the process of antigenic deletion and that of fortification may occur simultaneously. In any event, replicating altered antigenic components of a neoplastic process may become self-perpetuating initiators of immunologic activity and thus result in an autoimmune mechanism.

Specific studies in erythremic myelosis have revealed enzymatic abnormalities (heme-synthetase and delta-aminolevulinic acid dehydrase, and chromosomal aberrations, suggesting a mutagenic effect reminiscent of acute leukemia. These changes may occur at the earliest stage of the disease which is manifested only by erythroblast proliferation. Hemoglobin H was found in two of our patients and has been reported by Beaven et al. Others have observed blood group changes in patients with myeloproliferative diseases.
Thus, there is suggestive evidence that the neoplastic bone marrow tissue differs immunologically from its normal counterpart, either because of differences in surface antigens or in DNA, or both.

These changes in the structural, chemical, and genetic patterns in erythremic myelosis may indicate the presence of an antigenic difference which could then stimulate the development of an immune response. Immunologic defense mechanisms conceivably operate against naturally occurring human neoplasms. At their most effective, these defenses may destroy mutant cells before neoplasia becomes clinically apparent. There is presently no way of determining the frequency of such events, although the natural history of some neoplasms suggests the influence of immune processes. All too often, the defenses of the host are apparently insufficient to contain the malignancy. If misdirected, such immune responses may further compound the situation by producing potentially damaging reactions, which are evident both in the laboratory and in the host patient. Perhaps some of the unexplained systemic manifestations of malignant diseases are caused by immunologic aberrations, with resultant injury to many tissues distant from the neoplasm.

**Summary and Conclusions**

Immunologic studies were performed in 49 patients with the Di Guglielmo syndrome. Although altered immune reactivity has not been previously thought to be a feature of myeloproliferative disorders, more than one-third of the cases showed immunologic aberrations. The abnormalities encountered included overproduction of antibody protein (hypergammaglobulinemia) and an increased tendency to form rheumatoid factor, LE factor (including one case with overt systemic lupus), positive serologic tests for syphilis, and erythrocyte autoantibodies and isoantibodies.

Possible pathogenetic mechanisms are considered. The underlying neoplastic process might directly involve the immunocytes, resulting in exaggerated and nonspecific responses, or in defective self-recognition and thus in the production of autoantibodies. Alternatively, preexisting but "hidden" antigens might be exposed by the proliferative disorder, thus stimulating an antibody response. Finally, and perhaps most likely, antigenic alteration of bone marrow tissue might accompany its neoplastic transformation. Such tissue could be recognized as "not-self" or "foreign" by a qualitatively normal immune system. This would result in the production of abnormal proteins, some of which would be immunologically effective.

**Summario in Interlingua**

Esseva effectuate studios immunologic in 49 patientes con le syndrome de Di Guglielmo. Ben que in le passato il non esseva cognoscite que alterationes del reactivitate immunologic es un caracteristica de disordines myeloproliferative, plus que un terto del casos manifestava aberrations immunologic. Le anormalitates incontrate includeva un excesso del production de proteina anticorporee (hypergammaglobulinemia) con un augmentate tendentia a formar factor rheumatoide, le presentia de factor de LE (incluse un caso con
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patente lupus disseminate) positive tests serologic pro syphilis, e autoanticorpo e isoanticorpo erythrocytic.

Possible mechanismos pathogenetic es commentate. Le subjacente processo neoplastic affice, secundo un interpretation possibile, le immunocytos directemente, con le resultato de exaggerate e nonspecific responsas o de defectos del auto-recognition e assi del production de autoanticorpo. Secundo un altere interpretation, preexistente sed "occulte" antigeno es exponite per le disordine proliferative, resultante in le stimulation de un responsa anticorpo. Finalmente, ile existe le possibilitate—forsan le plus probable—that un alteration antigenic del tissu del medulla ossee accompania su transformation neoplastic. Tal tissu esserea recognoscite como "alien" o "non-native" per un qualitatively normal systema immunologic. Isto resultarea in le production de proteinas anormal, incluse certes de efficacia immunologic.

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IMMUNOLOGIC ABERRATIONS IN THE DI GUGLIELMO SYNDROME


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HARVEY E. FINKEL, MARK J. BRAUER, ROBERT N. TAUB and WILLIAM DAMESHEK

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