Autoimmune Thrombocytopenic Purpura ("ITP" Type) with Chronic Lymphocytic Leukemia

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THROMBOCYTOPENIA occurring during the course of chronic lymphocytic leukemia is usually due to involvement of the bone marrow by the primary disease.1-4 As such, it represents a serious complication of the terminal phase of leukemia in which the bone marrow is crowded with proliferating or invading lymphocytes, and megakaryocytes are lacking. Involvement of the spleen with resultant hypersplenism has also been cited as a possible mechanism for the thrombocytopenia.1,3,5

Recently, some attention has been given to occasional cases of chronic lymphocytic leukemia4,6,7 and lymphosarcoma5 with thrombocytopenia despite an abundance of megakaryocytes in the bone marrow. Dameshek and Gunz5 refer to two patients with "auto-immune thrombocytopenia" which occurred during the course of chronic lymphocytic leukemia and which responded to treatment with cortisone or prednisone. These cases resembled idiopathic thrombocytopenic purpura (ITP) for which there is some evidence of an autoimmune disturbance.9 The similarity of this situation to autoimmune hemolytic anemia, a rather common complication of the lymphoproliferative disorders,10 is striking. Recognition of this type of purpura and its differentiation from the thrombocytopenia of marked bone marrow infiltration and megakaryocytopenia have both therapeutic and prognostic significance.

The present report deals with five cases of chronic lymphocytic leukemia complicated by thrombocytopenia of the "ITP" variety, i.e. platelet reduction in the presence of abundant megakaryocytes in the marrow. In four of the cases, purpura was a presenting manifestation.

METHODS

Hemoglobin and hematocrit determinations, red cell, white cell and reticulocyte counts, and direct Coombs' tests were done by standard procedures.11 Platelet counts were done by the indirect method described by Dameshek.12 With this method the normal values for men are 500,000 to 900,000 platelets/mm.3 Bleeding due to thrombocytopenia may occur when platelets number less than 100,000/mm.3; bleeding is a regular occurrence when platelets are less than 50,000/mm.3

To prepare bone marrow smears, 2-3 ml. of marrow were aspirated from the sternum or iliac crest into a syringe containing a small amount of a solution of heparin (100 mg. per cent in normal saline.) Marrow particles were immediately transferred to glass slides and smeared between two slides. The slides were stained with Wright and Giemsa stains.

Platelet agglutinins were determined by the method described by Stefanini et al.13

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The platelet survival time was measured in Case 1 by the infusion of polycythemic platelet rich plasma as described by Stefanini and Dameshek; the normal for this method is 4-5 days. In Cases 3 and 5 it was measured by infusing homologous platelets labeled with chromium\(^{51}\) as described by Aas and Gardner\(^{15}\) with slight modifications; the normal for this method is 8-11 days.

Bone marrow smears of Cases 4 and 5 were fixed in cold 95 per cent ethyl alcohol and stained with horse anti-human globulin conjugated with fluorescein isothiocyanate which had been absorbed with mouse liver powder and normal human platelets. After washing with phosphate buffer, pH 7.2, the smears were examined for fluorescence with an ultraviolet microscope.\(^{17}\)

**Case Reports**

**Case 1, S. R. (NECH #23-307),** a 43-year-old white male, sought medical attention because of enlarged submental lymph nodes in July 1947. There was generalized lymphadenopathy of marked degree and greatly hypertrophied tonsils. The liver and spleen each extended two fingersbreadth below their respective costal margins. Blood hemoglobin was 12.9 Gm. per cent, hematocrit 44 p.r cent, red cells 4.22 million/mm\(^3\), reticulocytes 0.8 per cent, platelets 866,000/mm\(^3\), and leukocytes 29,300/mm\(^3\) with 87 per cent lymphocytes, 8 per cent neutrophils, 4 per cent monocytes and 1 per cent eosinophils. The lymphocytes were of the small mature variety. Chronic lymphocytic leukemia was diagnosed, and x-ray therapy was administered to the cervical, axillary, and inguinal regions. There was a reduction in the size of the nodes and the leukocyte count became reduced to 10,450/mm\(^3\) with 72 per cent lymphocytes.

In October 1947, the patient underwent surgery for acute cholecystitis without complications; five hundred ml. of blood were given during the operation. In May 1948, lymphadenopathy recurred, and, despite additional x-ray therapy, the lymph nodes, liver, and spleen continued to enlarge, and the patient lost weight. In August 1948, spray radiation was attempted but discontinued because of a severe systemic reaction. By December 1948, there were marked body wasting, pronounced enlargement of lymph nodes, and enlargement of the liver and spleen. Blood hemoglobin was 9.7 Gm. per cent, leukocytes 4,100/mm\(^3\) with 59 per cent lymphocytes, 36 per cent neutrophils, and 5 per cent eosinophils, and platelets were moderately reduced on the smear. In January 1949, without therapy, a dramatic and steady improvement began with gradual increase in body weight, diminution of size of spleen and liver, and reversion of the blood count to normal. For two and one-half years, the patient did well without treatment. In June 1950, lymph nodes, liver, and spleen again began to enlarge, and lymphocytosis of the blood reappeared. By April 1951, there was marked lymphadenopathy, the liver and spleen were three and four fingersbreadth below the costal margins, respectively, and the leukocyte count was 27,000/mm\(^3\) with 89 per cent lymphocytes. Blood hemoglobin was 13.6 Gm. per cent, and the platelets appeared normal on smear. At this time and in October 1951, the administration of small doses of triethylene melamine (TEM), namely 2.5 mg., caused severe systemic reactions. The first reaction was associated with a marked but transient thrombocytopenia, the second was not. Examination of the blood in November 1951, revealed the hemoglobin to be 14.0 Gm. per cent and leukocytes 24,000/mm\(^3\) with 77 per cent lymphocytes; platelets were normal on the smear.

One month later, epistaxis, hemoptysis, and hematuria developed. The patient was hospitalized, and examination revealed pallor, icterus, blood in the nose, and petechiae of the palate and ankles. The lymph nodes were generally enlarged, and the liver and spleen were grossly enlarged. Blood hemoglobin was 10.1 Gm. per cent, red cells 3.30 million/mm\(^3\), reticulocytes, 7.4 per cent, platelets 13,200/mm\(^3\), and leukocytes 62,000/mm\(^3\) with 91 per cent lymphocytes, 7 per cent neutrophils, and 2 per cent monocytes. The smear showed anisocytosis, poikilocytosis, polychromasia, and spherocytosis. Bone marrow smears were hypercellular and showed a marked lymphocytosis, normoblastic erythroid hyperplasia, and diminished granulopoiesis. Megakaryocytes appeared normal in morphology and number; there were 1-6 per low power field, uniformly on two slides. Tourniquet test was
2-plus. Platelet survival time was determined by infusion of platelet rich plasma; virtually all the platelets had disappeared from his circulation within three hours after infusion. Serum bilirubin was 1.8 mg. per cent of which 1.6 mg. per cent was indirect reacting. The direct Coombs' test was positive, and erythrocyte auto- and isoagglutinins were demonstrated at 37 C. The blood Hinton test was negative, and serum proteins were normal. Thus, both autoimmune hemolytic anemia and “ITP” were present.

Within one week after starting adrenocorticotrophic hormone (ACTH) therapy, 80-120 units daily, bleeding stopped and the platelet count rose to 141,000/mm.³. Therapy was continued with cortisone, 37.5-100 mg. daily, for ten months, during which time the patient felt well; his hemoglobin rose to 15 Gm. per cent, the leukocyte count dropped and remained under 50,000/mm.³, and platelets continued to be abundant. In November 1952, steroids were discontinued, and the platelet count rose to 141,000/mm.³. Therapy was continued with cortisone, 37.5-100 mg. daily, for ten months, during which time the bone marrow was devoid of megakaryocytes, and steroid therapy was ineffective.

In June 1953, he began to lose weight, developed intense pruritis, and showed increasing splenomegaly. The blood hemoglobin decreased to 12 Gm. per cent, leukocyte count rose to 77,400/mm.³ with 87 per cent lymphocytes, and the platelets were diminished in the smear. Cortisone, 100 mg. daily, did not improve the hematologic picture. In February, 1954, epistaxis disappeared, platelets disappeared from the blood smear, and the leukocyte count rose to 198,000/mm.³. The following month he developed herpes zoster. In September 1954, the blood hemoglobin was 7.9 Gm. per cent, red cells 2.5 million/mm.³, reticulocytes 3.3 per cent, leukocytes 164,000/mm.³ with 98 per cent lymphocytes, and platelets 25,000/mm.³. The bone marrow consisted almost entirely of lymphocytes; no megakaryocytes were seen. Therapy, including transfusions, x-ray, and cortisone, was without effect. In January 1955, compression fractures of two vertebrae occurred. In April 1955, the patient died.

Autopsy revealed generalized lymphocytic leukemic involvement of the viscera, severe osteoporosis with collapse of thoracic vertebrae, adrenal cortical atrophy, and hemorrhagic pneumonia. The bone marrow was amegakaryocytic.

Summary: After having had chronic lymphocytic leukemia for four and one-half years, this patient developed autoimmune hemolytic anemia and megakaryocytic purpura (“ITP”) with a platelet life span of less than three hours. These disturbances came on about two months after reactions induced by TEM. Both complications and the leukemia responded to ACTH and cortisone therapy. Three years later, when purpura recurred, the bone marrow was devoid of megakaryocytes, and steroid therapy was ineffective.

Case 2, A. C. (NECH #118-793), a 52-year-old white male was first seen in March 1958, with complaints of cutaneous and oral petechiae and ecchymoses for one week. An upper respiratory infection with occasional bloodstreaked sputum had been present for one month. For several days he had had diarrhea, and for many years infrequent episodes of heartburn which were relieved by antacids or milk. The patient appeared thin, pale, and chronically ill. There were petechiae of the skin and mucous membranes and a blood blister in the mouth. Generalized lymphadenopathy was present, with lymph nodes measuring from 0.5 to 1 cm. in diameter. The liver was not palpable, but the edge of the spleen was three fingersbreadth below the costal margin. The blood hemoglobin concentration was 10.6 Gm. per cent, hematocrit 34 per cent, red cells 3.69 million/mm.³, reticulocytes 2.9 per cent, platelets 3,690/mm.³, and leukocytes 377,000/mm.³ with 97 per cent lymphocytes, 2 per cent neutrophils, and 1 per cent blasts. Bone marrow smears were hypercellular and consisted almost entirely of sheets of small, mature lymphocytes. Megakaryocytes were numerous; there were 2-10 in nearly every low power field. Many of the megakaryocytes had agranular, vacuolated cytoplasm with no apparent platelet formation. The bleeding time was more than 15 minutes, the tourniquet test was 3-plus, and there was no prothrombin consumption (serum prothrombin was 98 per cent); no other coagulation defect was demonstrable. The diagnosis of chronic lymphocytic leukemia complicated by megakaryocytic thrombocytopenia purpura (“ITP”) was made.
Four days later, the patient was hospitalized. He had continued to have diarrhea, developing dark stools, and had noticed weakness, anorexia, exertional dyspnea, palpitations, and tightness in his chest. Except for tachycardia, the physical examination was unchanged. Guaiac test of the stool gave a 4-plus reaction. The blood hemoglobin had dropped to 4.6 Gm. per cent with reticulocytes of 15 per cent. The direct Coombs' test was negative, serum proteins were normal, and platelet agglutinins were not demonstrable in his serum. An upper gastrointestinal x-ray examination revealed deformity of the duodenal bulb and splenic enlargement. The gastrointestinal hemorrhage was attributed to thrombocytopenia superimposed on a chronic duodenal ulcer.

Treatment was begun with prednisone, 50 mg. daily; concurrently, peptic ulcer therapy and blood transfusions were given. Ten days later, gastrointestinal bleeding had ceased, and the platelet count had risen to 100,000/mm³. The dose of prednisone was gradually diminished, and there was no recurrence of bleeding. Eighteen days after treatment was begun, the platelet count was 261,300/mm³, and the leukocyte count had dropped to 85,300/mm³ with 87 per cent lymphocytes and 13 per cent neutrophils.

Because of the peptic ulcer, prednisone was rapidly decreased and discontinued. During the next six months the patient felt well and had no further bleeding. However, the spleen, liver, and lymph nodes gradually enlarged, and the leukocyte count slowly increased to 150,000/mm³ while the platelets continued to be plentiful in the blood smears. On October 17, 1958, treatment of the leukemia was begun with TEM, 2.5 mg. twice weekly for three weeks.

On November 18, 1958, he was re-hospitalized as an emergency measure with a two-day history of rapidly progressive weakness, icterus, and dark urine. He was disoriented, restless, pale, and icteric. Pulse rate was 148/min. and blood pressure 96/60 mm Hg. Small petechiae were scattered over the lower legs and feet. Lymph nodes were generally enlarged, the edge of the spleen was 3 cm. below the umbilicus, but the liver was not palpable. The hemoglobin concentration was 3.8 Gm. per cent. After transfusion with three units of washed erythrocytes, the blood hemoglobin was 6.4 Gm. per cent, red cells 1.51 million/mm³, reticulocytes 11 per cent, platelets 42,280/mm³, and leukocytes 407,650/mm³ with 90 per cent lymphocytes, 2 per cent monocytes, 4 per cent neutrophils and 4 per cent myelocytes. The blood smear showed, in addition, spherocytosis and occasional nucleated red cells. The bone marrow consisted of sheets of small mature lymphocytes with only scattered normoblasts and cells of the granulocyte series; megakaryocytes were present (0–5 per low power field), and they appeared morphologically normal with the exception of a few cytoplasmic vacuoles. The direct Coombs' test was strongly positive. The benzidine test was positive on both the sediment and supernatant of the urine. He had, at this time, developed severe autoimmune hemolytic anemia and recurrence of megakaryocytic thrombocytopenia. Treatment with transfusions of washed red cells, Dextran, and hydrocortisone did not improve his condition; heart failure developed, and he died 32 hours after admission to the hospital. Autopsy showed involvement of lymph nodes, liver, spleen and other organs by lymphocytic leukemia. Also, there were hemorrhages into the skin and the pelvis of the left kidney and bilateral pulmonary edema. Bone marrow sections, unfortunately, were not made.

Summary: The initial symptoms in this man were those of thrombocytopenic purpura. He was found to have chronic lymphocytic leukemia with involvement of the lymph nodes, spleen, and bone marrow; the marrow, nevertheless, contained many megakaryocytes. A platelet agglutinin was not demonstrable. Treatment with prednisone controlled bleeding and was associated with a striking increase in the platelet count and a temporary decrease in the lymphocytosis of the blood. When the leukemia progressed, the patient was given TEM therapy. Shortly thereafter he developed severe autoimmune hemo-
lytic anemia and recurrence of the thrombocytopenic purpura. Therapy at this time was without avail and the patient expired.

Case 3, T. S. (NECH # 124-536), a 57-year-old white male, was first seen in January 1959, because of a hemorrhagic condition. For two years there had been mild oral bleeding and slight epistaxis; the skin had bruised easily for about eighteen months. Dental repair work had produced severe gingival bleeding six months previously. There had been no other hemorrhagic manifestations or systemic symptoms. In 1954, the patient had undergone radical resection of the prostate gland for carcinoma without excessive bleeding; no evidences of metastases had been discovered at operation or subsequently. The patient looked well, but had gingival bleeding and numerous cutaneous ecchymoses and petechiae. The parotid and submaxillary glands were not palpable. Blood hemoglobin was 13.3 Gm. per cent, red cells 5.19 million/mm.³, and leukocytes 14,900/mm.³ with 53 per cent lymphocytes, 43 per cent neutrophils, and 4 per cent monocytes. Bone marrow aspiration revealed moderate hypercellularity, generalized lymphocytosis, and several foci of mature lymphocytes and lymphoblasts; the myeloid and erythroid series were normal. Megakaryocytes were abundant and uniformly distributed on five slides. Most low power fields contained 1–4 megakaryocytes with as many as 15; some appeared small, vacuolated, or non-productive of platelets. Tourniquet test was 2-pls, bleeding time was more than 15 minutes, and prothrombin consumption was 46 per cent of normal; other coagulation tests were normal. The serum demonstrated a platelet agglutinating factor. Blood sedimentation rate, direct Coombs' test, Hinton test, and serum proteins were normal. The diagnosis of chronic lymphocytic leukemia complicated by the Mikulicz syndrome and megakaryocytic thrombocytopenic purpura was made.

Within three days after starting treatment with prednisone, 100 mg. daily, mucosal bleeding ceased, and purpura diminished. One week later, however, epigastric distress, melena, and gingival bleeding occurred. No peptic ulcer was seen on gastrointestinal x-rays, and the symptoms responded to reduction of prednisone dosage, blood transfusions, and antacids. During the next ten weeks he was maintained on prednisone, 25–37.5 mg. daily; mild purpura continued, and the blood counts did not change. There was a striking diminution in the lymphocytes of the bone marrow, and megakaryocytes remained plentiful (1–10 per low power field). The platelet agglutination reaction continued to be positive.

Because of failure of prednisone to affect the purpura, manifested chiefly as marked gingival bleeding, Leukeran, 8 mg. daily, was given in combination with prednisone, 21.5 mg. daily, for four weeks. There was suppression of lymphocytosis in the blood but no change in the platelet count or bone marrow. After an additional month of prednisone therapy without improvement, the patient was hospitalized in June 1959, for splenectomy. Physical examination was unchanged; the trunk and extremities were covered with ecchymoses and petechiae, and blood blisters were present in the mouth. The liver, spleen, and lymph nodes were not enlarged. The blood hemoglobin was 10.8 Gm. per cent, reticulocytes 3.5 per cent, platelets 36,320/mm.³, and leukocytes 22,000/mm.³ with 51 per cent lymphocytes, 45 per cent neutrophils, 3 per cent monocytes, 1 per cent basophils. Coagulation studies were as before. The chromium⁵¹-labeled platelet survival time was less than three hours.¹ Blood sedimentation rate was 22 mm. in one hour. Coombs' test, serum bilirubin, fecal urobilinogen, and serum proteins were normal.

Splenectomy was performed on June 11, 1959. The spleen weighed 235 Gm.; there was diffuse infiltration of the red pulp with mature lymphocytes and polymorphonuclear leukocytes and atrophy of the Malpighian bodies. Liver biopsy showed a rare portal tract containing small numbers of lymphocytes. There was no improvement of the thrombocytopenia after splenectomy; two weeks after surgery, the platelet survival was as before, but the platelet agglutinin was no longer demonstrable. One week later, bleeding from mucous membranes recurred. Two months after splenectomy, the patient died from hemorrhage into
the peritoneum and gastrointestinal tract, peritonitis from gangrene of the bowel secondary to hemorrhage, and massive left hydrothorax. Sections of lymph nodes made at autopsy were consistent with treated lymphoproliferative disease. Bone marrow sections revealed lymphocytic infiltration and abundant megakaryocytes.

**Summary:** Presenting with chronic, mild bleeding from the oral and nasal mucous membranes, this patient proved to have chronic lymphocytic leukemia with severe thrombocytopenia. Although the marrow was involved by considerable lymphoid proliferation, megakaryocytes were abundant. An extremely short platelet survival time and a platelet agglutinin were demonstrated. There was no improvement from treatment with prednisone or splenectomy.

**Case 4, F. G.** (BRL #56-315), a 51-year-old white male, was found to have chronic lymphocytic leukemia and thrombocytopenia in May 1956. For three months he had had ecchymoses and petechiae of the skin and epistaxis after minimal trauma to the nose. Since childhood he had bruised easily and bled readily following slight trauma; however, an appendectomy at the age of 19 years had not been associated with excessive bleeding. When 43 years old he had had three myocardial infarctions, following which he had taken Dicumarol daily without difficulty. On four occasions during the previous thirteen years the patient had received courses of x-ray therapy; 375 r for chronic dermatitis of the lower abdomen in 1938, an undetermined amount for an infection of his right arm in 1941, 1200 r for folliculitis of the right axilla in 1943, and 225 r for bursitis of the left shoulder in 1947. The family history revealed the presence of coronary artery disease and diabetes mellitus. He was a healthy appearing, moderately obese man with ecchymoses of the abdominal wall and thighs and numerous petechiae on the shoulders and legs. Lymph nodes were generally enlarged. The liver and spleen were not palpable. The blood hemoglobin concentration was 15 Gm. per cent, red cells 4.8 million/mm.³, hematocrit 46 per cent, reticulocytes 0.4 per cent, platelets 106,000/mm.³, and leukocytes 18,700/mm.³ with 69 per cent lymphocytes, 30 per cent neutrophils, and 1 per cent eosinophils. Large, bizarre platelets were seen on the blood smears. Bone marrow aspiration revealed hypercellularity consisting primarily of sheets of small lymphocytes. Megakaryocytes were normal in number (0–4 per low power field); however, many contained vacuoles, and no platelet formation was evident. Direct Coombs' test was negative as were tests for serum hemolysins and red cell agglutinins. A platelet agglutinin was not demonstrable. The tourniquet test was positive, and the bleeding time was 14 minutes; other coagulation tests were normal. The diagnosis of chronic lymphocytic leukemia was confirmed; the hemorrhagic phenomena was attributed to thrombocytopenia in the presence of adequate numbers of megakaryocytes.

After one week of treatment with prednisone, 50 mg. daily, purpura had diminished, lymphadenopathy had regressed, the leukocyte count was 21,400/mm.³ with 34 per cent lymphocytes, but the platelets had dropped to 19,000/mm.³. Prednisone was increased to 100 mg. daily; nine days later lymph nodes were no longer palpable but the blood count remained unchanged. Mild purpura continued as the drug dosage was decreased to 10 mg. daily, but the bleeding tendency became worse when prednisone was temporarily discontinued. In March 1959, a respiratory infection was associated with enlargement of cervical lymph nodes and increasing lymphocytosis in the blood.

In May 1959, he was re-evaluated. He appeared well, but had petechiae and ecchymoses of the skin and mucous membranes. One lymph node was palpable in the neck, but the rest of the physical examination was normal. The blood hemoglobin concentration was 13.8 Gm. per cent, hematocrit 44 per cent, red cells 5.19 million/mm.³, reticulocytes 2.8 per cent, platelets 109,000/mm.³, and leukocytes 97,500/mm.³ with 91 per cent lymphocytes, 7 per cent neutrophils, 1 per cent monocytes, and 1 per cent myelocytes. The bone marrow was unchanged; it was crowded with small lymphocytes, and megakaryocytes were plentiful (0–11 per low power field). Blood sedimentation rate, direct Coombs' test, serum bilirubin
and serum proteins were normal; the serum globulin concentration was at the lower limit of normal. Again, no platelet agglutinin was demonstrable. The bleeding time was more than 15 minutes, tourniquet test was positive, and prothrombin consumption was impaired. The diagnosis remained as before, and treatment was continued with small doses of prednisone.

In July 1960, his clinical condition and blood counts remained essentially unchanged. The blood hemoglobin was 12 Gm. per cent, leukocytes were 152,500/mm.³ of which 90 per cent were lymphocytes, and the platelets were 78,200/mm.³ The bone marrow again revealed a sheet of small, mature lymphocytes; megakaryocytes were adequate, with as many as 13 per low power field. After staining the bone marrow with fluorescein-labeled anti-human globulin, the megakaryocytes were fluorescent, but the lymphocytes were not. Prior incubation of the marrow with non-fluorescent anti-human gamma globulin blocked subsequent staining of the megakaryocytes with the fluorescent material.¹⁷

Summary: This patient had mild chronic lymphocytic leukemia with megakaryocytic thrombocytopenic purpura for at least four years. He had neither splenomegaly nor a demonstrable platelet agglutinin. His megakaryocytes were, however, apparently coated with gamma globulin. Prednisone therapy reduced the amount of purpura but had no demonstrable effect on the blood platelet level.

Case 5., E. B. (NECH #138-302), a 76-year-old white male, was admitted to the hospital on December 22, 1960. He complained of fatigue and exertional dyspnea which had been of increasing severity for six months. He had lost ten pounds of weight, and for a few weeks had had night sweats and spontaneous ecchymoses without other evidence of bleeding. The patient denied other symptoms or significant previous illnesses.

Physical examination revealed pallor and scattered petechiae. Small cervical lymph nodes were palpable as was a 2 x 2 cm. left axillary node. The liver edge was 2 fingersbreadth below the right costal margin; the spleen filled the left upper quadrant of the abdomen. Vital signs were normal. Mild pulmonary emphysema with basilar rales and a grade-2 precordial systolic murmur were present.

The blood hemoglobin was 5.6 Gm. per cent, hematocrit 18 per cent, red cells 1.54 million/mm.³, reticulocytes 10.9 per cent, platelets 18,490/mm.³, and leukocytes 440,000/mm.³ of which 97 per cent were lymphocytes and 3 per cent neutrophils. The bone marrow was hypercellular and consisted almost entirely of small mature lymphocytes; however, megakaryocytes were easily found in the marrow, with as many as eleven megakaryocytes per low power field. Erythrocyte and granulocyte precursors were diminished in number. The direct Coombs' test was 2-plus positive. After staining the bone marrow with fluorescein-labeled anti-human globulin, there was no fluorescence of the megakaryocytes or lymphocytes.¹⁷ The serum bilirubin concentration was 1.8 Gm. per cent of which 1.6 mg. per cent was of the indirect-reacting type. Serum uric acid was slightly elevated at 7.1 mg. per cent; blood sugar, blood urea nitrogen and serum proteins were normal. A complete coagulation survey revealed only poor prothrombin consumption consistent with thrombocytopenia. An x-ray of the abdomen confirmed the splenic enlargement.

It was felt that this patient had chronic lymphocytic leukemia with both autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura.

After transfusion of two units of washed, platelet-free red cells, survival of isologous, chromium⁵¹-labeled platelets was determined. The survival time of these platelets was only 5–6 hours. Biopsy of the axillary lymph node yielded histologic evidence of lymphocytic leukemia or lymphosarcoma in that there was uniform replacement of the node by lymphocytes and lymphoblasts.

Treatment was begun with prednisolone, 50 mg. daily for four days, then 75 mg. daily. Two days after starting treatment the cervical nodes were smaller, and after six days
the spleen was definitely smaller. After eight days of therapy, the hemoglobin value stabilized at 6.5–7.0 Gm. per cent and the leukocyte count had risen from a pretreatment value of 360,000/mm.\(^3\) (99 per cent lymphocytes) to 564,000/mm.\(^3\) (98 per cent lymphocytes). However, no significant change had occurred in the platelet count. After 16 days of therapy, the hemoglobin had risen to 9.2 Gm. per cent, leukocytes had decreased to 334,500/mm.\(^3\) (99 per cent lymphocytes), and the platelets had increased to 94,720/mm.\(^3\)

**Summary:** This 76-year-old man presented with symptoms primarily of anemia. This was found to be of the autoimmune type associated with previously undiagnosed chronic lymphocytic leukemia. In addition, he had a minor bleeding tendency due to marked thrombocytopenia of the ITP type, i.e., megakaryocytes in the bone marrow and a very short platelet survival time.

**Discussion**

These five patients with chronic lymphocytic leukemia developed thrombocytopenic purpura. Instead of showing the customary picture of marked lymphocytic infiltration of the bone marrow with greatly reduced numbers of megakaryocytes, the megakaryocytes were abundant, as in typical examples of idiopathic thrombocytopenic purpura (ITP) (fig. 1). This finding appeared to exclude megakaryocytopenia as the cause of the thrombocytopenic state. Although marrow samples were not aspirated from several sites at one time, the cellular distribution was uniform on several slides prepared from each patient; Cases 2, 3, and 4 had comparable numbers of megakaryocytes at different times. Post-mortem sections of bone marrow confirmed the terminal absence

![Fig. 1.—Representative bone marrow smears showing numerous megakaryocytes in marrow which is otherwise crowded with lymphocytes. Original magnification x 100.](image-url)
of megakaryocytes in Case 1 and their abundance in Case 3. Three of the five patients had increases of platelet counts associated with adrenal cortical steroid therapy, indicating the presence of functional, or potentially functional, megakaryocytes. It appeared, therefore, that these patients differed from the conventional cases of thrombocytopenic purpura with leukemia in that they had plentiful numbers of platelet-forming cells in their bone marrows.

Hypersplenism due to involvement of the spleen by the leukemic process may be associated with thrombocytopenia. The presence of hypersplenism may be associated with thrombocytopenia. The presence of hypersplenism may be said to be indicated by: (1) splenomegaly, (2) isolated or multiple cytopenias, (3) cellular bone marrow and (4) remission of the cytopenias after splenectomy.19 Three of the five patients had grossly enlarged spleens; all presumably had leukemic involvement of the spleen whether palpably enlarged or not. All had low platelet counts, but other cytopenias were not present except for the anemia which was related either to blood loss or to increased hemolysis. The bone marrows were cellular in that there were abundant lymphocytes and megakaryocytes, but the diffuse hyperplasia of hypersplenism was not seen; this response, however, could have been prevented by the underlying proliferative disease. One patient was subjected to splenectomy after failure to improve with steroid therapy; no improvement in circulating platelet levels resulted from this procedure. In three cases prompt increases in platelet levels took place following steroid therapy, despite the continued presence of splenomegaly.

In three of our patients, the platelet survival time was demonstrated to be between two and six hours (fig 2). Such short survival times are almost invariably associated with thrombocytopenia of the ITP type3 and cannot be attributed solely to rapid utilization of platelets in platelet-depleted individuals, as patients with amegakaryocytic thrombocytopenia have been shown to have platelet survival times which are considerably longer.3 The rapid loss of platelets from the circulation implies an extrinsic mechanism acting to destroy platelets. Two possibilities for this mechanism are (1) hypersplenism and (2) an antiplatelet antibody. Both in man20,21 and in experimental animals,22-24 hypersplenism has been shown to produce primarily a marrow-suppressive effect with only slightly accelerated destruction of the involved blood cells. On the other hand, patients with uncomplicated idiopathic thrombocytopenic purpura, without splenomegaly, have had platelet survival times of only a few hours.3,25 The platelet survival times observed in the patients presented here are of the latter type.

Various agents used in the treatment of leukemia may, at times, be responsible for the induction of thrombocytopenia. X-ray, which was administered to two patients several years before development of thrombocytopenia, did not appear to be etiologically related to the platelet reduction. One patient (Case 1) received small doses of triethylene melamine (TEM) nine months and two months preceding the onset of thrombocytopenia; it was thus of possible etiologic significance. However, TEM toxicity is almost invariably associated with bone marrow damage and amegakaryocytosis, which were not seen in this patient. In Case 2, the time relationship between administration of
Fig. 2.—Survival of fresh, chromium$^{51}$-labeled platelets in normal recipients and in Cases 3 and 5. The platelet survival in Case 1 was also very short (3 hours), but was done by a different method for which the normal survival is 96–120 hours.

TEM and development of the terminal phase with autoimmune hemolysis and recurrent thrombocytopenia suggested a relationship between the drug and the autoimmune disease. Possibly, rapid destruction of lymphoid tissue by TEM caused a massive release of antibody protein with its subsequent attack on erythrocytes and platelets, or the drug modified the antibody producing cells with resultant production of autoantibodies to these blood cells.$^{26}$

In two of our patients, the bone marrows were stained with fluorescein-labeled anti-human globulin. In one patient the megakaryocytes exhibited fluorescence, indicating the attachment to them of a globulin; this staining was blocked by incubation of the marrow slides with unlabeled anti-human
gamma globulin prior to staining with fluorescent anti-human globulin. In the other patient, the megakaryocytes did not become fluorescent, and in neither patient did the lymphocytes fluoresce. These results are compatible with the report by Pisciotta and McKenna\textsuperscript{27} in which they found that only half of their patients with chronic ITP had fluorescence of the megakaryocytes when their bone marrows were stained in a similar fashion. However, from preliminary investigations it appears that the fluorescent staining of megakaryocytes may be a nonspecific reaction, as fluorescence of apparently normal megakaryocytes has been repeatedly observed.\textsuperscript{17} Further studies are in progress to determine the true value of this test. The lack of fluorescence of lymphocytes in these two cases is puzzling, as these cells have been presumed to be the site of antibody formation.\textsuperscript{28}

In two of our five patients, the megakaryocytes displayed morphologic changes as described in ITP,\textsuperscript{29} with immaturity, vacuolization, lack of cytoplasmic granularity, and no evidence of platelet production. A platelet agglutinin was demonstrable in one of the three sera tested. Of seven similar patients with chronic lymphocytic leukemia and megakaryocytic thrombocytopenia recently cited by Harrington and Arimura,\textsuperscript{7} five had platelet agglutinins.

Three of our five cases had autoimmune hemolytic anemia with positive Coombs' tests; in two this occurred simultaneously with the thrombocytopenia and in the other several months later with recurrent thrombocytopenia. The coincidence of autoimmune hemolytic anemia and thrombocytopenic purpura has been cited as evidence of an autoimmune mechanism for both disorders;\textsuperscript{30} it is reasonable to suspect that antiplatelet as well as antieythrocyte antibodies were present in these cases or that possibly a single autoantibody was reactive with both tissue components. That hemolysis itself was responsible for the low platelet counts does not seem likely, since other types of hemolytic disease are not, as a rule, associated with thrombocytopenia.\textsuperscript{1} None of the patients had other evidences of immune disease; the serum proteins were normal, with the tests performed, in all, and none had leukopenia or vasculitis.

Lymphocytes are known to be involved with production and/or storage of antibody proteins,\textsuperscript{31-33} and such lymphoproliferative diseases as lymphocytic leukemia, lymphosarcoma and infectious mononucleosis have been associated with several apparently immunologic disorders.\textsuperscript{10,26,34-40} Specifically, platelet agglutinins have been described\textsuperscript{19} as well as the clinical syndrome of ITP.\textsuperscript{2,4,6,41,42} One of the current concepts of autoimmune disease\textsuperscript{43-45} proposes that the abnormality lies in the presence of a population of abnormal but immunologically competent cells which are capable of producing antibody against self-components without being rejected themselves by the host. Perhaps, then, the patients with megakaryocytic thrombocytopenia associated with lymphoproliferative disorders represent one aspect of a potentially broad spectrum of immunologic abnormalities in which, for some reason, platelets are the self-components which act as either (1) stimulus for antibody production by the proliferating cells or (2) target organs for an abnormal protein produced by these cells.

Upon reviewing the data from these five patients (table 1), it appeared
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Table 1.—Summary of Findings Suggesting an Autoimmune Mechanism for Megakaryocytic Thrombocytopenic Purpura Complicating Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th></th>
<th>CASE 1</th>
<th>CASE 2</th>
<th>CASE 3</th>
<th>CASE 4</th>
<th>CASE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megakaryocytes Plentiful</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Platelet Agglutinin in Serum</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Platelet Survival Very Short (2–6 Hours)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Staining of Megakaryocytes With Fluorescein-Labeled Anti-Human Globulin</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Auto-Immune Hemolytic Anemia</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Response of Platelet Count to Steroid Therapy</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present, O = Absent, □ = Test Not Done

that they developed thrombocytopenia secondarily to chronic lymphocytic leukemia, thus making the situation analogous to the autoimmune hemolytic anemia which is a rather frequent complication of this disease. Recognition of this disorder depended on identification of thrombocytopenia in the presence of lymphoproliferative disease with the bone marrow containing a normal or increased number of megakaryocytes. Suggestive of the presence of an extrinsic antiplatelet factor, presumably of antibody type, were the very short platelet life span in the peripheral bloods of three cases, a platelet agglutinin in the serum of one case, staining of the megakaryocytes by fluorescein-labeled anti-human globulin in one case, and favorable responses to steroid therapy in three cases. Three patients had evidence of other autoantibodies in the form of autoimmune hemolytic anemia with positive Coombs’ tests. In addition, all patients had a lymphoproliferative disease known to be associated with autoimmunization. Although this syndrome has been mentioned by other authors, it has not previously been emphasized as another of the varied immunologic abnormalities which may be associated with lymphoproliferative disorders.

The prognosis in this circumstance is perhaps somewhat better than in those instances of leukemia in which the marrow is so crowded with lymphocytes that megakaryocytes are lacking. Clinical improvement from adrenal cortical steroids may be anticipated with an ITP-like picture, whereas replacement therapy with platelet transfusions and/or control of the underlying leukemia are necessary in the amegakaryocytic type. Steroid therapy induced remissions in the thrombocytopenic state in three patients but was ineffective in the others.* From these results and the experience of others, it appears

*In patients with lymphoproliferative disorders and amegakaryocytic thrombocytopenia, large doses of corticosteroids may increase the platelet level in association with rather marked regression of the proliferative process. (Kyle, R. A.; McParland, C. E., and Dameshek, W.: The malignant lymphoproliferative disorders: Treatment with corticosteroids in large doses. In preparation.)
THROMBOCYOPENIA WITH LYMPHOCYTIC LEUKEMIA

that steroid therapy is effective in about half of such cases. The value of
splenectomy cannot be judged from this group of cases. In the literature,
splenectomy produced a remission in one of four patients;6,12 the one of our
patients who had this procedure did not respond. The use of x-ray therapy
or alkylating agents in this type of patient is inadvisable because of the risks
deroasing platelet production and of releasing large amounts of antibody,
thereby further increasing the rate of platelet destruction.

SUMMARY

Five cases of chronic lymphocytic leukemia complicated by thrombocyto-
epenic purpura are presented. They differed from the usual cases with this
complication in that megakaryocytes were plentiful in spite of leukemic in-
volveinent of the bone marrow. Hypersplenism did not appear to be a factor.
The evidence suggests that the thrombocytopenia was of an autoimmune
ature, due to antiplatelet antibodies. Brief platelet survival times, the presence
of a platelet agglutinin, staining of megakaryocytes by fluorescein-labeled
anti-human globulin, and responses to corticosteroids were demonstrated.

SUMMARY IN INTERLINGUA

Es presentate cinque casos de chronic leucemia lymphocytic, complicate
per purpura thrombocytopenic. Iste casos differeva ab le casos usual de iste
complication in tanto que in illos megakaryocytos esseva abundante in especto
del affection leucemic del medulla ossee. Hypersplenismo non pareva esser un
factor in le situation.

Le datos pare indicar que le thrombocytopenia esseva de natura auto-immun,
causate per anticorpore anti placettas. Esseva demonstrate curte
temores de superviventia del placettas, le presentia de un agglutinina de
placettas, tincturation del megakaryocytos per globulina anti human marcate
con fluoresceina, e responsas a corticosteroides.

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Autoimmune Thrombocytopenic Purpura ("ITP" Type) with Chronic Lymphocytic Leukemia

SHIRLEY EBBE, BENJAMIN WITTELS and WILLIAM DAMESHEK

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