Tn Polyagglutination Preceding Acute Leukemia

By P. M. Ness, G. Garratty, P. A. Morel, and H. A. Perkins

Tn polyagglutination (persistent mixed-field polyagglutination) was detected in the blood of a 66-yr-old male laborer at the time of a splenectomy for life-threatening thrombocytopenia. Confirmation that the polyagglutination was caused by Tn activation was established by the use of lectins, by failure of the patient’s red cells to react with sera from other patients with Tn polyagglutination, by weak aggregation with polybrene, by low red cell sialic acid levels, and by the persistence of polyagglutination over several years of testing. Two years after the discovery of the Tn polyagglutination, the patient developed acute myelomonocytic leukemia. Vigorous chemotherapy regimens resulted in clinical remission of the leukemia and the Tn polyagglutination. This report describes the first known case of Tn polyagglutination preceding the development of acute myelogenous leukemia.

POLYAGGLUTINATION of erythrocytes is observed when most normal ABO-compatible human sera agglutinate the tested red cells. Unless this is recognized and handled appropriately in the blood bank, blood grouping and cross-match problems usually occur. Tn polyagglutination, or persistent mixed-field polyagglutination, is a type of polyagglutination commonly associated with hematologic abnormalities, including leukopenia, thrombocytopenia, and hemolytic anemia. It has also been detected in healthy blood donors who have appeared to change their blood grouping on later donations. In 1976 Bird and associates described a patient with acute myelocytic leukemia noted to have Tn activation of his red cells. In the present case, Tn polyagglutination was detected prior to the development of acute myelocytic leukemia.

MATERIALS AND METHODS

Case Report

F.G., a 66-yr-old white laborer, had hematologic problems first discovered in 1966 when he was hospitalized for fever. Initial blood examinations revealed leukopenia (WBC 3.1 x 10^9/liter, with 58% segmented cells, 2% bands, 36% lymphocytes, and 4% monocytes) and mild thrombocytopenia (platelet count 120 x 10^9/liter). Bone marrow examination demonstrated a left shift in the myeloid series, with occasional giant band forms and some atypical multinucleated erythroid forms. Splenomegaly was suggested by abdominal x-rays. He was discharged without a specific diagnosis.

In 1972 an enlarged spleen was detected 10 cm below the costal margin. Leukopenia (WBC 2.9 x 10^9/liter) and thrombocytopenia (56 x 10^9/liter) were noted again. The bone marrow was moderately hypercellular, with myeloid hyperplasia and normal megakaryocytes. A liver-spleen scan suggested a diffuse infiltrative process, but liver biopsy was refused.

In January 1974 his platelet count fell to 22 x 10^9/liter. On admission for splenectomy he had hepatosplenomegaly without lymphadenopathy. Blood studies revealed a hematocrit of 43%, a WBC count of 2.3 x 10^9/liter, with 44% polys, and hypercellular bone marrow with normal megakaryocytes and M:E ratio of 6:1. Platelet kinetic studies revealed hypersplenism (shortened survival, with rapid...
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splenic sequestration) and ineffective thrombopoiesis (low platelet turnover rate). It was predicted that splenectomy would improve the platelet count, but to a subnormal level, because of apparent bone marrow dysfunction. During the preoperative investigations he was found to have polyagglutinable red cells caused by Tn activation.

At surgery the spleen weighed 860 g and was speckled with nodules. The liver was enlarged but appeared normal. Microscopic examination revealed congestive splenomegaly with focal extramedullary hematopoiesis and moderate fatty metamorphosis of the liver. Postoperatively the platelet count rose to $143 \times 10^9$/liter, and in September 1974 it was $185 \times 10^9$/liter. By December 1975 the platelet count had fallen to $53 \times 10^9$/liter. In January 1976 the hematocrit was 41%, platelet count $20 \times 10^9$/liter, and WBC count $43.5 \times 10^9$/liter, with a marked left shift and several nucleated red cells.

On readmission, his bone marrow was hypercellular, with mostly myelomonoblasts and promyelocytes and reduced megakaryocytes with atypical multinucleated forms. The leukoerythroblastic peripheral blood and marrow were diagnostic of acute myelomonocytic leukemia. He was treated with cytosine arabinoside and daunomycin. The platelet count increased, but the marrow demonstrated persistent leukemia. He was discharged with an apparent partial remission.

The persistent leukopenia improved to $2.3 \times 10^9$/liter, with 24% polys, in March 1976. A course of cytosine arabinoside and 6-thioguanine was administered as consolidation chemotherapy in May 1976. The WBC count gradually became normal, and the platelet count stabilized at $70-100 \times 10^9$/liter.

In January 1977 he was readmitted with increasing fatigue. An elevated WBC count of $32.6 \times 10^9$/liter and a decreased platelet count of $9 \times 10^9$/liter were noted. Despite chemotherapy, he became febrile and comatose and died on the fifth hospital day. Postmortem examination revealed acute myelomonocytic leukemia with widespread tissue infiltration.

Methods

Standard serologic methods were used. Lectins were prepared as described in the AABB technical manual. To measure the Tn activity on serial samples of the patient's red cells, the red cells were tested against serial dilutions of a normal group A serum as a source of human anti-Tn and Salvia sclarea as a lectin anti-Tn. The titration reactions were expressed as agglutination scores.

RESULTS

The patient's red cells were found to react with anti-A, anti-B, and anti-A,B. Agglutination showed a mixed-field appearance. The patient's serum reacted with A cells and B cells but did not react with O cells or the patient's own cells. The patient's saliva contained H substance, but no A or B substance. The patient's cells reacted with all donor sera containing anti-A or anti-A,B (2+ to 3+ reactions). Some donor sera containing anti-B reacted 1+, whereas others failed to react. Some AB sera reacted (1+ to 3+), but others were nonreactive. All cord sera failed to react with the patient's cells.

To determine if T or Tn activation was the cause of the polyagglutination, the patient's red cells were tested with lectins. The cells reacted strongly with lectins from Dolichos biflorus, Ulex europaeus, S. horminum, and S. sclarea, but failed to react with Arachis hypogaea. The patient's red cells thus appeared to be demonstrating Tn polyagglutination. Further tests confirmed that the red cells failed to react with serum from another patient with Tn polyagglutination and that the patient's serum lacked anti-Tn. When the patient's red cells were treated with papain, they failed to react with anti-A or the Salvia lectins.

The patient's red cells were aggregated only weakly by polybrene, but were strongly agglutinated by soybean lectin. Red cell sialic acid content was 11.1 $\mu g/10^8$RBC (normal 13-17 $\mu g/10^8$RBC).

Samples of peripheral blood from the patient and from a normal control were studied for Tn activity of the leukocytes and platelets by inhibiton studies using...
Table 1. Tn Activity of Patient’s Red Cells

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<th>Mean Agglutination Score Using Serial Dilutions</th>
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<tr>
<td></td>
<td>Group A</td>
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<td></td>
<td>Normal Serum</td>
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<td>January</td>
<td>9</td>
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*Chemotherapy given.

Salvia lectin. The red-cell-free buffy coat from the patient’s blood showed Tn polyagglutination, whereas the normal buffy coat did not demonstrate polyagglutination. Details of these studies were reported elsewhere by Beck et al.5

The Tn status of the patient’s red cells was tested at weekly intervals during 1976 after the diagnosis of acute myelomonocytic leukemia. The cells were tested against dilutions of normal group A serum containing anti-Tn and against S. sclarea. The agglutination scores remained elevated until May 1976, when the polyagglutination weakened considerably. Reactions became weaker over the next few months, until the polyagglutination disappeared completely in November 1976 (Table 1). At the time of his death with fulminant leukemia, Tn activation was not detectable.

DISCUSSION

Polyagglutinability occurs when erythrocyte membrane receptors, normally unexposed or absent, become exposed or present de novo. Five such receptors have been described: T, Tn, Cad, Tk, and VA. The corresponding antibodies are normally present in human adult sera.6 The two most commonly encountered are T and Tn. The T receptor is concealed in normal erythrocytes and exposed by bacterial neuraminidase enzymes either in vitro in contaminated blood samples or in vivo. In vivo T polyagglutination is transient and disappears when the causative infection clears. T activation is usually not associated with hematologic abnormalities. Tn polyagglutination is an acquired phenomenon whose etiology is uncertain, although an acquired defect of bone marrow stem cells has been suggested.1 The fact that Beck et al.5 demonstrated that our patient’s white cells and platelets were Tn-activated would imply a mutation of a pluripotent hematopoietic stem cell. The absence of Tn activation of buffy coat cells in other cases suggests variable sites of the somatic mutation.

We have presented conclusive evidence that our patient had Tn activation: the reactions with D. biflorus, S. sclarea, and S. horminum and the failure to react with A. hypogoea;7 the failure of the patient’s cells to react with sera from another patient with Tn activation; the weak aggregation with polynbrene; the low red cell sialic acid levels;8 and the persistence of the polyagglutination.

Concurrent diagnosis of acute myelogenous leukemia with Tn polyagglutination was reported by Bird and associates.2 Our case differs from theirs in two important
aspects. First, our patient had polyagglutinable red cells for at least 2 yr prior to the diagnosis of acute myelogenous leukemia. The Tn polyagglutination was discovered by chance when routine blood grouping was necessary. His long history of leukopenia and thrombocytopenia, commonly associated with Tn polyagglutination, suggests that his Tn activation occurred even earlier. Second, the case described by Bird et al. was not classic acute myelogenous leukemia. The lymphadenopathy, the inability to aspirate bone marrow, the low leukocyte alkaline phosphatase score, and the elevated vitamin B₁₂ level favor the diagnosis of Philadelphia-chromosome-negative chronic myelogenous leukemia. Our case was more typical of acute myelogenous leukemia in adults.

The duration of Tn polyagglutination is controversial. It has been called both persistent and permanent. In Bird's case, the Tn activation disappeared 5 mo after cytotoxic chemotherapy. Other reported cases have shown variable effects of chemotherapy. Our patient lost Tn polyagglutination concomitant with cytotoxic chemotherapy. These observations imply that the Tn disappeared as a chemotherapy effect, and they further support the description of Tn polyagglutination as persistent, not permanent.

Retrospective analyses of acute myelogenous leukemia have led to the recognition of a constellation of hematologic findings in patients who develop leukemia later. These preleukemia findings commonly include pancytopenia with hypercellular marrow. In retrospect, our patient had a preleukemic syndrome. The similarities between the preleukemic syndrome and the hematologic findings with Tn activation suggest a possible relationship. The somatic cell mutation causing the Tn activation may also have been responsible for his preleukemic state or a preleukemic marker. The development of acute myelogenous leukemia in patients with Tn polyagglutination may be analogous to the situation in patients with PNH who develop leukemia; both disorders are marked by red cell membrane defects resulting from proposed somatic cell mutation and often resulting in hemolytic anemia and various cytopenias. Several observations detract from this attractive hypothesis: (1) we do not know if the Tn activation occurred concurrently with his hematologic problems; (2) the Tn disappeared when the leukemia relapsed; (3) many other patients with Tn activation have not developed leukemia.

Despite the fact that we cannot prove that the Tn activation had any etiologic role in his development of acute myelogenous leukemia, the association of Tn and acute leukemia in this case and one other case justifies careful clinical observation of any patient or blood donor with Tn polyagglutination.

ACKNOWLEDGMENT

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REFERENCES

4. Miller WV (ed): American Association of


tic and serological aspects. Vox Sang 10:7, 1971


vation. Transfusion 10:43, 1970

Harbour, Miami Beach, Fla, 1973


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