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The Use of Leukocyte Acid Phosphatase in the Diagnosis of Malignant Disease

Case Report and Review of Literature

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ABSTRACT

Tartrate resistant acid phosphatase (TRAP) has been demonstrated during relapse in the cells in the cerebral spinal fluid (CSF) in a patient with the clinical features of acute T cell lymphocytic leukemia which suggests this isozyme may be a marker for malignant transformation of some types of lymphocytes. The presence of TRAP may be helpful in establishing the diagnosis of a lymphocytic malignancy since normal lymphocytes appear to have tartrate sensitive acid phosphatase. The presence of TRAP can no longer be considered specific for hairy cell leukemia, since children with acute lymphocytic leukemia are being found with this isozyme in their malignant blasts. The presence of TRAP does not appear to be specific for T or B cell lines of malignant lymphocytes since it has been described in cells with either type of cell markers. The presence of TRAP seems most useful in differentiating lymphocytic malignancies from monocytic and histiocytic malignancies and from benign lymphocytoses when the cells of the peripheral blood and marrow may have similar morphologic features with routine staining.

Introduction

Acid phosphatase, an enzyme found in blood cells and prostatic tissue, was noted by Gutman et al to be increased in serum in patients with invasive and metastatic carcinoma of the prostate.[16] This observation has remained valid for forty years and recently attempts have been made to provide a more sensitive measurement of serum acid phosphatase by radioimmunoassay.[12] Abul-Fadl and King[1] demonstrated inhibition of prostatic acid phosphatase by tartrate in 1949. This was confirmed in 1959 by Nigam et al.[28]

By 1972, Li, Yam and Lam[24] and Katayama[22] had demonstrated seven different tissue acid phosphatase isoenzymes by acrylamide gel electrophoresis and showed activity in individual cells by histochemical staining using naphthol AS-BI phosphoric acid as substrate. Their studies on a variety of patients with different diseases of the blood showed that

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isozyme 0 was found only in the macrophage storage cells of Gaucher's disease. Isozymes 1, 2 and 4 were prominent in neutrophils, 1 and 4 in monocytes, 3 in lymphocytes and platelets, 3b in primitive blasts, respectively. All the preceding isozymes proved to be inhibited by tartrate. Isozyme 5, on the other hand, was tartrate resistant and was found most prominently in the large mononuclear cells of leukemic reticuloendotheliosis or so called "hairy cell leukemia" (HCL).

Katayama[22,23] suggested that the presence of tartrate resistant acid phosphatase (TRAP) might be a helpful diagnostic tool in differentiating HCL from monocytic leukemia and from lymphosarcoma. Subsequently, in 1974 Catovsky et al[6,7] published studies which suggested acid phosphatase was present in the lymphocytes of children with acute T cell leukemia. These authors did not utilize tartrate inhibition to distinguish specific isoenzymes. A further summary of their work,[4] published in 1977, indicated the absence of acid phosphatase in the lymphocytes of children with acute null cell leukemia and suggested acid phosphatase might be a marker for T cell leukemia.

The present study was stimulated by the presentation of a patient with central nervous system relapse of acute lymphocytic leukemia whose initial clinical presentation had suggested a T cell type of acute leukemia.[10]

Case Presentation

The patient, a white male child, had acute lymphocytic leukemia diagnosed 21 months previously when he was 4½ years old. He presented with lymphadenopathy and hepatosplenomegaly. His initial white count was 43,000/mm³ with 68 percent blasts. His chest X-ray revealed widening of the superior mediastinum and hilar adenopathy. Remission was induced with adriamycin (40 mg per m²) on day one, and prednisone (40 mg per m² per day) and vincristine (1.5 mg per m² per week) for four weeks. Central nervous system (CNS) prophylaxis was then instituted. The patient received 2,500 rads to the cranium over a 2½ week period and five doses of intrathecal methotrexate (12 mg per m² bi-weekly). He was continued on maintenance therapy receiving 6-mercaptopurine daily, methotrexate weekly and pulses of vincristine and prednisone monthly. He did well for 14 months and then developed jaundice which was thought to be due to 6-mercaptopurine (6-MP) hepatotoxicity. With reduction in 6 MP, the jaundice resolved and the patient continued to do well until 21 months after diagnosis when he developed severe headaches and vomiting. His physical examination was normal. A spinal tap was performed. The opening pressure was 380 mm H_2O and the cell count was 1,940 white cells per mm³. Cytocentrifuge preparations of the CSF revealed that almost all of the white cells were lymphoblasts. The CSF protein was 41 mg per dl and the glucose was 39 mg per dl. Bone marrow aspirate showed continued marrow remission. Special stains for acid phosphatase were performed.

Methods

Air dried smears of bone marrow, peripheral blood, buffy coat or centrifuged spinal fluid were fixed by dipping them for 30 seconds into a solution of 10 percent methanol in 60 percent acetone containing 0.03 M citrate maintained at 4°. Subsequently, the slides were incubated at 37° for 45 minutes in coplin jars containing 40 ml of the substrate prepared as follows: 0.5 ml of N, N-dimethyl formamide containing naphthol AS-BI phosphoric acid (20 mg per ml) was added to 100 ml of 0.1 M acetate buffer pH 5.0 with 20 mg Fast Garnet GBC for staining control slides. Tartrate resistance of the white cell acid phosphatase was determined by incubating a duplicate slide in the same substrate but containing 300 mg of L (+) tartaric acid. The substrate was filtered just before use. After the incubation, the slides were counterstained while still wet in Mayer's haematoxylin for 30 seconds. The slides were then rinsed in distilled water and mounted wet in glycerine jelly.

Control slides (substrate without tartrate) showed red-brown granules in all cells while with tartrate added the neutrophils were negative. If lymphocytes stain positive in the presence of tartrate, this is considered consistant with hairy cell leukemia according to Katayama, Li, Yam and Lam.[22,23,24] Rosetting with sheep erythrocytes or antibody-complement coated sheep red cells (EAC) was carried out by published procedures.[2,10,32]

Results

The cerebrospinal fluid cells were virtually all lymphoblasts which stained both in the presence and in the absence of tartrate (figure 1). Control blood smears stained in the substrate solutions at the same time showed positive staining leukocytes in the absence of tartrate and negative staining in the presence of tartrate (figure 1). Thus, this patient, who had clinical features suggesting T cell leukemia, had lymphoblasts in the spinal fluid staining positive for TRAP. None of the morphologic or clinical features of hairy cell leukemia were present.

Examination of the cerebrospinal fluid (CSF) cells by rosetting with washed sheep erythrocytes showed 2.7 percent rosetting, while with EAC, 65.5 percent of the cells formed rosettes. Studies for surface IgG could not be performed. These studies demonstrate that the receptors of mature T cells were not present on the leukemic cells in the cerebrospinal fluid, although complement receptors were present. The patient attained remission quickly with a repeat course of intrathecal methotrexate.

Discussion

These observations suggest TRAP may not be a specific marker for hairy cell leukemia but may have broader specificity within the lymphoproliferative disorders. One other child has been seen with acute lymphocytic leukemia (ALL) in relapse whose marrow cells were positive for TRAP. Another child with ALL and TRAP positive cells has been reported by Loeffler in 1977.[26] The presence of leukocyte acid phosphatase has been associated with T cell leukemia as reported by Catovsky in a series of pediatric patients.[6,7] His study revealed that all eight children with T cell ALL, as judged by rosetting of blood lymphocytes with sheep cells and lack of surface IgG, showed lymphoblasts that stained for acid phosphatase. On the other hand, only one of 51 patients with null cell leukemia and none of two patients with B cell ALL showed any staining for acid phosphatase. These differences are statistically significant by χ^2, but the series was small. Unfortunately, tartrate resistance was not determined in these patients.

The present report describes a patient with clinical features of T cell acute lymphocytic leukemia whose leukemic cells in the spinal fluid had receptors for C3. C3 receptors are most often found in lymphocytes of bone marrow origin.[32] They may also be on T cells, especially if the cells are malignant.[18,25,32] C3 receptors have also been found on fetal thymocytes[33] and on immature lymphocytes of thymic origin.[15] Their appearance on the cell surface precedes that of the sheep/erythrocyte receptor.[11] Our patient may have a type of T cell malignancy in which the cells are too immature to have sheep erythrocyte receptors. Similar patients with ALL and lymphoblastic lymphoma have been described by other investigators who considered them to have malignancies of thymic origin.[9,20]

It would be attractive to consider TRAP an isozyme marker for T cell type lymphocytic malignancies. Indeed, Saxon et al[30] and Cawley et al[8] have reported cases of HCL positive for TRAP with T cell characteristics, including spontaneous rosetting of the lymphocytes with sheep erythrocytes and lack of surface Ig. However, many more of the patients with HCL positive for TRAP have been shown to have features of B cells with surface Ig and lack of sheep cell rosettes.[2,5,22,23,24]

Still more intriguing are reports of leukemias with both B & T cell characteristics [17,18,21,27] that suggest malignant transformation may sometimes result in hybrid forms which also demonstrate the presence of acid phosphatase. In a case reported by Shevach,[32] 92 percent of the cells stained for acid phosphatase, but only 10 percent showed tartrate resistant acid phosphatase.

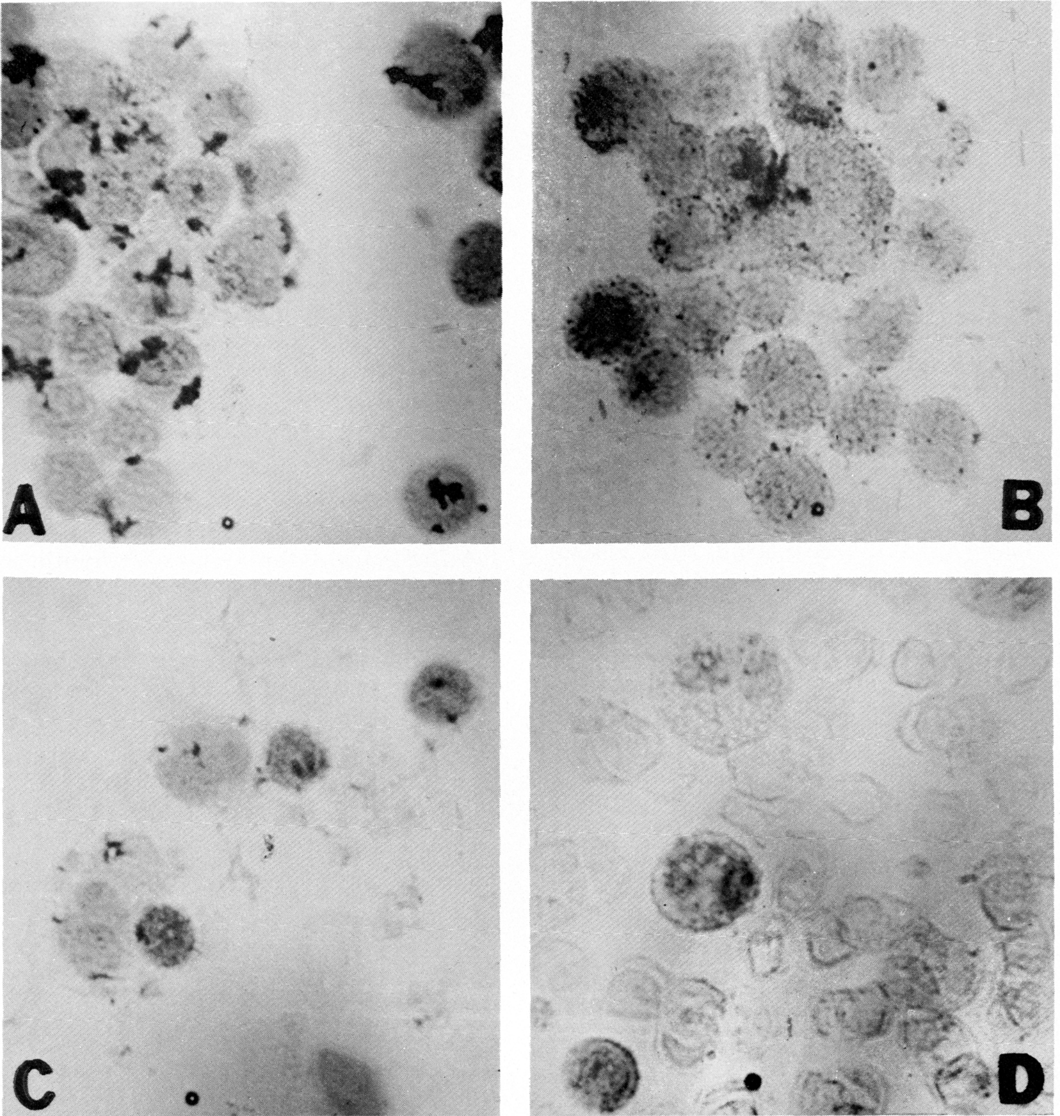

FIGURE 1. A. CSF lymphoblasts showing marked staining for acid phosphatase in absence of tartrate. B. CSF lymphoblasts showing moderate staining for acid phosphatase in presence of tartrate. C. Control blood smear showing moderate staining for acid phosphatase in absence of tartrate. D. Control blood smear showing no staining for acid phosphatase in presence of tartrate.

Golomb et al[13] reported 12 patients with HCL and demonstrated synthesis of monoclonal Ig in all, establishing beyond doubt the B cell nature of these cells. TRAP staining was variable but 8/12 showed more than 50 percent of the cells positive.

Brouet and Prieur[3] studied a patient with chronic lymphocytic leukemia whose lymphocytes had surface IgM with anti sheep cell activity causing rosette formation. This suggests that search for markers by multiple techniques is necessary before identification of B or T cells is certain. Brouet et al also demonstrated that acid phosphatase was found in the lymphocytes of over 80 percent of patients with T cell acute leukemia but in only 10

percent of patients with null cell acute leukemia.[4] They did not determine resistance to tartrate.

Huhn et al[19] have recently reported a study of 27 patients with acute lymphocytic leukemia whose lymphocytes were differentiated into: (1) T cells which formed rosettes with sheep red cells and had a T antigen; (2) T cells without rosette formation but reacting positively for the presence of T antigen; and (3) cells neither rosetting with sheep erythrocytes nor having the T antigen. No differences were observed in overall activity of acid phosphatase in these three situations. The comment was made that in cases where cells showed very strong enzyme activity (index > 250) T cells showed qualitative differences from non T cells.

Pangalis, Waldman and Rappaport[29] have demonstrated that normal B and T lymphocyte populations contained comparable acid phosphatase activity which was entirely inhibited by addition of tartaric acid. These observations show that normal lymphoid cells have acid phosphatase isozyme 3, which is tartrate inhibitable, while previously quoted studies and the patient studied by the present authors show that some malignant B or T lymphocytes may have acid phosphatase isozyme 5 which is resistant to tartrate.

Thus, staining for the tartrate resistant acid phosphatase fraction may be more meaningful in differentiating malignant lymphocytic disease from benign lymphocytic disease than staining for total or nonspecific acid phosphatase. Caution should be used in diagnosing hairy cell leukemia on the basis of the presence of TRAP until the more exact specificity of isozyme 5 is known in other lymphocytic malignancies.

Summary

1. Tartrate resistant acid phosphatase has been demonstrated during relapse in the cells in the CSF in a patient with the clinical features of acute T cell lymphocytic leukemia which suggests this isozyme may be a marker for malignant transformation of some types of lymphocytes.

2. The presence of TRAP may be helpful in establishing the diagnosis of a lymphocytic malignancy since normal lymphocytes appear to have tartrate sensitive acid phosphatase.[24,29]

3. The presence of TRAP can no longer be considered specific for hairy cell leukemia, since children with acute lymphocytic leukemia are being found with this isozyme in their malignant blasts[19,26] (and present study).

4. The presence of TRAP does not appear to be specific for T or B cell lines of malignant lymphocytes since it has been described in cells with either type of cell markers.[5,8,12,27,30,32,34]

5. The presence of TRAP seems most useful in differentiating lymphocytic malignancies from monocytic and histiocytic malignancies and from benign lymphocytoses when the cells of the peripheral blood and marrow may have similar morphologic features with routine staining.[2,5,12,23,24]

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