Relationship of a Leukemia-Associated Antigen to the Presence of Lymphoblasts in the Peripheral Blood in Children with Acute Lymphocytic Leukemia

By Elaine Morgan and Clement C.S. Hsu

Peripheral blood samples from 57 children with newly diagnosed E-rosette-negative, surface-immunoglobulin-negative acute lymphocytic leukemia (ALL) were studied for the presence of a leukemia-associated antigen (ALLA). Ficoll-Hypaque separated cells were tested using a rabbit antisera to human null lymphoblasts and an indirect immunofluorescent assay. The percentage of ALLA-positive cells was compared to the percentage of lymphoblasts determined by differential counts of a Wright-Giemsa-stained smear of a concurrently obtained peripheral blood sample. The mean ratio of percentage of lymphoblasts to percentage of ALLA-positive cells was 0.90. However, in 13 patients, the ratio of percent of ALLA-positive cells to percent of lymphoblasts was equal to or greater than 2:1. In the blood of 6 additional patients (with newly diagnosed, 1 relapsed patient) in whom no morphologically identifiable lymphoblasts were detected, ALLA-positive cells were present (7%-49%). These results indicate that testing for ALLA-positive cells is a sensitive technique for detection of leukemic cells in children with ALLA-positive ALL.

Following initial demonstration of the prognostic significance of T-cell (E-rosette positive) and B-cell (surface immunoglobulin [SIg] positive) membrane markers in childhood acute lymphocytic leukemia (ALL),1 many investigators have developed heteroantisera to a variety of membrane antigens on lymphoblasts in an attempt to further refine subclassification of this disease. Specifically, antisera to ALL cells (anti-ALL) that reacted with the leukemic cells of approximately 70% of children with "null" (E-rosette negative, SIg negative) ALL in relapse was initially described by Greaves et al.7 These antisera also showed reactivity to cells from some patients with acute undifferentiated leukemia (AUL) and from several patients with chronic myelogenous leukemia (CML) in blast crisis. There was minimal cross-reactivity to cells of a few patients with acute myelocytic leukemia and to a small proportion of cells in normal bone marrow and spleen and fetal liver. The majority of normal blood and bone marrow cells from patients with T-cell ALL and other lymphoproliferative disorders did not react to the antisera.7 Sallan et al. have described an antisera to a common ALL antigen (cALLA) that has a similar spectrum of reactivity.8

In our laboratory we have developed antisera to "null" ALL cells (anti-ALLA) according to the method described by Greaves that has a spectrum of reactivity similar to that of Greaves' anti-ALL.9-11 Using these antisera we have attempted to quantify the percentage of ALL-associated antigen (ALLA) bearing cells in children with ALL and to correlate this with the percentage of circulating lymphoblasts.

MATERIALS AND METHODS

Peripheral blood samples from newly diagnosed children with ALL were obtained prior to initiation of therapy. The mononuclear cells were separated by a Ficoll-Hypaque gradient technique.12 Several blood samples were also studied without the cell fractionation, using an ammonium chloride solution (9 parts 0.93% NH4Cl, 1 part Tris-HCl, pH 7.65) to lyse the red blood cells.9 Anti-ALL antisera (anti-ALLA) were prepared as described previously9 by immunizing rabbits with 5 x 107 null ALL cells that had been coated with rabbit anti-human lymphocyte serum. The antisera were incubated at 56°C for 30 min, absorbed with human tonsil cells (2 ml packed cells/ml serum), and lyophilized human liver powder (50 mg/ml serum), centrifuged at 100,000g for 90 min, and finally diluted to an appropriate concentration.

The specificity of our ALL antisera for null ALL cells has been documented previously.9 Specifically, peripheral blood samples from 28 normal persons, 10 patients with acute nonlymphocytic leukemia, 23 patients with chronic lymphocytic leukemia, 2 patients with chronic granulocytic leukemia, 4 patients with lymphoma, and 25 patients with nonneoplastic disorders have shown no reactivity or minimal (0%-4%) nonspecific staining that was also present on control slides prepared using serum from an unimmunized rabbit.9 Two additional patients with CGL in blast crisis were noted to have ALLA-positive cells.

Nonreactivity of the anti-ALL antisera in bone marrow samples has also been demonstrated. Examination of 19 remission bone marrow samples from 15 patients who were known to be previously ALLA-positive showed 0%-1% reactive cells. Bone marrow aspirates from 2 children with ANLL and uninvolved bone marrow aspirates of 4 patients with lymphoma and 2 patients with malignant histiocytosis were nonreactive with the anti-ALLA, as were the bone marrow samples from one patient with Gaucher's disease and one normal bone marrow.

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An indirect immunofluorescent technique was used for identification of cells carrying ALLA, using the anti-ALLA antisera. Control slides were prepared for each sample using test cells, normal rabbit serum and fluorescein isothiocyanate conjugated goat anti-rabbit IgG. All slides were examined with a microscope equipped with a phase-contrast lens, and the percentage of immunofluorescent cells was determined, based on 200 cells counted.

Simultaneously obtained peripheral blood samples were stained with Wright-Giemsa stain and the percentage of lymphoblasts determined by two or more independent experienced observers. In each case, a differential count was performed on a minimum of 100 leukocytes, and the smear was subsequently scanned under low power magnification (100X) for the presence of large mononuclear cells or cells having a large nuclear:cytoplasm ratio. The percentage of blast cells was expressed as a ratio of blasts to mononuclear cells for direct comparison to the results of immunofluorescent staining performed on samples of mononuclear cells. Lymphoblasts were identified by standard French-American-British (FAB) criteria.

**RESULTS**

Peripheral blood samples from 57 newly diagnosed children with sheep erythrocyte receptor negative, surface immunoglobulin negative ALLA-positive ALL were studied. The mean percentage of lymphoblasts in these patients was 42% with a range of 0%-99%. The mean percentage of ALLA-positive cells was 50% with a range of 0%-99%. The mean ratio of percentage of lymphoblasts to percentage of ALLA-positive cells was 0.90. In 44 of the 57 samples studied, the percentage of antigen-positive cells was equal to or greater than the percentage of lymphoblasts (Fig. 1). In 13 cases, the ratio of percentage of ALLA-positive cells to percentage of lymphoblasts was 2:1 or greater (Table 1).

Five peripheral blood samples were examined for percentage of ALLA-positive cells using the technique of ammonium chloride lysis of red blood cells, in order to compare directly the percentage of ALLA-positive cells and the percentage of blasts in the blood (Table 2).

In two of these cases (patients P.P. and K.B.), the percentages of ALLA-positive cells in ammonium chloride treated samples were more than 15% lower than those of Ficoll-Hypaque separated samples, suggesting ALLA-positive cell concentration by the density gradient centrifugation. Nevertheless, in two other cases (patients A.B. and K.R.), the percentages of ALLA-positive cells in ammonium chloride treated samples were more than 19% higher than the percentages of blast cells in the blood. In 4 of the 5 cases studied, the percentage of ALLA-positive cells in the ammonium chloride treated samples was equal to or greater than the percentage of lymphoblasts in the corresponding Wright-Giemsa-stained blood sample.

Furthermore, we noted the presence of ALLA-positive cells in the peripheral blood samples of 6 patients with ALL in whom no morphologically identifiable blasts were detected (Table 3). The first five of
these patients were newly diagnosed, the last patient (F) was a child studied at the time of an asymptomatic bone marrow relapse. The percentage of ALLA-positive cells in these samples varied from 7% to 49%. The percentage of lymphoblasts in the bone marrow aspirates of the newly diagnosed patients varied from 58% to 98% (as indicated in Table 3) and was 24% in the bone marrow of the patient studied at relapse (patient F). It was technically impossible to obtain adequate samples of bone marrow from the 5 newly diagnosed patients for testing with the anti-ALL antiserum. The bone marrow aspirate of patient F was positive for ALLA (24% lymphoblasts; 48% ALLA-positive cells). Subsequent to this study, two additional patients with ALL with no identifiable peripheral blast cells were studied (patients G and H, Table 3). Bone marrow aspirates in these children also revealed ALLA-positive cells.

DISCUSSION

These studies demonstrate that the immunofluorescent technique for detection of cells carrying the ALLA is a sensitive method for detection of leukemic cells in the peripheral blood of patients who are ALLA-positive. The results of studies using ammonium chloride lysis of red blood cells suggest that the discrepancy in some patients between percentage of lymphoblasts in the blood smear and percentage of antigen-positive cells in the Ficoll-Hypaque separated sample was not entirely the result of selective cell concentration by the density gradient. It is likely that normal-appearing lymphocytes carry the ALLA. It is known that the morphological identification of leukemic cells is imperfect, particularly when large numbers of microlymphoblasts are present. This immunologic technique at least partially overcomes this difficulty.

Conceivably, the presence of ALLA on normal-appearing lymphocytes could result from the adsorption of circulating antigen onto a small percentage or specific subset of normal lymphocytes. However, overnight incubation of several of our patient’s samples in the absence of serum has not resulted in decreased numbers of ALLA-positive cells, suggesting that the antigen is probably not of exogenous origin.14

These findings potentially carry theoretical and practical clinical implications. Many observers have documented the prognostic significance of the height of the total white blood cell count at the time of diagnosis in patients with ALL. In some patients, the number of circulating lymphoblasts does not parallel the total white cell count because of a relatively low percentage of blasts. Our findings suggest that a partial explanation for the documented significance of total white cell count, despite the apparent discrepancy with numbers of lymphoblasts in some patients, may be the relatively normal appearance of circulating leukemic cells in such patients.

The presence or absence of ALLA on lymphoblasts in children with null ALL has been shown to have prognostic significance.6,10,12 In some patients it is technically difficult to obtain an adequate volume of aspirated bone marrow at the time of diagnosis to test for the presence of this antigen. In some of these patients, antigen-bearing cells may be identified in the peripheral blood despite the absence of morphologically identifiable leukemic cells. Our observations suggest that routine examination of peripheral blood in children with newly diagnosed null ALL may aid in accurate subclassification even in the absence of circulating lymphoblasts.

Finally, this technique is an attractive method for serial follow-up studies in antigen-positive patients between infrequent bone marrow examinations. The detection of small numbers of antigen-positive cells in the peripheral blood of such patients might enable early detection of relapse and possibly could reduce the necessary frequency of bone marrow examinations in the surveillance of these patients. Prospective studies in a large number of children with ALL will be needed to determine the clinical applicability of this approach.
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REFERENCES

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