CONCISE REPORT

Recategorizing Childhood Acute Lymphoblastic Leukemia with Monoclonal Antibodies to Human T Cells

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The lymphoblasts of three patients with childhood acute lymphoblastic leukemia (ALL) were analyzed for their immunologic surface markers. Blasts from two of these patients did not form rosettes with sheep erythrocytes and the third did so marginally, suggesting these patients had non-T-cell leukemia. These blasts were also tested with monoclonal antibodies that detect thymocyte differentiation markers, and all three patients were highly reactive with at least two of these reagents. We anticipate the availability of multiple standardized monoclonal reagents will necessitate a recategorization of ALL phenotypes. Some of these leukemic phenotypes may not correspond to normal stages of lymphoid differentiation. Therefore, we suggest that it may be inappropriate to attempt to identify and categorize leukemic cells by the pathways of normal differentiation.

CHILDHOOD acute lymphoblastic leukemia (ALL) has been subjected to detailed immunologic assessment, now designated immune phenotyping. The initial impetus for this approach resulted from studies that showed patients with T-cell ALL (as defined by the ability to form rosettes with sheep erythrocytes) had a poorer prognosis than patients with non-T-cell ALL. The T-cell leukemias have been further subdivided by Reinherz et al. using monoclonal antisera directed against discrete stages of differentiating thymocytes. In their study of 25 patients with T-ALL, all but 4 had lymphoblasts that clearly corresponded to normal thymocytes at some stage of differentiation.

However, the great majority of children with ALL have “null” blasts that do not bear surface immunoglobulin, nor do they form sheep rosettes. Most of these do bear human La antigen (p 23,30) and express a common antigenic determinant designated “cALL antigen.” The cellular origin of “null” or “common” ALL remains controversial. One-third of these may have cytoplasmic Ig, suggesting they are “pre-B” cells. We report here that some “null” ALL patients bear an unusual array of markers detected by monoclonal antibodies to human thymocytes.

MATERIALS AND METHODS

Lymphocytes and Lymphoblasts

All patients and controls were evaluated in the clinical immunology laboratory of the University of Wisconsin Clinical Science Center. Leukemic lymphoblasts (cases I, II, III) were obtained by bone marrow aspiration on the day of diagnosis prior to any chemotherapy. Lymphoma lymphoblasts were obtained by gentle dissection and mincing of a diagnostic (pretherapy) biopsy specimen. Human thymocytes, derived from thymic fragments being prepared for transplantation, were obtained from immunologically normal donors at the time of corrective cardiac surgery. Peripheral blood from volunteers was obtained by venipuncture. Lymphocytes and lymphoblasts were purified by Ficoll-Hypaque flotation and dissection and mincing of a diagnostic (pretherapy) biopsy specimen.

Sheep erythrocyte rosettes. Spontaneous rosettes with sheep erythrocytes (GIBCO Labs, Madison, Wisc.) at 4°C was assayed by the method of Jondal.

Rhesus erythrocyte rosettes. Spontaneous rosettes with rhesus erythrocytes (Flow Labs, McLean, Va.) at 37°C were assayed by the method of Chiao et al.

Surface membrane immunoglobulin (SmIg). Goat antibody to human immunoglobulin light chains (λ and κ) was directly conjugated with fluorescein isothiocyanate. Cells were incubated with this reagent, thrice washed, and examined by fluorescent microscopy.

Detection of thymocyte markers with monoclonal antisera. Monoclonal antibodies to human thymocytes were obtained from Ortho Pharmaceuticals (Raritan, N.J.). Antibodies utilized were OKT3, reactive with peripheral T lymphocytes; OKT4, reactive with inducer/helper T lymphocytes; OKT6, reactive with common thymocytes; and OKT8 reactive with suppressor/cytotoxic T lymphocytes. For each test, 10^6 cells were incubated with 5 μl of the monoclonal reagent for 30 min. After washing, biotinylated goat anti-mouse IgG (Vector Labs, Burlingame, Calif.) was added for 30 min. Cells were washed again and 10 μg of fluorescein-labeled Avidin-D (Vector Labs, Burlingame, Calif.) was added for 30 min. Following subsequent washing, cells were examined and counted by fluorescent microscopy.

RESULTS

Cell surface markers on our four most recently diagnosed patients with lymphoid malignancies are shown in Table 1. Cases I and II were considered “null” ALL by the absence of SmIg and the inability to form sheep rosettes. Nevertheless, they each formed rhesus rosettes, suggestive of thymocyte lineage.

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Furthermore, they showed near complete reactivity (>95%) with all 4 of the OKT monoclonal reagents tested. While this suggests thymocyte lineage, no normal thymocyte bears the OKT3 (mature thymocyte) marker and still simultaneously expresses both OKT4 and OKT8 as seen on "common thymocytes." Case III is more complex immunologically. Thirty-eight percent of her blasts formed SRFCs; because of the poor cell yield in the bone marrow sample, this was initially felt to reflect contamination of the marrow by peripheral blood T cells. Her low white count, and the absence of any mediastinal enlargement, appeared consistent with null cell as opposed to T-cell ALL. Nevertheless, >90% of her bone marrow was OKT4+ and OKT8+, consistent with T-cell lineage. Of interest here is the absent reactivity with OKT6; no normal thymocyte in the Reinherz and Schlossman schema is OKT6 negative and positive for both OKT4 and OKT8. Thus, case III as well does not appear to correspond to a cell in a normal stage of thymocyte differentiation.

Case IV represents a surface immunoglobulin positive lymphoma. The nonreactivity of these lymphoblasts with the monoclonal antibodies provides a negative control for the OKT reactions in cases I, II, and III. The presence of SmIg would categorize this lymphoma as "B-cell," however, this is not consistent with the 77% positivity for rhesus rosettes. It is possible that the surface markers tested are not adequately specific to completely discriminate normal B cells from cells of thymocyte lineage. Alternatively, this lymphoma has both T- and B-cell characteristics; certainly not a "normal" phenotype.

The last 2 columns of Table I are representative samples of thymocytes and peripheral blood lymphocytes from healthy donors. These and other control tissues in our laboratory have shown markers consistent with previously published results using these identical monoclonal reagents. The only deviation we observe is a slight increase in thymocyte reactivity with OKT3.

**DISCUSSION**

These data demonstrate that at least some "null cell" ALL lymphoblasts bear surface markers that are ordinarily present only on normal thymocytes, indicating that these ALL patients might have a leukemia of thymocyte origin. However, the presence of T markers on cells that would have been considered null cells by sheep erythrocyte rosetting criteria does not imply these individuals have the same disease or prognosis as classical rosette-positive "T-cell" leukemic patients. Moreover, the lymphoblasts in cases I, II, III, and IV each bear an abnormal array of differentiation markers. They do not appear to be normal lymphoid cells "stuck in differentiation" by neoplastic transformation. Thus, it may be inappropriate for us to force neoplastic lymphoid blasts into these normal cell categories by attempting to label them as "B-cell, pre-B-cell, T-cell, or pre-T-cell."

We therefore suggest that lymphoid malignancies be identified and categorized operationally by virtue of their immunologic (and enzymatic) properties. What has previously been referred to as "T-cell ALL" could easily be designated sheep rosette positive ALL, to differentiate it from the sheep rosette negative, OKT 3, 4, 6, 8, positive ALL described here. The commercial availability of many of these reagents may enable reference labs to phenotype large numbers of ALL patients with reproducible and comparable assays. Analysis of these data might then delineate the prognostic and therapeutic implications of each distinct leukemic phenotype.
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NOTE ADDED IN PROOF

Since submission of this manuscript, we have occasionally noted some nonspecific binding of avidin to monocytes. To further corroborate the results presented here, we have repeated these studies using fluoresceinated goat antimouse immunoglobulin to detect the binding of monoclonal mouse immunoglobulin to the surface of human cells. Our results with this system are comparable to those shown in Table I. Specifically, cryopreserved cells from case II showed the following markers, OKT3—88%, OKT4—89%, OKT5—88%, OKT8—91%, OKT9—2%, OKT11a—0%. Furthermore, 83% of this same patient’s blasts were reactive with J-5, a mouse monoclonal antibody directed against CALLA (kindly given to us by Dr. Jerome Ritz of the Sidney Farber Cancer Center, Boston, Mass.).

REFERENCES

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