CONCISE REPORT
Recategorizing Childhood Acute Lymphoblastic Leukemia with Monoclonal Antibodies to Human T Cells
By Paul M. Sondel, Wayne Borcherding, Nasrollah T. Shahidi, Dorothy J. Ganick, John C. Schultz, and Richard Hong

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.

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 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.7

Rhesus erythrocyte rosettes. Spontaneous rosettes with rhesus erythrocytes (Flow Labs, McLean, Va.) at 37°C were assayed by the method of Chiao et al.8

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.7

Detection of thymocyte markers with monoclonal antibodies. Monoclonal antibodies to human thymocytes were obtained from Ortho Pharmaceuticals9 (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.3 For each test, 106 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.11 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.
**Table 1. Surface Markers on Neoplastic and Normal Lymphoid Cells**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Case I</th>
<th>Case II</th>
<th>Case III</th>
<th>Case IV</th>
<th>Thymus</th>
<th>Peripheral Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Yr)</td>
<td>3</td>
<td>2</td>
<td>14</td>
<td>80</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>WBC*</td>
<td>95,000</td>
<td>13,000</td>
<td>4,600</td>
<td>Female</td>
<td>77</td>
<td>98</td>
</tr>
<tr>
<td>Rhesus†</td>
<td>92</td>
<td>96</td>
<td>45</td>
<td>8</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>Sheep†</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>93</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>SMlg</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>OKT3‡</td>
<td>95</td>
<td>97</td>
<td>29</td>
<td>5</td>
<td>75</td>
<td>65</td>
</tr>
<tr>
<td>OKT4‡</td>
<td>96</td>
<td>96</td>
<td>93</td>
<td>3</td>
<td>82</td>
<td>42</td>
</tr>
<tr>
<td>OKT6‡</td>
<td>98</td>
<td>96</td>
<td>4</td>
<td>0</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>OKT8‡</td>
<td>95</td>
<td>98</td>
<td>95</td>
<td>2</td>
<td>78</td>
<td>30</td>
</tr>
</tbody>
</table>

*Peripheral blood leukocyte count at diagnosis.
†Percent rosette-forming cells with sheep or rhesus erythrocytes.
‡Percent fluorescent cells (see Materials and Methods).

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."3

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.12 Her low white count, and the absence of any mediastinal enlargement, appeared consistent with null cell as opposed to T-cell ALL.4 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 OKT8.13 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 prognostic14 and therapeutic15 implications of each distinct leukemic phenotype.
ACKNOWLEDGMENT

Our thanks to Dr. P. Dvorak for patient referral and to Grace Baums and Mona Moran for preparation of this manuscript.

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 1. 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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