Receptors for Peanut Agglutinin (Arachus hypogea) in Childhood Acute Lymphoblastic Leukemia: Possible Clinical Significance

By S. Levin, E. C. Russell, D. Blanchard, N. B. McWilliams, H. M. Maurer, and T. Mohanakumar

The presence of lymphocyte receptors for peanut agglutinin in significant numbers (>15%) was identified on leukemic cells from T-cell acute lymphoblastic leukemia (T-ALL) [3/4], B-cell ALL (B-ALL) [2/4], null cell ALL [8/17], and on normal fetal thymic lymphocytes but not on normal human peripheral blood lymphocytes. Peanut agglutinin (PNA) binding was blocked specifically on leukemic lymphoblasts and thymic lymphocytes by the addition of galactose to the medium. When all immunologic subgroups of ALL are combined, preliminary data suggest that of the 13 ALL patients having greater than 15% PNA-positive lymphoblasts, 8 had relapsed, whereas none of the 12 ALL patients with less than 15% PNA-positive cells have recurrent disease at this time. It is likely that analysis of PNA receptors on ALL lymphoblasts may be a useful adjunct to the existing clinical and immunologic prognostic indicators.

Materials and Methods

Patients and Test Cells

Lymphocytes were prepared from heparinized (25 U/ml) human blood using Ficoll-Hypaque density gradient centrifugation at 400 g for 20 min. Twenty-five patients with childhood ALL were examined in this study. Blast cells were obtained from diagnostic bone marrow aspirations at initial diagnosis (20 patients) or at relapse (5 patients), and all samples contained >90% blasts. (For clinical characteristics see Table 2.)
percentages of lymphocytes with receptors for PNA. Of the 17 null cell ALL patients, 8 had >15% PNA-positive lymphoblasts. While none of the null cell ALL patients with less than 15% PNA+ lymphoblasts have relapsed, 5 of the 8 with greater than 15% PNA+ lymphoblasts have relapsed. Of 4 patients with B-ALL, 2 had high numbers of PNA+ lymphoblasts (33% and 48%), and both of these patients have had relapses and died. The remaining 2 B-ALL patients with low values of PNA+ lymphoblasts have shown no evidence of relapse. Although the follow-up times have been short in this study as a whole, it is noteworthy to point out that there was no significant differences between the follow-up times of PNA+ and PNA− groups. When all ALL subgroups are combined, 13 had >15% PNA+ lymphoblasts, and 8 of these have relapsed, whereas only 1 of 12 patients with <15% PNA+ lymphoblasts have had recurrent disease at this time.

The sugar specificity of the PNA lectin used in this study was analyzed using both lymphoblasts from a null cell ALL patient and thymic lymphocytes, which

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**Table 1. Determination of PNA Receptor on Lymphocytes Prepared From Normal Human Peripheral Blood and Fetal Thymus Tissue**

<table>
<thead>
<tr>
<th>Target</th>
<th>Percent Immunofluorescence With Fluorescein Isothiocyanate-Conjugated PNA</th>
<th>Average Percent of Fluorescent Cells</th>
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</thead>
<tbody>
<tr>
<td>Peripheral blood lymphocytes</td>
<td>20</td>
<td>6.1%</td>
</tr>
<tr>
<td>Fetal thymus</td>
<td>5</td>
<td>34%-78%</td>
</tr>
</tbody>
</table>

Peripheral blood lymphocytes, with an average value of 6.1%. Except one, all other values were below 10%. Thymic lymphocytes, however, had a high percentage of PNA binding sites—34%–68%, with an average of 51%.

The detailed immunologic and clinical findings of the ALL patients studied are given in Table 2. ALL patients were subgrouped into T, B, and null based on receptors for E and immunoglobulins. Three of 4 patients with T-ALL (having mediastinal mass and receptors for sheep erythrocytes) also had high percentages of lymphocytes with receptors for PNA. Of the 17 null cell ALL patients, 8 had >15% PNA-positive lymphoblasts. While none of the null cell ALL patients with less than 15% PNA+ lymphoblasts have relapsed, 5 of the 8 with greater than 15% PNA+ lymphoblasts have relapsed. Of 4 patients with B-ALL, 2 had high numbers of PNA+ lymphoblasts (33% and 48%), and both of these patients have had relapses and died. The remaining 2 B-ALL patients with low values of PNA+ lymphoblasts have shown no evidence of relapse. Although the follow-up times have been short in this study as a whole, it is noteworthy to point out that there was no significant differences between the follow-up times of PNA+ and PNA− groups. When all ALL subgroups are combined, 13 had >15% PNA+ lymphoblasts, and 8 of these have relapsed, whereas only 1 of 12 patients with <15% PNA+ lymphoblasts have had recurrent disease at this time.

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**Table 2. Results of Clinical Immunologic Analysis of the ALL Patients**

<table>
<thead>
<tr>
<th>Patients (&gt;90% Blasts)</th>
<th>Clinical Course</th>
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<tbody>
<tr>
<td>Age at Diagnosis (yr)</td>
<td>Relapse (Site)</td>
</tr>
<tr>
<td>Race, Sex</td>
<td>Time to Relapse (mo)</td>
</tr>
<tr>
<td>WBC at Diagnosis (cu mm)</td>
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</tr>
</tbody>
</table>

PNA, peanut agglutinin; median follow-up 9 mo for both PNA+ and PNA− groups; E, sheep erythrocyte rosette; Slg, surface immunoglobulin; Ia, Human B-cell antigen; BM, bone marrow; CNS, central nervous system.

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*Cells tested at relapse, all others were analyzed at initial diagnosis.
†Deceased due to leukemia.
‡Died due to causes unrelated to leukemia.
had high numbers of PNA binding cells (92% and 78%, respectively). When galactose was added to the medium, blast cells and thymic lymphocytes showed a 50% reduction in their binding to PNA at 10% concentration and completely abolished the binding at a 30% sugar concentration. In contrast, glucose at a similar concentration had no significant effect on the percentage of cells binding PNA.

**DISCUSSION**

Determination of clinical findings such as initial WBC, age, tumor burden at diagnosis, race, sex, etc., and subgroups based on the immunologic markers (E, surface Ig) has become an integral part to the clinical management of children with ALL. Recently, subgroups of ALL within the null cell category have been identified, and those having T-lymphocyte antigens seem to have a poorer prognosis when compared to others. Thus, the immunologic classification of ALL is far from complete, and it is likely that further elucidation of immunologic markers may enable pretreatment predictions to correlate more closely with subsequent clinical course.

In a search for other cell surface markers with specificity for leukemic cells, PNA binding was examined. Our observation of receptors for PNA in significant numbers only on human fetal thymic lymphocytes but not on normal human blood lymphocytes (Table 1) correlates well with the experimental animal studies. Choice of PNA +/− threshold among ALL patients was made arbitrarily based on the observation that all of the normal values except one fell near 10% or below, and only one instance of 18% was noted. Analysis of ALL patients indicated that lymphoblasts of ¼ patients with T-ALL, some patients with the B-ALL (¼), and some with null cell ALL (¼) possessed significantly higher percentage of PNA+ cells. Three of the T-ALL patients are receiving intensive chemotherapy based on their expected poor prognosis and are currently in complete hematologic remission, although one patient has had a marrow relapse. A fourth patient with T-ALL has died following multiple relapses. In contrast, the presence of receptors for PNA among B and null cell ALL was a bit surprising. In this group of 10 patients with PNA+ lymphoblasts, 7 have relapsed and all have subsequently died. Only 1 of 12 patients with less than 15% PNA+ lymphoblasts has had a relapse. However, since the number of patients analyzed is small and follow-up time is relatively short, no meaningful statistical analysis could be done at this time, especially because all observations among the PNA-negative null ALL group are same. Among the null and B-cell ALL, no correlation was noted between the presence of PNA+ lymphoblasts and other clinical prognostic indicators. Human Ia-like antigens were detected on all the null and B-cell ALL and also on one of the two T-ALL analyzed. Cultured normal human T lymphoblasts have been shown to express Ia-like antigens, and it is likely that some of the T-ALL may also express Ia-like antigens. Based on PNA receptor studies on other tumor models, it is likely that the presence of PNA receptors on ALL lymphoblasts may be related to the cellular maturity. However, preliminary studies presented here indicate the potential usefulness of PNA receptor analysis of ALL lymphoblasts as an adjunct to other existing prognostic indicators.

**ACKNOWLEDGMENT**

We thank Rae Spivey and Fay Akers for their expert secretarial assistance in the preparation of the manuscript.

**REFERENCES**

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