Southwest Oncology Group Experience with Immunological Phenotyping in Acute Lymphocytic Leukemia of Childhood

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Abstract

The Pediatric Division of the Southwest Oncology Group is attempting to define subgroups of acute lymphoblastic leukemia (ALL) by laboratory delineation of marrow lymphoblast characteristics at diagnosis. Immunological subclassification studies include erythrocyte-forming rosette (E-rosette), C3 receptor, Fc receptor, surface immunoglobulin (Slg) and cytoplasmic immunoglobulin (Clg) determinations, as well as peripheral T (PT), thymocyte, la, and common ALL membrane antigen testing. Four subgroups of ALL have thus far been defined: null (Slg+, Clg", PT+); pre-B (Slg", Clg", PT+); B-cell (Slg+, Clg-, PT+); and T-cell (Slg-, Clg-, PT+). A group of 118 patients have had complete testing, and an additional 121 patients have had partial testing. Thirty-five patients with pre-B ALL and 27 patients with T-cell ALL have thus far been studied. Pre-B and null types of ALL appear closely related in multiple subclassification parameters (age, sex, physical findings, white blood cell count, and lymphoblast la and common ALL antigen positivity). Pre-B ALL patients treated with the same treatment regimens as were null ALL patients appear to have shorter durations of complete remission, although the difference has not yet reached statistical significance. Results suggest that the male predominance and older age distribution previously recognized for E-rosette-positive T-cell leukemia can also be expected in the E-rosette-intermediate and E-rosette-negative T-cell ALL subsets, although statistically higher white blood cell counts at diagnosis are seen in the E-rosette-positive subset. Mediastinal mass at diagnosis is most likely in the patient with E-rosette-positive, thymocyte antigen-positive lymphoblasts. The E-rosette-intermediate and E-rosette-negative, as well as the E-rosette-positive, T-cell ALL subsets appear to constitute poor prognosis categories. Correlation with treatment results is preliminary in all groups but suggests that stratification of ALL at diagnosis according to several immunological subgroupings may prove helpful in planning therapy.

Introduction

The Pediatric Division of the SWOG in May 1978 initiated the Pilot ALinC 13 Classification protocol (SWOG 7865), which utilizes multiple laboratory tests at the time of diagnosis of ALL. The purpose of the protocol is to establish subsets of ALL according to laboratory delineation of malignant cell characteristics. The premise is that increasing attention to laboratory description of subgroups of ALL will eventually result in better definition of the disease and thus serve to sharpen prognostic categories in ALL. The present report describes some of the findings to date in the 7865 protocol, with special emphasis on the immunological subclassification studies.

Prior to initiation of the 7865 classification protocol, the SWOG had previously had experience with more limited immunological laboratory evaluation for its new pediatric ALL patients. This consisted of E-rosette testing, which had been performed since 1976 on aspirated bone marrow cells from each newly diagnosed pediatric ALL patient (14).

Materials and Methods

Since May 1978, all pediatric patients at each member institution of the Pediatric Division of the SWOG have been studied at the time of diagnosis of ALL using the SWOG 7865 classification protocol. The first 10 months constituted an implementation period for establishing methodologies at each institution, so that some patients initially had incomplete testing. Since April 1979, all studies have been required for each patient registered.

The patients described in this report had complete information recorded at diagnosis concerning physical examination and X-ray findings. Complete peripheral blood counts and marrow differential counts were recorded. Only patients who had greater than 60% blasts in the marrow differential count are being utilized in this report. Marrow samples for study were obtained prior to transfusion of blood products and prior to initiation of chemotherapy. Bone marrow aspirates were stained with Wright’s-Giemsa, Sudan black, PAS, acid phosphatase, and nonspecific and chloroacetate esterase techniques. Patients with blasts showing Sudan black or esterase positivity were considered ineligible for the diagnosis of ALL. FAB classification was assigned by the majority vote of a 5-member morphology review committee, with each reviewer independently evaluating the slides (5).

E-rosette studies were performed at each institution utilizing a previously described standard methodological protocol (SWOG 7675), which evaluates bone marrow and peripheral blood samples at both 4°C and at 37°C (14). Bone marrow samples were studied at each institution for complement receptors using a standard zymosan methodology (20, 24) and more recently also for Fc receptors with a standard method using aggregated human γ-globulin (3). Levels of IgG, IgA, and IgM were quantitated in serum samples from each patient.

lymphoblastic leukemia; E-rosette, erythrocyte-forming rosette; PAS, periodic acid-Schiff; CALL, common acute lymphoblastic leukemia; PT antigen, peripheral T-antigen; Thy antigen, thymocyte antigen; CNS, central nervous system; Slg, surface immunoglobulin; Clg, cytoplasmic immunoglobulin.
After partial processing of marrow samples at each institution, preparations were sent to the University of Alabama immunology reference laboratory for testing to detect Slg and Clg. The immunofluorescent antisera staining techniques used for Slg and Clg determinations at the University of Alabama have been previously described (8, 31). Also, a bone marrow sample was sent immediately to the Duke University Immunology reference laboratory for testing to detect membrane antigens using xenoantisera. Cytotoxicity antisera testing, as previously reported by Metzgar, was utilized to identify the La, CALL, PT, and Thy antigens (21, 23). The preparation and utilization of the antisera in this study have been reviewed by Metzgar et al. (22). The membrane antigen termed PT antigen in this paper has previously been called T-lymphotocyte-associated antigen in other manuscripts (1, 2).

Treatment in the Pediatric Division of the SWOG for children with E-rosette-positive T-cell leukemia has been with intensive chemotherapy protocols. [The SWOG 7615 protocol, modeled after the Sloan-Kettering LSA-Lp protocol, was used from 1976 until April 1979 (32). The SWOG 7837 “T-cell 2” protocol was activated in April 1979.] Children with null or pre-B ALL or with E-rosette-negative T-cell leukemia have been treated with more standard ALL treatment regimens (SWOG 7823, ALinC 12). Before treatment randomization, the ALinC 12 protocol uses stratification on the basis of prognostic risk groups as defined primarily by age and WBC. All treatment arms include vincristine-prednisone induction, with or without asparaginase; CNS prophylaxis; and maintenance therapy with daily 6-mercaptopurine, weekly methotrexate, and periodic reinforcement with vincristine and prednisone. Consolidation therapy and the level of WBC maintained during remission maintenance therapy vary with the treatment arms.

Definitions utilized in this report as applied to marrow determinations are as follows: B-cell ALL, ≥10% Slg-positive blasts; pre-B cell ALL, ≥10% Clg-positive blasts; T-cell ALL, ≥40% PT antigen-positive blasts (i.e., 40% above the percentage of control lysis in cytotoxicity testing). “Null” cell ALL is the remaining group of patients.

E-rosette testing results are defined as follows: ‘‘definite positivity,’’ ≥40% E-rosette positive at 4°; ‘‘intermediate results,’’ >20% E-rosette positive but <40% E-rosette positive at 4°; ‘‘negative results,’’ ≤20% E-rosette positive at 4°; ‘‘Heat stable’’ indicates that the E-rosetting cells remain ≥40% at 37° incubation, while ‘‘heat labile’’ indicates that the E-rosetting cells decrease from ≥40% at 4° to <20% at 37°. ALinC 12 risk groups as used to delineate good and poor prognostic categories in the SWOG 7623 protocol are defined in Table 1.

The following statistical analysis methods were used. In comparing the means for the age and WBC data, the nonparametric Kruskal-Wallis one-way analysis-of-variance procedure was used (28). Except for the data involving remission times, all other analyses were of the usual x² contingency type with Fisher’s exact test used when necessary (27). The remission curves were plotted using standard life table procedures. Wilcoxon-Gehan tests were used to make comparisons between the curves (11). Table 3 shows the immunological subclasses identified within these 3 groups. This report will deal primarily with the patients who had complete testing (Group 1). Group 2 will be utilized to give additional information concerning pre-B leukemia, and Group 3 will be utilized to provide additional information concerning T-cell leukemia subsets.

Seventy % of the 35 pre-B ALL patients in Group 2 had >50% Clg-containing blasts; 91% of the patients had >25% Clg-containing blasts. One patient who had Slg and Clg testing is not shown in the tables because of difficulty with classification. This patient had 96.1% Clg-containing blasts but also had Slg demonstrated on 49.2% of the blasts.

Classification Results

Age and Sex. Tables 3 and 4 summarize classification findings for Groups 1 and 2, respectively, according to accepted prognostic risk factors. These tables demonstrate that the pre-B ALL patients did not differ from the null ALL patients in age or in sex distribution. However, the T-cell ALL patients were significantly older at diagnosis, and there was a significantly higher proportion of boys than in the null and pre-B groups.

WBC. Table 3 shows that the T-cell ALL patients had significantly higher WBC levels at diagnosis than did the null and pre-B groups. Tables 3 and 4 show that the pre-B patients had slightly higher WBC at diagnosis than did the null patients. This difference is not statistically significant in the smaller group of patients (Table 3) but does attain statistical significance in the expanded group (Table 4).
Mediastinal Mass and Extramedullary Disease. No pre-B or null ALL patient had a mediastinal mass at diagnosis, whereas 42% of T-cell ALL patients presented with a mediastinal mass (Table 3). Table 4 demonstrates that pre-B patients did not have a higher incidence of CNS or kidney involvement at diagnosis than did null patients. No pre-B or null patient had skin involvement or a leukemic mass present at diagnosis. Marked lymphadenopathy was present at diagnosis in only a small proportion of the patients in both the null and pre-B groups.

**ALinC 12 Risk Group.** No significant difference was demonstrated between pre-B and null ALL patients as to the percentage classified in the poorer prognostic risk group, i.e., Risk Group B, ALinC 12 definition (Tables 3 and 4). Table 3 shows that the T-cell leukemia group is approaching significance as having a higher proportion of patients in Risk Group B than do the null and pre-B groups.

**FAB Classification.** Table 5 shows the FAB classification assigned for Group 1 patients by the majority of the 5 members of the review committee. Some patients’ slides were determined by the committee to be of inadequate quality for FAB classification assignment. No correlation between FAB morphology and surface marker results was found in the null, pre-B, and T-cell ALL groups.

**PAS and Acid Phosphatase Staining.** Null, pre-B, and T-cell leukemia patients did not differ significantly in narrow PAS staining. T-cell leukemia was associated with a higher incidence of acid phosphatase positivity than were the null and pre-B groups (Table 5).

**Immunoglobulin Levels.** Table 6 shows serum IgG, IgM, and IgA levels at diagnosis in Group 1 patients. There was no significant difference in the proportion of patients showing decreased IgM or IgA in the null, pre-B, and T groups. Pre-B and null ALL patients did not differ significantly from each other in the proportion having decreased IgG, but together they were more likely to have decreased IgM than were the T-cell leukemia patients.

**Complement Receptors.** Null, pre-B, and T-cell ALL patients did not differ significantly in the percentage of blasts with complement receptors (Table 6). Only 3 of the 97 null and pre-B ALL patients tested had >20% of blasts with complement receptors (21.7, 24, and 29.7%, respectively).

**Fc Receptors.** Results for Group 1 patients who had Fc receptor testing are shown in Table 6. No significant difference was demonstrated in the percentage of blasts with Fc receptors in the null, pre-B, and T-cell groups.

**Ia and CALL Antigen Testing.** Results of Ia and CALL antigen testing for Group 1 null and pre-B patients are shown in Table 7. All null and pre-B patients tested had blasts which were Ia antigen positive. Ninety-four % of null patients and 90.5% of pre-B patients had blasts which were CALL antigen positive. As shown in Table 7, the patients in the smaller CALL-negative, null, and pre-B group had significantly higher WBC at diagnosis than did the larger CALL-positive group of patients. (Four of 7 CALL-negative, null, and pre-B patients in Group 1 had WBC >200,000/cu mm at diagnosis, whereas only 4 of 97 of the CALL-positive, null, and pre-B patients had WBC >100,000/cu mm.)

Analysis for CALL-negative relationships in the expanded group of patients who had antisera testing done (Group 3) showed that 6 of the 10 CALL-negative, non-T patients had WBC at diagnosis >200,000/cu mm; whereas only 12 of the 156 CALL-positive, non-T patients had WBC >100,000/cu mm. Another observation is that 4 of 10 of the CALL-negative, non-T patients were less than 12 months old at diagnosis, whereas only 2 of the 156 CALL-positive, non-T patients were less than 12 months old.

**T-Cell Leukemia Subsets.** Table 8 summarizes some of the classification findings in the Group 3 patients which includes all patients who had cytotoxicity antisera testing with techni-
cally satisfactory results. This group incorporates 27 T-cell leukemia, i.e., PT antigen-positive, patients and will be utilized to analyze T-cell leukemia subsets as defined by E-rosette testing. These subgroups are E-rosetting positive (16 patients),

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**Table 3**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Sex (M:F)</th>
<th>Mediastinal mass present</th>
<th>Age (yr)</th>
<th>WBC/cu mm (x 10^3)</th>
<th>ALinC 12 risk group (A:B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>83</td>
<td>46:37</td>
<td>0</td>
<td>5.97</td>
<td>0.08-16.17</td>
</tr>
<tr>
<td>Pre-B</td>
<td>21</td>
<td>12:9</td>
<td>0</td>
<td>5.94</td>
<td>0.92-13.08</td>
</tr>
<tr>
<td>B-cell</td>
<td>2</td>
<td>1:1</td>
<td>0</td>
<td>8.42</td>
<td>2.92-13.92</td>
</tr>
<tr>
<td>T-cell</td>
<td>10:2</td>
<td>5</td>
<td>5</td>
<td>9.51</td>
<td>3.58-17.42</td>
</tr>
</tbody>
</table>

**Table 4**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>WBC/cu mm (x 10^3)</th>
<th>Medialstitial mass</th>
<th>Extramedullary disease (CNS, Kidney, Other)</th>
<th>Risk group (A:B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>117</td>
<td>6.2</td>
<td>4.5</td>
<td>0.08-17.5</td>
<td>42.1</td>
<td>9.4</td>
<td>4:2</td>
</tr>
<tr>
<td>Pre-B</td>
<td>35</td>
<td>5.5</td>
<td>4.3</td>
<td>0.42-14.7</td>
<td>63.7</td>
<td>33.2</td>
<td>2:1</td>
</tr>
<tr>
<td>B-cell</td>
<td>3</td>
<td>8.8</td>
<td>9.4</td>
<td>2.9-13.9</td>
<td>35.5</td>
<td>29.7</td>
<td>0:2</td>
</tr>
</tbody>
</table>

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Table 5
Patients who had E-rosette, Slg, Clg, and cytotoxicity antisera testing (Group 1) classification according to lymphoblast-staining qualities

<table>
<thead>
<tr>
<th>Lymphoblast-staining qualities</th>
<th>No. of patients</th>
<th>FAB&lt;sup&gt;a&lt;/sup&gt; Positive</th>
<th>Weakly positive</th>
<th>Acid phosphatase stain&lt;sup&gt;b&lt;/sup&gt; Positive + negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>83</td>
<td>18</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td>Pre-B</td>
<td>21</td>
<td>6</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>B-cell</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T-cell</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not significant.
<sup>b</sup> Pre-B versus null, not significant; T versus pre-B and null, 0.025 < p < 0.05.

Table 6
Patients who had E-rosette, Slg, Clg, and cytotoxicity antisera testing (Group 1) classification according to immunoglobulin levels, zymosan complement receptors, and Fc receptors

<table>
<thead>
<tr>
<th>Serum IgG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serum IgM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serum IgA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Zymosan complement receptors (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Fc receptors (%)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inc&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NL</td>
<td>Dec</td>
<td>No. tested</td>
<td>Mean</td>
</tr>
<tr>
<td>Null</td>
<td>3</td>
<td>66</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Pre-B</td>
<td>0</td>
<td>15</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>B-cell</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>T-cell</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Decreased IgG: null versus pre-B, not significant; (null + pre-B) versus T, p = 0.0026.
<sup>b</sup> Decreased IgM; IgA: null versus pre-B versus T, not significant.
<sup>c</sup> Not significant.
<sup>d</sup> Inc, increased; NL, normal for age; Dec, decreased.

Table 7
Patients who had E-rosette, Slg, Clg, and cytotoxicity antisera testing (Group 1) classification according to la and CALL antigen testing (pre-B and null ALL patients)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of patients&lt;sup&gt;e&lt;/sup&gt;</th>
<th>WBC &lt;sup&gt;10&lt;sup&gt;12&lt;/sup&gt;&lt;/sup&gt;</th>
<th>% of Risk Group&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>la&lt;sup&gt;d&lt;/sup&gt;, CALL&lt;sup&gt;+&lt;/sup&gt;</td>
<td>78 (94.5)</td>
<td>35.9</td>
<td>12.3</td>
</tr>
<tr>
<td>la&lt;sup&gt;d&lt;/sup&gt;, CALL&lt;sup&gt;-&lt;/sup&gt;</td>
<td>5 (6.5)</td>
<td>319.1</td>
<td>241.0</td>
</tr>
</tbody>
</table>

<sup>d</sup> Pre-B CALL<sup>-</sup> versus null CALL<sup>-</sup>, not significant.
<sup>e</sup> CALL<sup>+</sup> versus CALL<sup>-</sup>, 0.005 < p < 0.01.
<sup>f</sup> CALL<sup>+</sup> versus CALL<sup>-</sup>, 0.025 < p < 0.05.
<sup>g</sup> Numbers in parentheses, percentage.

Table 8
Patients who had E-rosette and cytotoxicity antisera testing (Group 3) classification according to E-rosette-defined subgroups

| E-rosette-defined subgroup | WBC <sup>10^12</sup> | Acid phosphatase staining<sup>g</sup> Slightly positive + negative Risk Group<sup>f</sup> |
|---------------------------|-----------------------|-------------------------------------------------------------------|----------------------|
| Non-T                     | 158                   | 56.57 | 12.25 | 0.5–1000.0 | 26 | 117 | 74:82 |
| E<sup>a</sup>             | 16                    | 217.38 | 115.70 | 5.7–775.0 | 6 | 8 | 2:14 |
| E<sup>b</sup>             | 5                     | 112.36 | 4.0 | 2.4–290.0 | 0 | 4 | 2:3 |
| T-Cell                    | 27                    | 158.42 | 45.4 | 2.4–775.0 | 8 | 14 | 6:21 |

<sup>a</sup> T versus non-T, 0.01 < p < 0.025; E<sup>a</sup> versus E<sup>b</sup> versus E<sup>c</sup>, NS.
<sup>b</sup> T versus non-T, 0.005 < p < 0.01; E<sup>b</sup> versus E<sup>c</sup> versus E<sup>a</sup>, NS.
<sup>c</sup> T versus non-T, 0.025 < p < 0.05; E<sup>c</sup> versus E<sup>b</sup> versus E<sup>a</sup>, NS.
<sup>d</sup> Numbers in parentheses, percentage.

E-rosette intermediate (6 patients), and E-rosette negative (5 patients).

Table 9 shows the percentage of each E-rosette-defined subgroup which showed Th2 antigen positivity as well as PT antigen positivity. (Altogether, there were 12 Th2 antigen-positive patients and 15 Th2 antigen-negative patients.) Table 9 also shows the percentage of patients in each subgroup who demonstrated the usual T-cell blast characteristics of la antigen negativity and CALL antigen negativity. Table 8 shows again the preponderance of males in the entire T-cell group as compared to the non-T ALL group. No significant difference in male:female ratio was noted between
the 3 E-rosette subgroups. The T-cell ALL group as a whole had a significantly higher age at presentation than did the non-T group, with no significant differences in age among the E-rosette-defined subgroups.

The patients in the E-rosette-positive group had significantly higher WBC than did the E-rosette-intermediate and E-rosette-negative groups. The acid phosphatase stain was more often strongly positive in the T-cell group than in the non-T group, with no difference demonstrated among the E-rosette-defined subgroups as to acid phosphatase staining (Table 8).

A mediastinal mass was present in a significantly higher proportion of E-rosette-positive patients than in the E-rosette-intermediate and E-rosette-negative subgroups. However, the E-rosette-intermediate and E-rosette-negative subgroups did have a significantly higher incidence of mediastinal mass than did the non-T group (Table 8). Ten of the 12 Thy antigen-positive T-cell ALL patients had a mediastinal mass, while only 2 of the 15 Thy-negative patients had a mediastinal mass. It appears that the T-cell leukemia patients most likely to have a mediastinal mass at diagnosis are those whose blasts are E-rosette positive and Thy antigen positive.

ALiNC 12 prognosis risk group classification for the T-cell ALL patients is shown in Table 8. The T-cell group as a whole has a significantly higher proportion of patients in the poor prognosis group than does the non-T group, but there was no difference demonstrated between the E-rosette subgroups.

E-rosettes demonstrated heat stability at 37° in 13 of 16 (81%) of the E-rosette-positive ALL patients and showed heat lability in 3 of 16 (19%) patients. All 3 of the patients who had heat-labile E-rosettes had la-negative, CALL-negative blasts. Two of the 3 had Thy antigen positivity. One of the 3 had a mediastinal mass.

**Treatment Results**

Treatment results are preliminary since the range of follow-up for all patients is from 1 to 18 months as of January 28, 1980. Median duration of follow-up is 8.0 months. Treatment failure is defined as either failing to achieve remission or as developing relapse.

Treatment failures for Group 1 are 13% for the null group, 30% for the pre-B group, 100% for the B-cell group, and 33% for the T-cell group. Group 2 treatment failures are 12.7% for the null group, 26.4% for the pre-B group, and 100% for the B-cell group. Group 3 had 17% failures in the non-T group and 32% failures in the T-cell group. Within the T-cell group, there have been 27% failures in the E-rosette-positive subgroup, 40% failures in the E-rosette-intermediate subgroup, and 40% failures in the E-rosette-negative subgroup.

Charts 1 through 4 show the probability of maintaining initial complete remission with time for each of the groups and subgroups of patients. Pre-B patients now appear to be doing less well than the null group; however, the difference has not yet reached statistical significance (Charts 1 and 2). All of the relapses in the pre-B group have occurred in Risk Group B patients, so that it is within the poorer-prognosis subgroup that pre-B patients appear to be faring less well than the null patients. The T-cell ALL patients in Groups 1 and 3 are doing significantly less well than the non-T patients (p = 0.02 (Charts 1 and 3). There is no significant difference as yet in the duration...
of complete remission among the 3 E-rosette-defined subgroups (Chart 4).

No significant difference has been demonstrated as yet in the duration of complete remission of patients with decreased IgG, IgM, or IgA as compared to patients with normal immunoglobulin levels at diagnosis. The 3 patients from the null and pre-B groups who had >20% of blasts showing complement receptor positivity currently remain in complete remission at 10, 12, and 13 months, respectively.

Seven of the 10 CALL-negative, null, and pre-B patients continue in complete remission at intervals of from 4 to 17 months. One of the CALL-negative patients died at diagnosis; one did not attain remission; and one developed CNS and testicular relapse at 2 months.

Two of the 3 T-cell ALL patients who had heat-labile E-rosette positivity remain in complete remission at 2 and 8 months of follow-up. One developed bone marrow and CNS relapse at 4 months.

The relationship of the Thy antigen to treatment response has been as follows. The PT-positive, Thy-positive subgroup of 12 patients has had no induction failures and has had 2 relapses, with 10 patients continuing in remission at present. The PT-positive, Thy-negative subgroup of 15 patients has had 3 induction failures, 3 relapses, 2 early deaths, and 7 patients continuing in complete remission at present.

Discussion

The use of immunological markers in classification of ALL has been reviewed recently by several groups of investigators (13, 15, 17, 18, 29). The present report describes findings to date in the immunological subclassification studies of the SWOG 7865 leukemia classification protocol.

Pre-B ALL was initially described by Vogler et al. (31) in 1978, at the University of Alabama. Further reports have followed by Crist et al. (9) and Vogler et al., describing the collaborative efforts of the University of Alabama investigators and the SWOG in studying pre-B cell leukemia patients. The present report describes only those pre-B ALL patients studied since the SWOG 7865 protocol opened and therefore does not include the original group of patients described at Alabama. As suggested in the earlier reports, pre-B and null types of ALL appear very closely related as to age and sex distribution, physical findings at presentation, cytochemical staining characteristics, FAB classification, WBC at diagnosis, and early treatment response. The present report suggests that pre-B leukemia patients may have shorter durations of complete remission than do null ALL patients when treated with the same regimens. However, this suggested difference has not yet reached statistical significance.

One of the first pre-B ALL patients described at Alabama presented with a large scalp mass, which on biopsy showed leukemic infiltrate (31). Since the SWOG 7865 study opened, however, no additional pre-B patient has thus far been noted to have an extramedullary leukemic mass at the time of diagnosis of ALL. A recent report by Brouet et al. (6) described parotid involvement at diagnosis in 2 of 6 pre-B ALL patients. Parotid enlargement has not been noted to date in the pre-B patients registered on the SWOG 7865 study.

Studies done independently at Duke University and in collaboration with the SWOG have attempted to delineate the characteristics of the subgroups of T-cell leukemia (2, 19, 23). Although the numbers in each E-rosette-defined subgroup are small in the present study, the results suggest that the male predominance and older age distribution previously recognized for E-rosette-positive T-cell ALL patients can also be expected in the E-rosette-intermediate and -negative, PT antigen-positive subgroups. However, the high WBC often seen in E-rosette-positive T-cell ALL patients at diagnosis appears to be significantly less likely in the E-rosette-intermediate and E-rosette-negative subgroups of T-cell leukemia. The T-cell leukemia subgroup most likely to demonstrate a mediastinal mass at diagnosis appears to be the E-rosette-positive, T-antigen-positive subgroup.

E-rosette-positive T-cell leukemia has been previously demonstrated to have a decidedly worse prognosis than the non-T ALL group, with approximately 80% of patients relapsing within 12 months (4, 7, 12, 26, 30). Although the present report shows the T-cell leukemia group to be faring significantly worse than the non-T group, the E-rosette-positive T-cell leukemia patients still appear to be faring better during the first year of therapy than has been previously reported. This finding correlates with improved results demonstrated in preliminary analysis of the T-cell leukemia portion of the SWOG 7615 protocol. This suggests that more intensive treatment is improving the outlook for this poor-prognosis immunological subgroup of ALL patients.

The E-rosette-negative, PT-positive group of T-cell leukemia patients and most of the E-rosette-intermediate group have been treated in the SWOG with the same treatment regimens as those used for null cell leukemia patients. At the present time, the duration of remission curves for these small groups of patients appears to follow closely the E-rosette-positive curve.

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It remains to be seen whether therapy specifically designed for T-cell leukemia should be utilized for these small groups of patients.

The anti-PT serum used by the investigators at Duke University appears to be defining a broader group of T-cell leukemia patients than does the anti-Thy serum. The PT-positive, Thy-negative group of patients has had more treatment failures to date than has the PT-positive, Thy-positive group, so that Thy antigen negativity in the PT-positive patient does not appear to be a favorable prognostic sign.

Heat lability of E-rosette-positive blasts has been reported previously by SWOG investigators as representing a possibly favorable prognostic finding in T-cell leukemia (16). In the present report, the number of patients with E-rosette-positive, heat-labile blasts is too small and follow-up too short to determine whether E-rosette heat lability is a favorable finding for T-cell leukemia patients.

Both complement receptor positivity and Fc receptor positivity of blasts in null cell ALL have been suggested previously by Richie et al. (24) and by Esber et al. (10) as constituting possibly poor prognostic findings. In the present report, there were too few null ALL patients with complement receptor or with Fc receptor positivity and too small a follow-up of these patients to add information concerning this observation.

The fact that all null and pre-B ALL patients in this study had blasts which expressed the la antigen plus the demonstrated close relationship in multiple parameters of the null and pre-B groups suggest that the majority of childhood ALL patients have blasts which may be of the B-cell lineage. The patient whose blasts showed cytoplasmic and surface immunoglobulin was interpreted as having a pre-B, transitional to B-cell type ALL.

Greaves et al. have shown that CALL antigen testing in non-T, non-B ALL patients defines a small group of patients with CALL-negative blasts which differs in many respects from the larger CALL-positive group (17, 25). The present report shows that the null and pre-B ALL patients with CALL-negative blasts have had significantly higher WBC at diagnosis than have the null and pre-B ALL patients with CALL-positive blasts. In addition, a significantly higher proportion of patients in the CALL-negative, null, and pre-B ALL group has been less than 12 months old at the time of diagnosis.

The SWOG is demonstrating that detailed immunological laboratory testing of all newly diagnosed pediatric ALL patients is possible within the cooperative group structure. Results to date in the SWOG 7865 study appear to be leading to more definitive classification of types of pediatric ALL. Correlation with treatment results is preliminary but suggests that stratification of ALL at diagnosis according to additional immunological subgroups may prove helpful in planning therapy.

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Southwest Oncology Group Experience with Immunological Phenotyping in Acute Lymphocytic Leukemia of Childhood


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