Clinical Features and Outcome in Childhood T-Cell Leukemia-Lymphoma
According to Stage of Thymocyte Differentiation:
A Pediatric Oncology Group Study

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The immunophenotypes of lymphoblasts from children with newly diagnosed T-cell acute lymphoid leukemia (T-ALL; n = 101) or T-cell non-Hodgkin lymphoma (T-NHL; n = 31) were analyzed to correlate stage of thymocyte differentiation with clinical features and outcome. The 67 boys and 34 girls with T-ALL were 1 month to 14 years old (median, 6 years) with leukocyte counts ranging from 2 to 810 x 10^9/L (median, 55 x 10^9/L). Eighteen of these patients were black, and 70 had a mediastinal mass. Twenty-six boys and five girls with a median age of 9 years (range, 1 to 20 years) had T-NHL. Seven of these patients were black, and 24 had a mediastinal mass. The distributions of thymocyte developmental stages (early [CD7+], intermediate [CD1+ and/or CD4+ and/or CD8+], and mature [CD3+] in cases of T-ALL and T-NHL were significantly different: 34%, 43%, and 23% vs 6%, 62%, and 32% (P = .02). A comparison of the patients’ clinical features according to the maturational stage of thymocytes failed to disclose significant differences in the majority of characteristics studied. However, patients with mature-stage T-NHL, with or without the addition of subjects with mature-stage T-ALL, were less likely to have a mediastinal mass (P = .02 for both comparisons). Those with intermediate-stage T-cell malignancy (T-ALL and T-NHL combined) were the subgroup most likely to have a mediastinal mass (P = .01). Response to remission induction therapy was significantly worse in the T-ALL subgroup with an early-stage phenotype; a failure rate of 21% vs 0% and 6% for the two more differentiated phenotypic subgroups (P = .007). Event-free survival was not affected by thymocyte maturational stage in cases of either T-ALL or T-NHL. Despite evidence of clinical heterogeneity among the maturational stages of T-cell malignancies in children, these developmental subdivisions do not appear to be critical determinants of outcome once remission is achieved. We conclude that such phenotypes need not be included in the stratification plans for clinical trials using common induction treatment.

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T-ALL entered complete remission (CR) less often than did the others, there was no indication that thymocyte developmental stage influenced event-free survival.

MATERIALS AND METHODS

Clinical Material

T-ALL was diagnosed in 101 patients by the following criteria: infiltration of bone marrow by >25% malignant blasts and either >40% sheep erythrocyte rosette-forming blasts (ERFC) in marrow or blood and/or 40% above control lysis of marrow or peripheral blood blasts on cytotoxicity testing with heteroantisera against pan-T (pT) antigens.\textsuperscript{11,12} ERFC determinations were made at local institutions with quality-controlled standard techniques; pT markers were assessed at the Duke University Reference Laboratory for all T-ALL cases diagnosed between December 1980 and January 1986 as part of the Pediatric Oncology Group (POG) 7865 and 8035 classification studies.\textsuperscript{13,14} Approximately one third of these cases also had a cell suspension submitted for thymocyte subclassification studies as a part of the POG 8080 study, and they are the subject of this report. Fifty-six percent of these patients had >85% marrow blasts, and 44% had from 25% to 85%. For patients with T-ALL, bone marrow aspirates (>25% blasts, n = 95), peripheral blood samples (>90% blast involvement, n = 6), or both (n = 21) obtained at the time of diagnosis were available for surface marker analysis.

Thirty-one patients were diagnosed as having NHL by histologic examination of tumor biopsy specimens. Specimens obtained at the time of initial diagnosis from peripheral or mediastinal lymph nodes and/or malignant cells from other sites (eg, pleural effusion, spinal fluid, and skin) were subclassified as a part of the POG 8080 study. Staging was performed according to the system of Murphy.\textsuperscript{15}

The T-cell origin of lymphomas was indicated if >50% of the cells formed rosettes with sheep erythrocytes and/or expressed CD2 and if <10% of the cells expressed sIg (B cells), C\textsubscript{\gamma} heavy chains (pre-B cells), or B-lineage-specific antigens (CD19, CD20, or both) in a single-cell suspension prepared from tumor specimens. Histologically, all tumors were characterized by complete effacement with malignant-appearing blastlike cells.

Lymphocyte Surface Antigens

Preparation of mononuclear cells. Heparinized marrow was treated with Tris-ammonium chloride-buffered lysing solution to remove RBCs. Single-cell suspensions of tumor biopsy specimens and heparinized venous blood samples were diluted with Hank's balanced salt solution and passed over a Ficoll-Hypaque density gradient. Mononuclear cells isolated by these methods were washed, incubated to remove cytophilic immunoglobulin, and resuspended for sheep erythrocyte-rosetting and immunofluorescence analyses.

Monoclonal antibody analysis. Leukemic cells were analyzed by indirect immunofluorescence with a panel of monoclonal antibodies for lineage assignment and maturational staging. The T-lymphocyte surface antigen phenotype was established with the use of monoclonal antibodies against antigens present on mature thymocytes and all peripheral blood T cells: CD7, CD5 (T1), CD2 (T11), the sheep erythrocyte receptor, CD3 (T3), CD4 (T4), CD8 (T8), CD1 (T6), the transferrin receptor (TR), and CD38 (T10). Antibodies were obtained from Becton Dickinson, Sunnyvale, CA, and Ortho Diagnostic Laboratories, Raritan, NJ. All cases had blasts lacking C\textsubscript{\gamma} and CD19 (B4) antigens. Mononuclear cells (5 \times 10\textsuperscript{5}) were incubated with 10 \muL of the specific monoclonal antibody for 15 minutes, washed, and incubated with 10 \muL of purified fluorescein-conjugated goat antimouse IgG. Results were scored as positive when greater than 30% of the cells stained above background levels with fluorochrome-labeled antibodies as detected by flow cytometric analysis (FACS-Analyzer, Becton Dickinson) and by fluorescence microscopy.

Criteria for Thymocyte Subclassification

Because the number of cells retrieved from patients varied widely, not all samples were studied with the entire panel of monoclonal antibodies. The criteria for classifying the developmental stages of T cells were modified slightly from those previously published by Reinherz et al.\textsuperscript{18} Cases considered to have the early thymocyte phenotype expressed CD7, CD2, CD5, CD38, and TR and were negative for CD1, CD4, CD8, and CD3. Those with a common or intermediate phenotype were characterized by the expression of CD7, CD5, CD1, and CD2 with variable expression of CD38, TR, CD4, and CD8. In many cases, the malignant cells expressed both CD4 and CD8. In the mature thymocyte subgroup, all samples were positive for CD3, CD5, CD7, and CD2. CD4 and CD8 were present in some cases but always appeared singly rather than in combination.

Treatment

The majority of patients with T-ALL (n = 79) received identical therapy, and our major conclusions regarding outcome are based solely on this group. These 79 patients were treated in the second part of POG 7837 as shown in Fig 1. This therapy adds intensified CNS prophylaxis to the LSA1L2-based protocol (POG 7615), which has been reported previously.\textsuperscript{17,18} Fifteen patients with T-ALL were treated in the first part of POG 7837 (unpublished POG T2 multidrug regimen), and seven patients were treated according to other combination chemotherapy protocols.

Twenty-five of the 31 patients with T-NHL were treated by the POG 7905 protocol, one arm of which used the LSA1L3 regimen as in POG 7615 (14 patients)\textsuperscript{19}, the other incorporated the ACOP+ regimen (11 patients).\textsuperscript{20} Six children were treated in other studies of combination chemotherapy.

Since enrollment in the T-cell subclassification study (POG 8080) was not mandatory for inclusion in the therapeutic studies, about two thirds of all children with T-cell malignancies treated during the 5-year span covered by the POG 8080 subclassification study (December 1980 through January 1986) were not included in this report. Although we cannot rule out a selection bias, the treatment outcome and clinical characteristics of T-ALL or T-NHL patients enrolled in the POG 8080 subclassification study and treated in POG 7837, 7905, or 7615 are not significantly different from those of similarly treated patients who were not registered in POG 8080 (comparison of event-free survival, P = .83 for T-ALL and P = .26 for T-NHL).

Definitions of Treatment Response

CR was defined as <5% bone marrow blasts and no evidence of extramedullary leukemia. CNS relapse was defined as >10 mononuclear cells/\muL CSF, with blasts demonstrated on cytospin examination. CR for patients with T-NHL was defined as the absence of signs of disease on physical examination, bone marrow aspirate, and normal imaging procedures (ultrasonography or computed axial tomography). Event-free survival is defined as the interval between diagnosis and any event including induction failure, relapse at any site, or death. Treatment was stopped after 3 years of CR for patients with T-ALL or after 2 years for those with T-NHL. All patients failing to achieve a CR or who developed recurrent disease at any site were removed from the study.
**T CELL SUBTYPING**

Remission induction: cyclophosphamide (CP), 1,200 mg/m² intravenously (IV) on day 1; vincristine (VCR), 2 mg/m² (maximum, 2 mg) IV weekly for four doses beginning on day 3 or 4; prednisone (PRED), 60 mg/m² (maximum, 60 mg) orally (PO) beginning with VCR and continuing for 28 days, with seven days of decremental dosage; daunorubicin (DNR), 60 mg/m² IV on two successive days beginning 2 weeks after CP. In patients with high WBC counts, CP following each dose of ARA-C; L-asparaginase (A-ASE), 6,000 dosage; daunorubicin (DNR), 60 mg/m² IV on successive days (five-day cycles with ARA-C), 1.500 mg/m² IV or intramuscularly (IM) for five consecutive days of each of four weeks; thioguanine (TG), 50 mg/m² PO eight to 12 hours following each dose of ARA-C; l-asparaginase (A-ASE), 6,000 U/m² IV or IM daily for 14 days; bis-nitrosourea (BCNU), 60 mg/m² IV within three days after the last dose of A-ASE. Treatment is delayed until ANC > 1,000/μL. Maintenance (five-day cycles with seven- to ten-day intervals between cycles): cycle I—TG, 300 mg/m² PO daily for four days, followed by CGP, 600 mg/m² IV on day 5; cycle II—hydroxyurea (HU), 2,400 mg/m² PO daily for four days, followed by DNR, 45 mg/m² IV on day 5 (when the maximum dose of 480 mg/m² is reached, substitute CP, 600 mg/m² IV on day 5); cycle III—methotrexate (MTX), 10 mg/m² PO daily for four days, followed by BCNU, 60 mg/m² IV on day 5; cycle IV—ARA-C, 150 mg/m² IV for four days, followed by VCR, 2 mg/m² IV (maximum, 2 mg) on day 5. Cycles repeated in this order throughout maintenance therapy—cycles I through IV, one course. CNS prophylaxis: Cranial irradiation (RT) beginning 1 week before the initiation of systemic consolidation therapy, 2,400 cGy for 12 treatment days (5 days each week); triple intrathecal therapy (TIT)—MTX, 15 mg/m² (maximum, 15 mg); hydrocortisone (HC), 15 mg/m² (maximum, 15 mg); ARA-C, 30 mg/m² (maximum, 30 mg), administered on day 1 of induction (or within the first week), twice weekly for four doses just after remission is achieved concomitantly with CNS RT, on the day BCNU is administered at end of consolidation, and on day 5 of each cycle IV during maintenance therapy.

**Statistical Considerations**

Quantitative clinical features such as age and WBC count among the three phenotypically defined subgroups of T-ALL patients were compared with the Kruskal-Wallis test. The same procedure was used to examine differences between the early plus intermediate or mature thymocyte subgroups of T-NHL. The early and intermediate classifications were combined because only two T-NHL patients had early-stage thymocytes. Qualitative clinical features such as sex, race, and mediastinal mass were compared by use of the Pearson chi-square test. If expected cell frequencies were deemed inadequate for the Pearson chi-square approximation, an exact conditional chi-square was used. Life tables were constructed by the method of Kaplan-Meier and analyzed with the Mantel-Haenszel (log rank) statistic.

**RESULTS**

Cell samples from 101 patients with T-ALL and from 31 with T-NHL were studied at the time of diagnosis. The distribution of thymocyte maturational stages disclosed by immunophenotyping together with the clinical characteristics of the patients is shown in Table 1.

**T-ALL**

Thirty-four of the 101 T-ALL samples analyzed were designated as the early thymocyte type. Tumor cells from these samples lacked expression of CD1, CD4, CD8, and CD3, but all expressed CD7 and CD2. Of 93 samples additionally analyzed for TR and/or CD38, 76 expressed one or both markers. Only six (24%) of 25 specimens tested were ERFC+.

Forty-three specimens were classified as intermediate-stage thymocytes. All samples in this group expressed CD1, and most were simultaneously positive for CD4 and CD8 or expressed a combination of these antigens. Thirty-five of 41 intermediate-stage thymocyte samples studied for TR and CD38 expressed one or both of these surface antigens. Twenty-nine (71%) of 41 cases with an intermediate-stage phenotype were ERFC+. By definition, all of these samples lacked CD3.

In the final subset of 24 clinical samples, designated as mature thymocytes, all cells expressed CD3 antigen but showed variable expression of CD4, CD8, TR, and CD38. CD4 and CD8 were always detected individually rather than in combination on the same cells; this was in contrast to the typical pattern in cases with an intermediate-stage phenotype where the two antigens were more often coexpressed. Eleven (55%) of 20 samples tested expressed the ERFC receptor. There was a significant difference in the proportion of cases expressing ERFC receptors among the three subtypes of T-ALL (P < .01); the lowest fraction, 24%, was associated with early-stage thymocytes.

**T-NHL**

By using the same criteria to define the pattern of surface antigen expression in 31 T-NHL samples, we identified two cases with an early-stage phenotype, 19 with an intermediate stage, and 10 with a mature stage. The majority of centrally reviewed cases were histologically classified as lymphoblastic lymphoma, regardless of maturational stage. However, one example of large-cell morphology and one of mixed lymphocytic-histiocytic morphology were observed. At least one case of each of the three maturational stages of malignancy was observed among the 22 children with lymphoblastic histology.

The distribution of patients according to clinical stage was: stage II, one; stage III, 17; and stage IV, eight. Staging was uncertain in five patients. The relatively high proportion of children without adequate tissue for immunophenotyping is: stage II, one; stage III, 17; and stage IV, eight. Staging was uncertain in five patients.
precluded any assessment of the relationship among clinical features and thymocyte maturational stage in patients with T-NHL.

Clinical Correlates of Thymocyte Maturational Stage

To determine whether clinical features and outcome could be associated with the level of T-cell differentiation, we divided patients with T-ALL and T-NHL into early, intermediate, and mature subgroups on the basis of their patterns of surface antigen expression. Comparison of selected clinical features including age, WBC level (T-ALL only), male-female ratio, and black-other ratio revealed no significant differences. However, children with T-ALL were significantly more likely to present with the early thymocyte phenotype than were children with T-NHL ($P = .02$). Patients with mature-stage T-NHL, with or without the addition of similar T-ALL cases, were significantly less likely to have a mediastinal mass than were such patients whose blasts were in the early or intermediate category ($P = .02$). The greatest likelihood of having a mediastinal mass was in the intermediate-stage subgroup (T-ALL and T-NHL combined) ($P = .01$).

Clinical Outcome

Three patients with T-ALL were excluded from analysis because they lacked adequate follow-up information. The CR rate for T-ALL patients treated on the LSA2L2 study (POG 7837) was 92% (73 of 79); for all T-ALL patients, regardless of treatment, it was 92% (93 of 101). The failure rate during induction chemotherapy was highest in patients with an early stage of thymocyte development: 18% as compared with 2% and 4% for subjects with the intermediate and mature stages, respectively ($P = .047$). This association was stronger when the analysis was repeated with the 79 children who had received identical therapy (LSA2L2, POG 7837, Fig 1): five of 24 (21%) for the early subgroup versus 0 of 38 and 1 of 17 (6%) for the others ($P = .007$). Regardless of whether patients with T-ALL were treated uniformly or on different protocols, thymocyte developmental stage had no appreciable influence on event-free survival ($P = .74$, Fig 2). Similarly, there were no apparent differences in clinical outcome among patients with T-NHL when grouped according to maturational stage (see Fig 3).

The sites of failure for all patients with T-ALL or T-NHL are shown in Table 2. For patients with T-ALL, the largest proportion of failures occurred in the marrow, with or without involvement of extramedullary sites. When compared by thymocyte maturational stage, patterns of failure after the attainment of CR were similar.

DISCUSSION

In this study of a large cohort of children with T-ALL, we were able to compare presenting clinical features and treatment outcome with recognized maturational stages of the patients' blast cells. The majority of characteristics showed no relationship to the early, intermediate, and mature classifications of thymocyte development; however, mediastinal masses were more often associated with an intermediate-stage phenotype in cases of T-NHL and cases of T-NHL and T-ALL combined. Further, patients with early thymocyte...
malignancies were less likely to achieve CR during aggressive induction therapy than were groups with other maturational phenotypes. Of greater interest was the apparent lack of influence of thymocyte maturational stage on event-free survival (Figs 2 and 3). It should be emphasized that this observation was based on relatively small numbers of intensively treated subjects, which may have precluded the demonstration of significant differences among the three phenotypic subgroups.

Our findings of clinical heterogeneity and differences in response to induction therapy among subgroups of patients with T-cell malignancies are not as striking as results for the B-lineage malignancies of childhood. In particular, clinical outcome in the latter group becomes progressively worse with increasing stage of cellular development (early pre-B → pre-B → B), and the pre-B immunophenotype has been shown to have an independent negative influence on prognosis. Further, specific karyotypic differences have been demonstrated among the developmental subtypes of B-lineage malignancies but not among those of T-ALL. Conceivably, the “windows” of blast cell maturation being used to subclassify cases of T-cell malignancy are less discriminating than are those being applied to B-lineage ALL. If so, one might expect considerable overlap or blurring of characteristics that would distinguish one T-cell subgroup from another. Alternatively, the power of level of cellular differentiation to predict outcome noted for the B-lineage leukemias simply does not hold for the T-cell malignancies.

The failure to show relationships between age, gender, race, or leukocyte count at diagnosis and stage of thymocyte maturation in either T-ALL or T-NHL parallels experience with the pre-B and early pre-B malignancies of childhood. However, a recent analysis of a large prospective randomized clinical trial has revealed significant differences in leukocyte count and the incidence and type of karyotypic abnormalities within the B-progenitor subset of patients with ALL that were not apparent in earlier studies, including relatively small numbers of patients.

Our demonstration that the early-stage thymocyte phenotype occurs more often in T-ALL than in T-NHL substantiates earlier impressions of differences in the distribution of maturational stages between these two disease presentations. Only two (6%) of 31 patients with T-NHL as compared with 34 (34%) of 101 with T-ALL had the early thymocyte phenotype. The most common maturational phenotype in both T-ALL and T-NHL was the intermediate stage (43% and 62%, respectively). Moreover, the proportion of T-NHL patients with the mature thymocyte phenotype exceeded that in the T-ALL group (32% vs 23%), which confirms previous reports. Malignant thymocytes with an early stage of maturation appeared to involve bone marrow and blood sooner than did cells in other stages. That a majority of cells within the thymus gland are intermediate-stage thymocytes would explain, simply on a stochastic basis, the high association between this developmental compartment and the presence of a mediastinal mass.

It was also possible in this study to define the relationship of histopathology to maturational stage in childhood T-NHL. Most cases were of the lymphoblastic type, which is consistent with previous general observations. Interestingly, we observed two cases of mature or intermediate thymocyte malignancy that had mixed lymphocytic-histiocytic or large-cell histologic features. Finally, there was a significant difference in the incidence of blast cell expression of ERFC receptors according to stage of thymocyte maturation: blasts in cases of T-ALL with an early thymocyte phenotype
expressed these receptors much less commonly than did cases with other phenotypes. No obvious explanation for this result is apparent since all three stages of normal thymocyte development are expected to express ERFC receptors.

Despite evidence for clinical diversity among the maturationally defined subgroups of T-ALL and T-NHL, the impact of these findings on treatment outcome appears small. Thus, unlike the situation in the B-lineage leukemias of childhood, the developmental stage of leukemic T lymphoblasts does not need to be considered in the stratification of patients for clinical trials that use uniform induction treatment.

REFERENCES

### Appendix. Principal Investigators of POG Participating in Study No. 8080

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