Subset Derivation of T-Cell Acute Lymphoblastic Leukemia in Man

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ABSTRACT Normal human peripheral blood T cells can be characterized as belonging to either the TH_2^+ or TH_2^- T-cell subset. Approximately 20% of T cells are TH_2^+, whereas 80% are TH_2^- utilizing specific heteroantisera. To determine whether human T-cell acute lymphoblastic leukemia (T-ALL) cells belong to one or another T-cell subset, cell surface phenotyping was performed on tumor populations from 25 patients with T-ALL. Tumor cells from these 25 individuals were either TH_2^+ or TH_2^-, but not both. 5 of 25 patients had TH_2^+ T-ALL cells. These TH_2^+ tumor populations were found exclusively in children and often without an accompanying thymic mass. TH_2^- T-ALL, in contrast, occurred in both children and adults and was almost always associated with thymic enlargement. Although children with TH_2^+ T-ALL had as high or higher peripheral blast counts on presentation than their TH_2^- T-ALL counterparts, overall survival was greater for the TH_2^+ group (>36 mo) than the TH_2^- group (<12 mo). These studies demonstrate that T-cell leukemias in man arise from distinct T-cell subsets and that cell surface characterization of T-cell malignancies may provide useful clinical data related to prognosis.

INTRODUCTION

Immunologic classification of lymphoid malignancies, especially acute lymphoblastic leukemia (ALL),[1] has been the subject of recent intense investigation. Insight into the cellular origin of ALL has resulted from the elucidation of normal lymphocyte differentiation antigens and their relationship to neoplastic cells (1–8). Approximately 20% of children with ALL have lymphoblasts which bear sheep erythrocyte rosette receptors and T-cell antigens identified with specific heteroantisera (9–17). Almost all of the remaining patients possess lymphoblasts which lack T-cell markers and conventional B-cell markers such as surface immunoglobulins but have either cell surface leukemia-associated antigens or HLA-D locus-related alloantigens (8, 18). Less than 5% of patients have tumor cells which display surface immunoglobulin (19). Correlative clinical studies in individuals with these lymphoid leukemias have shown that T-ALL patients are more often male and generally present with high blast cell counts and mediastinal enlargement secondary to tumor mass. More importantly, both disease-free intervals and overall survival are shorter in this group than in patients with non-T-ALL (16, 17, 20).

Considerable evidence now exists supporting the notion of T-cell heterogeneity as defined by cell surface markers in both human and murine systems (21–27). This heterogeneity has been useful in detecting distinct T-cell subsets with unique effector or regulatory function. For example, with the use of heteroantisera, two human peripheral blood T-cell populations have been defined, termed TH_2^+ and TH_2^- (24). The TH_2^+ subset, which accounts for ≅20% of peripheral blood T cells, contains both the cytotoxic effector and suppressor cell populations (24, 27). In contrast, the TH_2^- subset, comprising 80% of peripheral blood T cells, provides a helper function in a variety of systems.

Given the heterogeneity of normal T cells and the clinical heterogeneity of T-cell leukemias (28), it seemed important to test whether T-ALL arises from one or both T-cell subsets. Consequently, cell surface phenotyping was performed on T-ALL tumor populations with subset-specific heteroantisera. In the studies to be reported below, it is demonstrated that a single T-ALL tumor population may arise from either

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[1] *Abbreviations used in this paper*: ALL, acute lymphoblastic leukemia; E, erythrocyte; T-ALL, T-Cell ALL; TH_2^+, TH_2^-, human peripheral blood T-cell populations.

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the TH_2^+ or TH_2^- subset, but not both. More importantly, both the clinical features and course of patients with tumors arising from these distinct subsets of T cells appears to be different.

METHODS

Detection of ALL of T-cell lineage. Approximately 150 patients ranging in age from 1 mo to 90 yr were diagnosed as having ALL over a 3 yr period beginning in January 1975. This diagnosis was established utilizing standard cytologic and histochemical criteria (29). Tumor cells were obtained from peripheral blood and (or) bone marrow of all patients at the time of diagnosis. The heparinized bone marrow and blood samples were collected and tumor cells harvested by Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) density sedimentation (30). Isolated cells were stored at −196°C in vapor-phase liquid nitrogen in 10% dimethylsulphoxide and 20% AB human serum until the time of surface characterization. The tumor populations analyzed were >90% blasts by Wright-Giemsa morphology in all instances.

Initial characterization of the tumor population from 25 of these patients showed them to be spontaneous sheep erythrocyte (E) rosetting cells by standard technique (31). All tumor populations were ≧20% E rosette positive and therefore of T-cell lineage. In addition, these same 25 tumor cell populations were reactive with two T-cell-specific rabbit heteroantisera, termed anti-HTL (anti-B.K.) and A99, utilizing immunofluorescence on a fluorescence-activated cell sorter as previously described (8, 32). Anti-HTL reacts only with leukemic human T cells and normal human thymocytes. In contrast, A99 reacts with all human cells of T lineage including peripheral blood T lymphocytes, but it is unreactive with normal B cells, Null cells, macrophages, and non-T leukemics.

Treatment of the patients with T-ALL characterized in this study will be the subject of a later report.

Equine anti-TH_2^+ preparation and specificity. Preparation of equine anti-TH_2^+ has been the subject of a previous report (27). In brief, ATG_2 (lot 16138-18, The Upjohn Company, Kalamazoo, Mich.) was made T cell-specific after extensive absorptions with human AB^+ erythrocytes and human B cells. Subsequently, it was further absorbed with 4.5×10^9 TH_2^- T-ALL tumor cells per ml of sera. The resultant antisera reacted only with the TH_2^+ T-cell subset.

Preparation of normal human T cells. Human peripheral blood mononuclear cells were isolated from healthy volunteer adult donors by Ficoll-Hypaque density gradient centrifugation. Unfractionated cells were then separated into surface Ig^+ (B) and Ig^- (T plus Null) populations by Sephadex G-200 anti-$F(ab')_2$ column chromatography as previously described (33). T cells were recovered by E rosetting the Ig^- population with 5% sheep erythrocytes (Microbiological Associates, Walkersville, Md.). The rosetted mixture was then layered over Ficoll and the E^+ (T cells) pellet treated with 0.155 M NH_4Cl. This recovered T-cell population was >95% E rosette positive. T cells so obtained were cryopreserved in vapor-phase liquid nitrogen as above.

Analysis of normal T cells and T-ALL with heteroantisera. $1-2 \times 10^6$ normal T cells or T-ALL tumor cells were thawed and washed extensively. Viability exceeded 85% on all populations at the time of study. Cells were first treated with 0.15 ml equine anti-TH_2 at a dilution of 1/125, incubated at 4°C for 30 min, and then washed twice. The cells were subsequently reacted with 0.15 ml of a 1/50 dilution of rabbit IgG fraction anti-horse IgG (R/H fluorescein isothiocyanate, N. L. Cappel Laboratories Inc., Cochranville, Pa.) for 30 min, centrifuged, washed three times, and analyzed on the fluorescence-Activated Cell Sorter (Becton, Dickinson, FACS Systems, Mountain View, Calif.) as previously described (27). Background staining was obtained by substituting 0.15 ml of a 1/125 dilution normal horse IgG for specific antibody.

RESULTS

Normal human T cells or T-ALL tumor cells were reacted with equine anti/TH_2 and stained with R/H fluorescein isothiocyanate as described. Subsequently, the cells were characterized on the fluorescence-activated cell sorter by obtaining a fluorescence profile of 40,000 individual cells and the results expressed in a composite fluorescence histogram. As shown in Fig. 1, the normal peripheral blood T-cell compartment is heterogeneous because ≅80% of human T cells are nonreactive (TH_2^-), whereas 20% are reactive (TH_2^+) with anti-TH_2. In contrast, Fig. 2 shows that T-ALL tumor populations contain leukemic cells which are all either TH_2^+ (Fig. 2A) or TH_2^- (Fig. 2B). Both TH_2^+ and TH_2^- T-ALL cells are morphologically indistinguishable.

Table I compares the clinical features of children with TH_2^+ and TH_2^- T-ALL. As indicated, only 5 of 18 children with T-ALL had leukemic cells which were

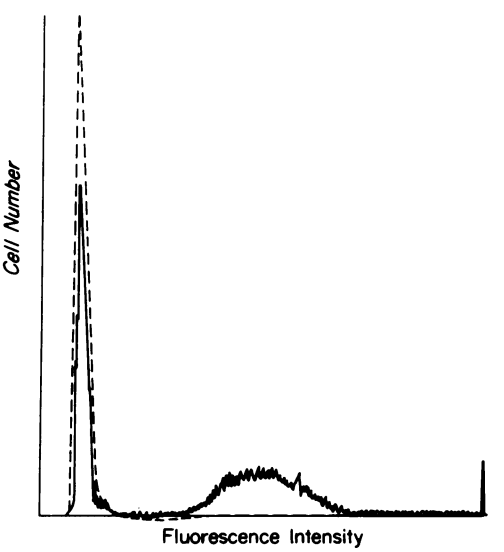

FIGURE 1 Reactivity of human peripheral blood T cells with anti-TH_2. Fluorescence-activated cell sorter histogram showing the fluorescence profile of 40,000 peripheral human T cells when stained with anti-TH_2 and R/H fluorescein isothiocyanate (solid line). As shown, the normal human peripheral blood T-cell compartment contains a subset of cells representing 20% of the population (≅8,000 cells) which is TH_2^+. The remaining 80% of peripheral T cells are nonreactive and TH_2^-. Background fluorescence staining (dashed line) was obtained by incubating cells with normal horse IgG and developing with R/H fluorescein isothiocyanate.

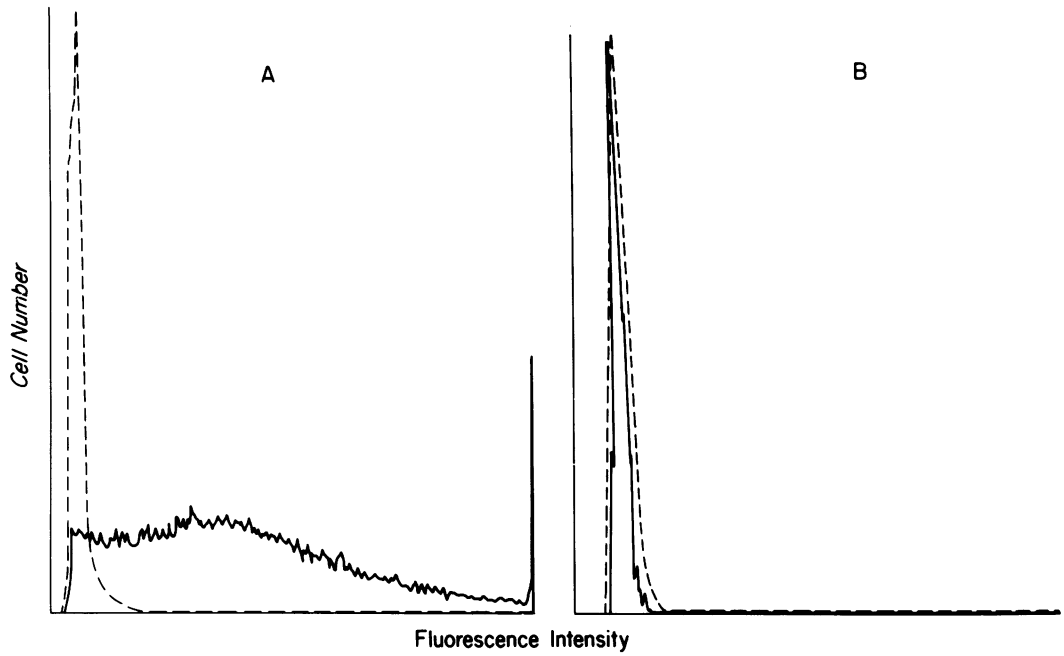

FIGURE 2 Reactivity of T-ALL blasts with anti-TH_2. Fluorescence-activated cell sorter histogram showing the fluorescence profile of tumor cells from representative patients with T-ALL. In A essentially all tumor cells were reactive with anti-TH_2 (TH_2^+). In contrast, B shows a T-ALL tumor population that is unreactive with anti-TH_2 (TH_2^-).

TH_2^+, similar to those cells shown in Fig. 2A. The median age of patients with TH_2^+ and TH_2^- T-cell ALL was 9.8 and 10.5 yr, respectively. 3 of 5 TH_2^+ patients were female, whereas 10 of 13 TH_2^- patients, in contrast, were male. Adenopathy and organomegaly were similar in both groups of patients. One difference of note between groups was the observation that only 2 of 5 TH_2^+ T-ALL patients, as compared to 11 of 13 TH_2^- patients, had mediastinal enlargement detectable on standard chest roentgenograms. It was of interest that all 5 TH_2^+ patients presented with lymphoblast counts >75,000/mm^3, whereas only 5 of 13 (<50%) of the TH_2^- patients presented with similar blast counts. In addition, none of the TH_2^+ T-ALL patients had a hematocrit >35% on presentation, whereas approximately one-third of the TH_2^- T-ALL patients had a hematocrit >35%. This latter observation was not simply a result of increased marrow involvement with leukemic infiltration in the TH_2^+ subgroup, since all patients with T-ALL had ≥80% marrow replacement on presentation with the exception of one TH_2^- patient.

To determine whether there were differences in clinical outcome among children with TH_2^+ and TH_2^- T-ALL, individual survival for patients with each type of T-ALL was compared. For analytic purposes, only individuals who were alive and followed for more than 12 mo or until death were considered. In addition, one TH_2^+ T-ALL patient died at the initiation of induction therapy as a consequence of congestive heart failure and severe anemia and was therefore not included in the survival data. As shown in Table I, children with TH_2^+ T-ALL did better than children with TH_2^- T-ALL. Three of four patients with TH_2^+ T-ALL are alive ≥33 mo after diagnosis. Two remain disease-free off therapy, whereas a third patient relapsed and died at 41 mo, and a fourth patient who relapsed at 33 mo is still alive. In contrast, no TH_2^- T-ALL children survived longer than 19 mo.

Not shown in Table I is clinical data on the TH_2^- T-ALL among adults (≥21 yr old). However, similar

TABLE I
Clinical Features of Children with T-ALL

	TH_2^+	TH_2^-
Number of patients	5	13
Median age, *yr*	9.8	10.5
Male/female ratio	0.66/1	3.3/1
Lymphadenopathy	4	10
Hepatomegaly	3	8
Splenomegaly	3	6
Mediastinal mass	2	11
Lymphoblasts >75,000/mm^3	5	5
Hematocrit >35	0	4
Mean survival, *mo*	36	11

to TH_2^- T-ALL in childhood, these patients had >80% incidence of mediastinal enlargement. Moreover, six of seven patients presented with a hematocrit of >35%. In addition, three of seven died within 6 mo of diagnosis on treatment. Of the remaining four patients, none are disease-free and all have had one or more relapses.

Differences in leukemic T-ALL tumor cells among adults and children were dissected further in Table II. This analysis shows that 5 of 18 children with T-ALL had TH_2^+ tumor cells. Thus, 25% of T-ALL in children results from malignant expansion of the TH_2^+ subset. Moreover, Table II shows that no TH_2^+ leukemias were detected among seven adult cases (ages 21–72).

DISCUSSION

In the present study, T-cell subset-specific heteroantisera were utilized to characterize tumor populations from 25 patients with T-ALL. These tumor cells were previously shown to be of T-cell lineage by reactivity with T-cell-specific heteroantisera and their capacity for spontaneous E rosette formation. Lymphoblasts from a given individual were subset restricted in that they all either reacted or were unreactive with anti-TH_2 antisera. 5 of 25 patients had blasts which bore the TH_2^+ antigen(s), whereas the remaining 20 patients lacked the antigen. Moreover, TH_2^+ T-ALL was found exclusively among childhood T-cell leukemia. Thus, 5 of 18 children and none of 7 adults had TH_2^+ T-ALL.

Despite the limited number of patients studied, the clinical presentation and course of children with TH_2^+ T-ALL appeared to differ from children with TH_2^- T-ALL. 3 of 5 TH_2^+ T-ALL patients were female, whereas 11 of 13 TH_2^- T-ALL patients were male. Hepatosplenomegaly and adenopathy were similar among both TH_2^+ and TH_2^- groups. In contrast, mediastinal enlargement was detectable in almost all cases of TH_2^- T-ALL although it was undetectable in three of five TH_2^+ T-ALL patients. In addition, the absolute lymphoblast counts in peripheral blood of TH_2^+ T-ALL cases were >75,000/mm³ in all cases. A similar blast level in peripheral blood was found in <50% of TH_2^- T-ALL patients. Despite the high leukocyte count, it would appear that the TH_2^+ T-ALL children had a longer overall survival than the TH_2^- T-ALL in children. All four TH_2^+ patients who received treatment have survived for >33 mo. Two of these patients are alive and disease-free >3 yr after diagnosis. The third patient died at 41 mo of recurrent disease, and the fourth patient relapsed at 33 mo and is still alive. In contrast, all children with TH_2^- T-ALL followed for >1 yr are dead. The longest survivor in this latter group lived for 19 mo after diagnosis.

TABLE II
Phenotypic Distinctions of Tumor Cells in Childhood and Adult T-ALL

	TH_2^+	TH_2^-	
Childhood*	5	13	18
Adult‡	0	7	7
	5	20	25

* Birth to 20 yr of age.
‡ Greater than 20 yr of age.

Prior studies of murine AKR strain T-cell lymphomas, induced with 1-ethyl-1-nitrosourea or spontaneously occurring, have shown these tumors to be either $Ly1^+$ (helper cell phenotype) or $Ly2,3^+$ (suppressor/cytotoxic cell phenotype) (34, 35). In this regard, the restricted expression of subset specific Ly antigens in the case of murine T-cell tumors and TH_2 antigens in the case of human T-ALL demonstrates obvious similarities between murine and human disease. Moreover, the restriction suggests that leukemogenesis can occur in a partially differentiated cell expressing, or destined to express, a mature T-cell phenotype and that human T-cell leukemia represents a malignant clonal expansion of cells deriving from a single T-cell subset. The present study would suggest that in children, T lymphocytes derived from both subsets are susceptible to lymphoblastic transformation, as are isotype restricted B-cell clones equally susceptible to transformation into myelomatous disease (36). To date, we have not found any adult patients with TH_2^+ leukemias. The observed restriction of T-ALL in adults to the TH_2^- subset may nevertheless be related to the small number of adult patients (seven cases) reported in this study. On the other hand, if borne out in larger series, it may reflect a difference in pathogenesis or alternatively, a difference in the susceptibility of the TH_2^+ subset to undergo leukemic transformation in children and adults.

The clear-cut heterogeneity in clinical response of T-ALL has, in past years, been difficult to interpret. However, there is now a significant literature supporting the notion of phenotypic, biologic, and functional T-cell heterogeneity among normal T-lymphocyte populations (22–27). Previous work has shown, for example, that the programming of specific cell function appears to be linked to the expression of a particular cell surface phenotype (24, 27). Thus, the functional repertoire of the human TH_2^+ cell is distinct from the TH_2^- cell. It would not be surprising to detect biological distinctions among tumor populations arising from normal T-cell subsets which are so functionally disparate.

An additional distinction between patients with TH_2^+ and TH_2^- T-cell ALL was that 10 patients (4 children and 6 adults) with TH_2^- T-cell ALL manifested no anemia, whereas all TH_2^+ T-ALL patients were

anemic on presentation. These 10 individuals presented with hematocrits >35% and averaging ≅40%, despite ≧80% bone marrow replacement in all cases. Since T cells have been shown to produce a factor(s) which is essential for induction of erythroid differentiation from the stem cell compartment in vitro, it is conceivable that absence of anemia could be due, in part, to production of such a helper factor by some malignant TH_2^- T-ALL cells (37). In this regard, it is of note that some human T-cell leukemias and lymphomas have been shown to retain functional properties including help and suppression of B-cell immunoglobulin production (38, 39). In the future, it will be of interest to determine whether some TH_2^+ and TH_2^- leukemias retain functions unique to their normal subsets.

The present study suggests that additional information can be gained by a precise phenotypic determination of cell surface antigens at the level of the component subsets of lymphoid cells. An elucidation of subsets which comprise the T-cell population in man will provide a means of understanding the clinical variability arising within T-cell malignancies which was not previously understood.

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