

## Immunologic Classification of Acute Lymphoblastic Leukemia in Korean<sup>†</sup>

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**= Abstract =**Recent advance in immunology has led to important insight into lymphocyte differentiation and the cellular origin of acute lymphoblastic leukemia(ALL). Leukemic cells from 68 children and 60 adults with ALL were analyzed utilizing highly specific monoclonal antibodies, terminal deoxynucleotidyl transferase and more traditional lymphocyte markers such as sheep erythrocyte receptor and surface membrane immunoglobulin. Monoclonal antibodies used were J5 detecting common ALL antigen (CALLA), OKT10, OKM1 and OKIa1 detecting HLA-DR. Four major subgroups of ALL were identified: in childhood ALL, common ALL (58.8%), null ALL (20.6%), T-ALL (19.1%) and B-ALL (1.5%) and in adult ALL, common ALL (60.0%), null ALL (25.0%), T-ALL (13.3%) and B-ALL (1.7%). The frequencies of expression of the markers within each of the four major subgroups were also evaluated. In T-ALL, 18% of childhood and 25% of adult cases expressed CALLA.

**Key words:** *Acute lymphoblastic leukemia, Immunologic classification*

### INTRODUCTION

Acute lymphoblastic leukemia(ALL) is characterized by a clonal proliferation of lymphoid cells and classified by morphologic and cytochemical criteria (Bennett *et al.* 1976). Morphologic classification of ALL into L1, L2, L3-group was not correlated among observers (Bennett *et al.* 1981), and it was difficult by cytochemistry alone to define whether lymphoblasts are T, B, or null, and to predict constantly the clinical course and prognosis (Miller *et al.* 1981; Greaves *et al.* 1981; Greaves and Lister 1981).

Recent developments in immunological technology have contributed new insight into the pathogenesis of leukemia. Utilizing classic surface membrane markers such as receptors for sheep

erythrocytes (E rosettes) to define T lymphocytes and surface membrane immunoglobulins (SmIg) to define B lymphocytes, the lymphoid leukemias can further be divided into immunological subsets. Especially the most important advance has been the development of monoclonal antibodies raised by the hybridoma technique (Kohler and Milstein 1975). These antibodies are becoming widely available and will allow a more uniform and clinically significant subclassification of the leukemias.

Because techniques for immunophenotyping by monoclonal antibodies have evolved over time and standardization of techniques has only recently begun to occur, few studies involving large numbers of patients have been published. In no instances have the ethnic differences been studied (Pendergrass 1985). We employed phenotypic analysis of ALL by monoclonal antibodies in combination with more conventional immunologic markers and intended to determine the frequency of each subgroup of ALL in Korean.

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**Table 1.** Monoclonal antibodies utilized for the determination of ALL phenotype

Monoclonal antibody	Isotype	Reactivity	Molecular weight of antigen
J5	IgG <sub>2a</sub>	CALLA	100,000
OKIa1	IgG <sub>2</sub>	HLA-DR	29,000 & 34,000
OKT10	IgG <sub>1</sub>	T10	12,000 & 46,000
OKM1	IgG <sub>2b</sub>	Myeloid cells and monocytes	170,000

### MATERIALS AND METHODS

**Patients:** This study included 128 patient who were 68 children ranging from 1 to 15 years and 60 adults from 16 to 89 years, untreated and first seen between March 1983 and May 1986 in Seoul National University Hospital.

The original diagnosis of ALL was based on cytologic examination of Wright-stained marrow and peripheral blood smears, and on myeloperoxidase or Sudan black B and nonspecific esterase negativity.

**Cytochemistry:** Leukemic cells were evaluated by standard cytochemical assays for periodic acid Schiff's stain, Sudan black B, myeloperoxidase and nonspecific esterase.

**Cells:** Leukemic cells were obtained from heparinized peripheral and bone marrow of patients at the time of diagnosis. Mononuclear cells were isolated from these samples by Ficoll-Hypaque density gradient centrifugation.

**Monoclonal antibodies:** OKT10, OKIa1 and OKM1 antibodies were supplied in a lyophilized form by Ortho Pharmaceutical Corporation and J5 antibody by UCLA (Table 1).

**Indirect immunofluorescent assay:** All cells were washed three times with RPMI-1640 at 4°C before analysis, 5 x 10<sup>6</sup>/mL cells were used for each individual assay. Cells were incubated 30 min at 4°C in 200 uL of tissue culture medium (RPMI-1640) containing 5 uL of monoclonal antibody solution. Cells were washed twice with phosphate buffered saline (PBS, pH 7.4) by centrifugation at 4°C and resuspended in 100 ul of appropriately diluted goat anti-mouse immunoglobulins (Cooper Biomedical). Following 30 min incubation at 4°C, the cells were washed twice with PBS and finally resuspended in one drop of 30% glycerol in PBS. Analysis was performed immediately or following overnight incubation at 4°C. Cells were ex-

amined on a fluorescent microscope equipped with epiillumination. A minimum of 200 cells were examined for surface immunofluorescence.

**Surface membrane immunoglobulin (Smlg) assay:** Smlg assay was used as a standard B cell marker. Cells were washed three times with warm PBS (37°C) before analysis. 100 uL of lymphocytes (2-4 x 10<sup>6</sup>/mL) were mixed with 100 uL of appropriately diluted fluorescein conjugated goat anti-human immunoglobulin F(ab')<sub>2</sub> fragment (Cooper Biomedical) and incubated 30 min at 0°C. The mixture was washed three times with cold PBS containing 0.02% NaN<sub>3</sub>, and finally resuspended with 30% glycerol in PBS. Analysis was performed immediately using fluorescence microscope.

**E-rosette assay:** Sheep erythrocyte(E) rosettes were used as a standard T cell marker. 200 uL of lymphocytes (4 x 10<sup>6</sup>/mL) were mixed 200 uL of 1% E and incubated 15 min at 37°C. The mixture was centrifuged for 5 min at 2500 rpm and left 16 to 18h at 4°C before gentle resuspension and counting.

**Terminal deoxynucleotidyl transferase (TdT) assay:** Assay for TdT activity were performed using reagents available from Bethesda Research Laboratories. The procedure was performed according to the manufacturer's directions.

**Definition of ALL subgroups:** Subgroups of ALL were characterized as Table 2. Phenotype positive in Smlg was B-ALL, positive in E rosette was T-ALL, and non-T, non-B ALL was classified by common ALL antigen (CALLA) which was positive in common ALL and negative in null ALL.

### RESULTS

#### 1. The ALL Subgroups

Between March 1983 and May 1986, membrane markers of blood or marrow cells from a total of 128 patients with newly diagnosed ALL in children (aged from 1 to 15) and in adults (aged from 16 to

**Table 2.** Major immunologic subgroups of ALL

Subgroup	Defining surface markers
Common ALL	CALLA <sup>+</sup> , Ia1 <sup>+/-</sup> , TdT <sup>+/-</sup> , T10 <sup>-/+</sup> , Smlg <sup>-</sup> , ER <sup>-</sup>
T-ALL	ER <sup>+</sup> , T10 <sup>+/-</sup> , TdT <sup>+/-</sup> , Ia1 <sup>-/+</sup> , CALLA <sup>-/+</sup> , Smlg <sup>-</sup>
B-ALL	Smlg <sup>+</sup> , Ia1 <sup>+</sup> , CALLA <sup>-/+</sup> , T10 <sup>-</sup> , TdT <sup>-</sup> , ER <sup>-</sup>
Null ALL	Smlg <sup>-</sup> , ER <sup>-</sup> , CALLA <sup>-</sup> , Ia1 <sup>-/+</sup> , T10 <sup>-/+</sup> , TdT <sup>-/+</sup>

**Table 3.** Subgroups and sex distribution of ALL

Subgroup	Childhood ALL (≤15 years)		Adult ALL (≥16 years)	
	Sex M:F (ratio)	No. typed with markers (%)	Sex M:F (ratio)	No. typed with markers (%)
Common ALL	25:15 (1.7)	40 (58.8)	20:16 (1.3)	36 (60.0)
Null ALL	6: 8 (0.8)	14 (20.6)	10: 5 (2.0)	15 (25.0)
T-ALL	10: 3 (3.3)	13 (19.1)	8: 0	8 (13.3)
B-ALL	1: 0	1 ( 1.5)	0: 1	1 ( 1.7)
Total	42:26 (1.62)	68 (100)	38:22 (1.73)	60 ( 100)

89) were analyzed. With use of three markers (Smlg, E rosettes, CALLA), the clinical entity of ALL could be divided into four major subgroups. These subgroups included common ALL (58.8%), null ALL (20.6%), T-ALL (19.1%), and B-ALL (1.5%) in 68 childhood ALL cases and common ALL (60.0%), null ALL (25.0%), T-ALL (13.3%), and B-ALL (1.7%) in 60 adult ALL cases (Table 3). Distribution of sex for each subgroup is also shown in Table 3. There were no significant differences with respect to sex distribution except male predominance in T-ALL.

### 2. Marker Expression in Major Immunologic Subgroups of ALL

The frequencies of expression of the markers evaluated within each of the four major subgroups of ALL are shown in Fig. 1 and 2. In childhood common ALL, 95% of cases tested were Ia1<sup>+</sup>, 62% were TdT<sup>+</sup>, and 36% were T10<sup>+</sup> and in adult common ALL, 86% were Ia1<sup>+</sup>, 82% were TdT<sup>+</sup>, and 43% were T10<sup>+</sup>. In childhood null ALL, 38% of cases tested were Ia1<sup>+</sup>, 50% were TdT<sup>+</sup>, and 29% were T10<sup>+</sup> and in adult null ALL, 53% were Ia1<sup>+</sup>, 60% were TdT<sup>+</sup>, and 54% were T10<sup>+</sup>.

In childhood T-ALL, 70% of cases tested were TdT<sup>+</sup>, 54% were T10<sup>+</sup>, 18% were CALLA<sup>+</sup>, and 8% were Ia1<sup>+</sup> and in adult T-ALL, 38% were TdT<sup>+</sup>, 25% were CALLA<sup>+</sup>, and 14% were T10<sup>+</sup>. Thus CALLA and Ia1 antigens were expressed by the leukemic T cells in a portion of the T-ALL cases.

### DISCUSSION

ALL represents the neoplastic expansion of a clone of cells that express most or all of the phenotypic features that characterize a normal lymphoid cell counterpart of specific lineage and maturational stage (Greaves and Janossy 1978; Greaves 1981). These phenotypic features have been and continue to be distinguished on the basis of cell morphology, histochemical evaluation, and clinical findings. The purpose of this study was to explore how the various forms of ALL in Korean can be subgrouped by surface antigen phenotype using highly specific monoclonal antibodies and the traditional cell surface markers.

Using the conventional surface markers of E rosette formation and Smlg, ALL can be divided into non-T, non-B ALL, T-ALL and B-ALL (Brouet *et al.* 1975). It is generally agreed that T- and B-ALL have a poor prognosis compared to non-T, non-B ALL (Sen and Borella 1975; Wolff *et al.* 1976). Non-T, non-B ALL can be further divided by expression of common ALL antigen (CALLA) which was originally defined by antisera produced in rabbits by immunization with Smlg-negative, E rosette-negative ALL cells (Greaves *et al.* 1975). Non-T, non-B, CALLA-negative ("null") ALL patients respond less favorably to treatment than patients who are CALLA positive.

ALL generally classified into the following four

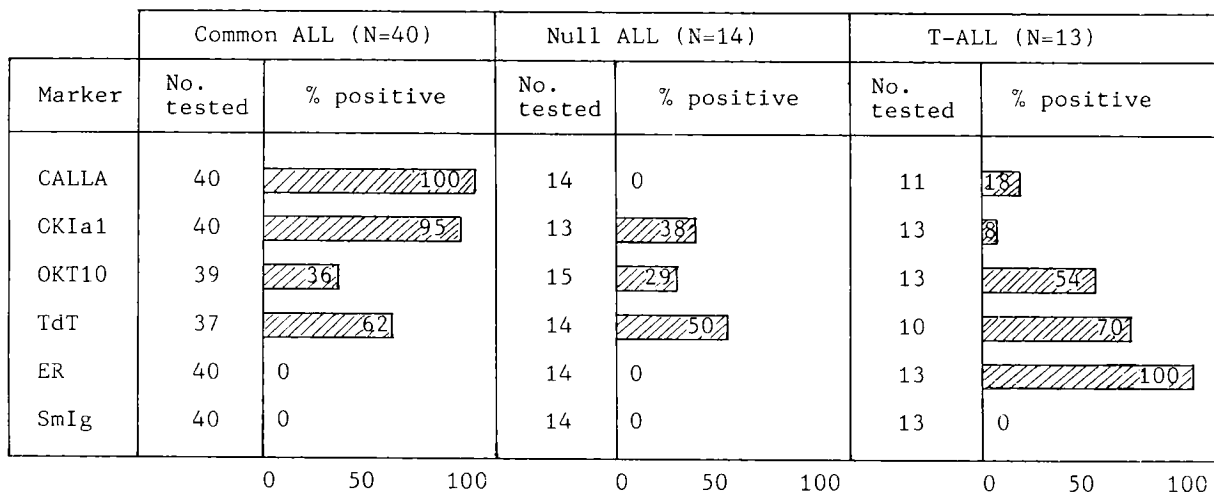


Fig. 1. Marker expression in major immunologic subgroups of childhood ALL.

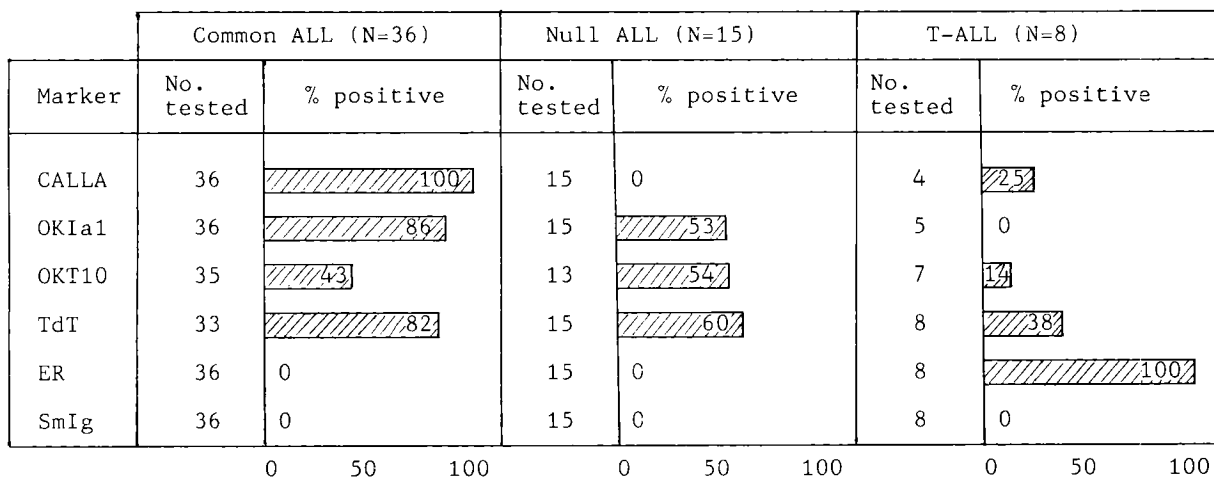


Fig. 2. Marker expression in major immunologic subgroups of adult ALL.

major subgroups: common ALL, null ALL, T-ALL and B-ALL. The close relationship between these immunologic subgroups and clinical manifestation is well accepted. Common ALL is the most frequently observed phenotype in Caucasian, representing approximately 70% of children and approximately 50% of adults with ALL (Greaves and Lister 1981; Catovsky *et al.* 1979). The principal difference between childhood and adult ALL is the considerably higher incidence of null ALL in adults. However, in this study investigating the phenotypes of ALL in Korean, the relative incidence of common ALL was approximately 60% both in childhood and adult ALL and there was no difference in the distribution of phenotypes between these. Particularly T-ALL was more frequently observed in Korean than in Caucasian. It is still obscure as to whether the higher incidence of T-ALL in Korean is related to HTLV-I which is a causative agent in adult T-cell leukemia and lymphoma being prevalent in

Japan. The exact magnitude of these differences and their relative incidence to other populations remains unclear.

CALLA is a 100,000 mol. wt. glycoprotein expressed on a variety of tissue including most cases of non-T, non-B ALL and some cases of T-ALL (Janossy *et al.* 1978; Sutherland *et al.* 1978; Clavell *et al.* 1981; Metzgar *et al.* 1981). In this study we found 18% of children and 25% of adults with T-ALL expressed CALLA. There are three possible explanations. (1) T cells possess the CALLA-defined antigen(s) in small amounts; (2) T cells possess a chemically distinct but serologically cross-reactive surface determinant; (3) T cells do not possess the major CALLA-defined antigen but do possess one or more of the unrelated antigens defined this antiserum. Clarification of this situation must await the availability of more specific antisera of high titer.

Although a very small proportion (approximately

1%) of ALL has been identified as B cell in origin, approximately 20% of cases of non-T, non-B ALL have been classified as pre-B cell ALL since a significant number of the leukemic cells express heavy chains within the cytoplasm (Pullen *et al.* 1984). Most of the remaining cases of non-T, non-B ALL have been thought to be of early pre-B cell lineage, based on the leukemic cell differentiation antigens (Balch *et al.* 1979; Nadler *et al.* 1983). Non-malignant counterparts to CALLA<sup>+</sup> non-T, non-B ALL have been identified in bone marrow and many of these cells expressed markers associated with cells early in B-lymphocyte differentiation (Greaves *et al.* 1980; Pullen *et al.* 1984). Non-malignant counterparts to CALLA<sup>+</sup> T-ALL have not been well characterized and thus the relationship between CALLA and T-lymphocyte differentiation pathways is unclear. Recently, however, CALLA<sup>+</sup> cells were detected within the cortex of thymus suggesting that CALLA may be expressed on some cells early in T-cell differentiation (Neudorf *et al.* 1984).

Leukemia cells generally reflect the phenotype of normal cells. Analysis of leukemic cell populations, therefore, can serve as a tool for studying the normal pathway of differentiation. Viewed in this light the cellular origin or developmental levels of maturation arrest with lymphoid lineages can be seen to be of considerable importance in influencing the possible outcome of treatment. Whether the substantial heterogeneity within a subgroup, e.g. common ALL, can be further linked to discrete maturation stages (e.g. pre-B ALL).

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= 국문초록 =

### 급성 림프구성 백혈병의 면역학적 분류

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서울대학교 병원에서 진단된 소아 급성림프아구성 백혈병 68예와 성인 급성림프구성 백혈병 60예 대하여 common ALL항원에 대한 항체, OKT10, OKM1, OKIa1 등 단세포군 항체와 terminal deoxynucleotidyl transferase를 이용하여 면역학적 분류를 실시하였다.

소아 급성림프아구성 백혈병은 common ALL 58.8% null ALL 20.6%, T-ALL 19.1%, B-ALL 1.5%로 분류되었고, 성인 급성림프아구성 백혈병은 common ALL이 60.0%, null ALL 25.0%, T-ALL 13.3%, B-ALL 1.7%로 분류되었다.