Analysis of T-Cell Differentiation Antigens in Acute Lymphatic Leukemia Using Monoclonal Antibodies

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Leukemic cells in 134 patients with ALL were analyzed by a panel of mouse monoclonal antibodies. Two antibodies are reactive with all peripheral blood T cells but define different surface antigens (Leu-1 and Leu-4). Two other antibodies react with antigens that are restricted to suppressor/cytotoxic T cells (Leu-2) and to helper T cells (Leu-3). We also used antibodies to the receptor for sheep red blood cells (SRBC) (Leu-5) and to a human “‘TL-like”’ antigen that is found on most thymocytes but not in peripheral T cells (Leu-6). An antibody to the human p29,34 “‘Ia-like’” molecule was also tested. Of the 134 ALL patients, 17 had a predominance of SRBC-rosetting (Leu-5+) lymphoblasts (“’T” ALL), expressing different surface phenotypes defined by this panel of monoclonal antibodies.

These phenotypes were not readily classifiable according to a scheme of sequential stages of normal differentiation proposed. Moreover, the lymphoblasts in 8 of 113 patients not expressing conventional B- or T-cell markers (“null” ALL) reacted with the monoclonal anti-T-cell antibodies. This study suggests that the classification of lymphoblasts in ALL based on the reactivities observed with this panel of mouse monoclonal antibodies is not easily reconciled with current models of normal T-cell differentiation. However, it should be emphasized that the precise sequence of antigenic expression by cells undergoing thymic differentiation is still not fully known, and further phenotypic analysis of ALL cells might contribute to an improved understanding of this malignancy.

DISSECTION OF THE phenotypic heterogeneity of ALL has resulted in the recognition of clinically significant prognostic categories of this disease1,2 and has been recently proposed to reflect normal maturational sequences in the thymic lineage in those cases expressing T-cell surface markers.3

However, the predominant number of ALL patients have leukemic cells that lack the conventional markers, surface immunoglobulin and receptor for sheep red blood cells (SRBC), and therefore cannot be assigned to the T- or B-cell lineages. These “null” lymphoblasts have high levels of terminal deoxynucleotidyld transferase (TdT)2,4 and usually are reactive with antibodies to human “‘Ia-like”” antigens.5 Moreover, the expression of TdT and la is frequently associated with the recognition of a surface antigen (CALLA) that is generally restricted to this ALL subtype.5 This antigen may also be found on a subpopulation of TdT+ lymphoblasts in normal bone marrow (BM), suggesting that it defines a stage of early lymphocyte differentiation.7 TdT+ “null” ALL cells have been conventionally distinguished from TdT+ T lymphoblasts by their failure to form SRBC rosettes.2,4

These studies were undertaken to determine the value of applying additional T-cell surface markers in the analysis of ALL lymphoblasts for the phenotypic classification of this disease. These markers are derived from studies in which human leukocyte antigens, which are preferentially expressed on thymocytes or T cells, were defined and characterized by mouse monoclonal antibodies.8-10 The panel of monoclonal antibodies used included (1) anti-Leu-1: reacts with a 67,000-70,000 dalton antigen, which is distributed on all thymocytes, all peripheral blood (PB) T cells, and 80%-90% of SRBC-rosetting cells; (2) anti-Leu-2: reacts with 25%-45% of SRBC-rosetting cells in the PB of normal individuals, and 55%-85% of thymocytes; (3) anti-Leu-3: reacts with 45%-75% of PB SRBC-rosetting cells, 80%-90% of thymocytes, and has not been found to react with leukocytes outside the thymic lineage. Whereas the majority of thymocytes are Leu-2+3+, each of these antigens belongs to one of the two non-overlapping T-cell subsets that together comprise the population of Leu-1- cells. As initially demonstrated with the original anti-TH3 serum,11,12 it has been shown using monoclonal antibodies of another series that Leu-2+3- (TH1+) cells have suppressor/cytotoxic functions and Leu-2-3+ (TH2-) cells have helper functions in a variety of assays in vitro; (4) anti-Leu-4: recognizes a 25,000-dalton molecule distributed to all PB SRBC-rosetting cells and about 30%-60% of thymocytes.13 It has not been found on leukocytes outside the thymic lineage; (5) anti-Leu-5: blocks SRBC rosette formation by T cells and non-T-cells and defines a 55,000-dalton antigen that has the same distribution as the SRBC receptor on lymphocytes; (6) anti-Leu-6: reacts with about 70% of thymocytes and is not found on PB
SRBC-rosetting cells or other subpopulations of leukocytes. Anti-Leu-6 exhibits competitive binding with the NA-1 antibody of McMichael et al., which precipitates two molecules of 44,000 and 12,000 daltons;\(^\text{15}\) (7) anti-Ia: recognizes a 29,000- and 34,000-dalton “Ia-like” antigen on human mononuclear cells.

**MATERIALS AND METHODS**

**Clinical Material**

Fresh cell suspensions of BM and/or PB lymphoblasts in 134 consecutive patients with ALL seen by the pediatric (83 cases) and adult (51 cases) Hematology Services at Memorial Hospital from January 1980 to December 1981 were subjected to surface marker analysis. All patients had active leukemia at the time of the study. Although a majority of patients had leukemic cell counts above 10,000 cells/cu mm, some had lower counts but with a predominance of leukemic elements confirmed by examination of cytocentrifuge smears of the cell suspensions analyzed. The FAB (French-American-British) scheme was used for the morphological classification of lymphoblasts.\(^\text{16}\)

**Marker Studies**

Lymphocyte preparation and immunofluorescent staining was performed as previously reported.\(^\text{17}\) A polyvalent rabbit antiserum to human immunoglobulin and fluorescein-conjugated F(ab')\(_2\) fragments of specific antisera to human surface markers was purchased from Kallestad Laboratories (Chaska, Minn.). The cells were examined with a Leitz Ortholux microscope equipped with fluorescent illumination. Spontaneous rosette formation with SRBC at 4°C and 37°C was determined following incubation at 37°C. Moreover, the leukemic cells in 13 of 16 patients (Table 1, 4 patients with “null” ALL had the Leu-1+ or -/+; 3- or +/--; 4- or +/-, 5-; 6-, Ia- phenotype (patients 1-4) and 4 were Leu-1-, 2-, 3- or +/-, 4- or +/-, 5- 6+, Ia+ (patients 5-8). When compared with the former, this latter phenotype characterized a younger group of patients (median age 8.5 versus 15.5 yr) with lower white counts (mean 19.8 versus 94.5 x 10\(^3\) cells/cu mm) (Table 2).

Seventeen of the 134 patients (14 untreated and 3 in relapse) had leukemic cells that rosetted with SRBC and are therefore provisionally classified as “T” ALL. Leukemic cells from these patients exhibited a striking heterogeneity in their quantitative and qualitative reactivity with Leu-1 through 6. As shown in Table 1, different phenotypic patterns resulted from the analysis of only 17 patients. Patient 9, who presented with a low leukemic count, L1 morphology, and no mediastinal mass, had leukemic cells only showing reactivity with anti-Ia and anti-Leu-5. The leukemic cells in patients 10 and 11 were reactive with anti-Leu-5 and anti-Leu-3 alone or combined with anti-Leu-2, but unreactive with other markers of thymocytes or mature T cells. Both were pediatric cases presenting with L1 morphology and mediastinal masses.

The lymphoblasts in patients 12–17 displayed reactivities suggestive of a thymocyte stage. However, in patients 12 and 13, although the cells expressed Leu-1 and Leu-6, they were reactive with Leu-2 but not with Leu-3. Moreover, the cells in patients 14–17 were not reactive with Leu-5 and did not coexpress Leu-2 and Leu-3 consistently. Although the lymphoblasts in patients 18–25 could be assigned to a mature T-cell stage because of Leu-4 antigen expression, a heterogeneity of phenotypic reactivities was detected not fully consistent with this developmental stage. For instance: (A) Leu-4 was coexpressed with Leu-6 in patients 22 and 23; (B) there was a lack of reactivity with anti-Leu-1, anti-Leu-2, and anti-Leu-3 in case 18 and only with the latter two antibodies in patients 19 and 24; (C) there was coexpression of Leu-2 and Leu-3 with Leu-4 and absence of Leu-6 in patients 20–22 and 24.

In only 10 of 17 patients with “T” ALL, the lymphoblasts formed SRBC rosettes that were heat stable at 37°C. Moreover, the leukemic cells in 13 of 16
patients studied formed a considerable proportion of EAC rosettes. Neither SRBC rosette at 37°C or EAC rosette formations correlated with any particular phenotypic expression as defined by the Leu monoclonal antibodies. Clinically, 16/17 of the “I” ALL patients studied had a predominance of LI lymphoblasts.

To interpret these data, the known distribution of Leu-1 through 6 on normal hematopoietic cells should first be summarized. Leu-1 is expressed by 100% of T cells and 95% thymocytes. Leu-2 and Leu-3 are each expressed by distinct, nonoverlapping subpopulations of T cells, but are both found on the majority of thymocytes. Leu-4 is found on 100% of T cells and approximately 30%–60% of thymocytes. Leu-5 is expressed by all T cells, 90% of thymocytes, and is also found on a small subpopulation of mononuclear cells that lack other T-cell surface markers. Leu-6 is expressed by the majority of thymocytes and is not found on T cells.

Studies in which other monoclonal antibodies to T-cell antigens have been used to analyze cell suspensions in “I” ALL have shown a marked heterogeneity of T-cell antigen expression. Reinherz et al. reported
on 25 cases of "T" ALL. Fifteen appeared to pertain to an early thymocyte stage by expression of OKT10 and/or OKT9. Five of the 25 cases were reactive with OKT6, equivalent to our Leu-6 reagent, suggesting that these cells arose from a thymic stage of development. In this group of patients there was variable expression of OKT4, OKT5, and OKT8, which correspond to the helper and suppressor/cytotoxic subsets, respectively. There was only one case with a mature thymocyte phenotype expressing OKT3 reactivity. Using the same panel of reagents, Bernard et al. have described a significant degree of phenotypic heterogeneity in the neoplastic T cells from 21 patients with lymphoblastic lymphoma. Moreover, one-third of these patients had malignant cells that were not readily classifiable according to their proposed scheme of intrathymic differentiation. This report by Bernard et al. and recent work by Greaves except for the expression of Leu-1 (elsewhere termed OKT1), which was suggested to be expressed only in late thymic differentiation and on T cells. One might therefore predict the following Leu phenotypes on malignant cells: Leu-1-, 2-, 3-, 4-, 5+, 6- (non-T-cells); Leu-1+, 2-, 3+, 4-, 5+, 6- (non-T-cells); Leu-1+, 2+, 3+, 4+, 5+, 6- (helper T cells); Leu-1+, 2+, 3+, 4+, 5+, 6- (suppressor T cells).

It should be emphasized that the precise sequences of appearance and disappearance of Leu-2, Leu-3, and Leu-6 on cells as they mature in the thymus are similar reactivity to the Leu reagents, further stressing the marked heterogeneity of phenotypic expression in "T" ALL. This information and evidence derived from other systems suggests that there are three major stages of thymic differentiation that are associated with the following phenotypes: Leu-1+, 2-, 3-, 4-, 5+, 6- (early thymic); Leu-1+, 2+, 3+, 4-, 5+, 6+ (thymic); Leu-1+, 2+/-, 3+/-, 4+, 5+, 6- (late thymic and T cell). This agrees with a model proposed by Reinherz et al. except for the expression of Leu-1 (elsewhere termed OKT1), which was suggested to be expressed only in late thymic differentiation and on T cells. One might therefore predict the following Leu phenotypes on malignant cells: Leu-1-, 2-, 3-, 4-, 5+, 6- (non-T-cells); Leu-1+, 2-, 3-, 4-, 5-, 6- (non-T-cells); Leu-1+, 2-, 3-, 4-, 5+, 6- (early thymocytes); Leu-1+, 2+, 3+, 4-, 5+, 6+ (thymocytes); Leu-1+, 2-, 3+, 4+, 5+, 6- (helper T cells); Leu-1+, 2+, 3-, 4+, 5+, 6- (suppressor T cells).
unknown, as is their relationship to the induction of Leu-4 on cells late in thymic differentiation. These considerations become significant if it is assumed that malignant transformation sometimes (?) occurs during transition of cells from one stage of differentiation to another. It is interesting, therefore, that the two markers that may appear in prethymic or in early thymic differentiation were generally present on cells that expressed markers associated with later stages of T-cell development. Thus, Leu-5 was expressed by lymphoblasts in 16/17 cases that expressed either Leu-2, 3, 4, or 6, and Leu-1 was expressed on the lymphoblasts of 12/17 of these cases. Moreover, it is questionable that the cases having cells of Leu-1+, 2−, 3−, 4−, 5−, 6+ phenotype were derived from the thymus, as the putative murine counterpart to this antigen, TL−, may be found on leukemic cells derived from normal cells which are TL−.23

The dissociation of Leu-2, 3, 4, and 6 from the phenotypes anticipated by the proposed sequences of T-cell development suggest, therefore, that these schemes are oversimplified, and/or that the oncogenic events take place during transition from one stage to the other when the phenotypic expression of these markers is being determined. This is further complicated by the consideration of evidence suggesting that Leu-2 and Leu-3 are antigen receptors of their respective subsets,10,24 which raises the possibility that at least a segment of both of these molecules undergoes somatic mutation in the thymus, and therefore is not determined until relatively late in thymic differentiation. Thus, the requirements for the generation of diversity in immunoglobulin V-gene-encoded T-cell receptors, proposed initially by Jerne,25 may be relevant to the apparent variability in the expression of these markers on T leukemic blasts. Interpretation of our results requires, therefore, much more information regarding the cell–cell signals that control thymic differentiation.

As previously described in another group of patients with T-ALL, not all of the ALL cases with a predominance of SRBC-rosetting lymphoblasts at 4°C preserved this capacity at 37°C. This temperature-dependent stability has been reported to be considered characteristic of T lymphoblasts and thymocytes, since it is not present in any other normal or neoplastic T-cell population.26 In this study, no relationship could be established between the heat stability of SRBC rosette formation on lymphoblasts and the different “T” ALL phenotypes identified by the Leu-T-cell monoclonal antibodies.

The finding that Leu-1 was the only antigen expressed on the cells of 2 cases (patients 1 and 2) that otherwise lacked other T-cell markers is interesting. Leu-1 is unique among human T-lymphocyte antigens thus far detected by monoclonal antibodies because it is expressed by surface immunoglobulin-bearing leukemic cells from most patients with chronic lymphocytic leukemia, but it has not been found in normal B cells.8 These findings may be relevant to the phenotype observed in these three cases of “null” ALL, as both malignant cell types could cells may represent the clonal expansion of neoplastic cells at an early stage of lymphocyte differentiation. Alternatively, expression of Leu-1 on ALL cells could be induced by leukemic transformation, which was raised as a possible explanation for the expression of this antigen by surface immunoglobulin-bearing CLL cells but not by normal B cells.8 In the “null” ALL category also, the lymphoblasts in patients 5–7 had the Leu-1−, 2−, 3−, 4−, 5−, 6+, Ia+ phenotype, argumentative of an early thymocyte stage.

This study suggests that the classification of lymphoblasts in ALL based on the reactivities observed with the panel of Leu mouse monoclonal antibodies is not easily reconciled with current models of normal T-cell differentiation. However, it should be emphasized that the precise sequence of antigenic expression by cells undergoing thymic differentiation is still not fully known and further phenotypic analysis of ALL cells might contribute to its improved understanding.

REFERENCES

ANALYSIS OF MONOCLONAL ANTIBODIES IN ALL


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