

A Unique Cell Surface Antigen Identifying Lymphoid Malignancies of B Cell Origin

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ABSTRACT A monoclonal antibody (anti-B1) specific for a unique B cell surface differentiation antigen was used to characterize the malignant cells from patients with leukemias or lymphomas. All tumor cells from patients with lymphomas or chronic lymphocytic leukemias, bearing either monoclonal κ or λ light chain, expressed the B1 antigen. In contrast, tumor cells from T cell leukemias and lymphomas or acute myeloblastic leukemias were unreactive. Approximately 50% of acute lymphoblastic leukemias (ALL) of non-T origin and 50% of chronic myelocytic leukemia in blast crisis were also anti-B1 reactive. Moreover, 21 of 28 patients with the common ALL antigen (CALLA) positive form of ALL were anti-B1 positive, whereas 0 of 13 patients with CALLA negative ALL were reactive.

These observations demonstrate that an antigen present on normal B cells is expressed on the vast majority of B cell lymphomas and on ~75% of CALLA positive ALL, suggesting that these tumors may share a common B cell lineage.

INTRODUCTION

Leukemias and lymphomas, which were not previously distinguishable by either morphologic or histochemical criteria, can now be subdivided into clinically and pathologically distinct subgroups by use of a number of cell surface markers expressed on normal lymphocytes (1-3). For example, both normal and malignant B cells are defined by their expression of cell surface immunoglobulin (4, 5). Other markers of the B cell membrane, including receptors for the Fc portion of human

immunoglobulin (6), receptors for components of the complement system (7), and HLA-D-related Ia-like antigens (8, 9) are less useful because they are not restricted to cells of B lineage and are also found on normal and malignant monocytes (10-12). In addition, Fc receptor-bound immunoglobulin may give spuriously positive results for cell surface immunoglobulin (13). Although T cells have been shown to be reactive with anti-T cell antisera (14, 15), or to form erythrocyte rosettes with sheep erythrocytes (16), they, too, may express Fc or C3 receptors or Ia-like antigens (17-19). Finally, Null cells, which lack the conventional markers of T and B cells (20), also have been shown to express C3, Fc, or Ia-like antigens (21-23). Given the extent of overlap of many of these cell surface markers, considerable attention has been directed at defining unique cell surface antigens present on normal T, B, and Null cells, which can then be used to identify and classify leukemias and lymphomas.

In a recent study (24), we described the development and characterization of a monoclonal antibody (anti-B1) that is reactive with a differentiation antigen expressed on all human B cells and on those cells destined to differentiate into immunoglobulin-secreting cells under pokeweed mitogen stimulation. The B1 antigen has been shown to be distinct from other known phenotypic markers of B cells, including surface immunoglobulin, Fc and C3 receptors, and Ia-like antigens. More importantly, anti-B1 was unreactive with normal T lymphocytes, Null cells, and granulocytes. In the present study, we have used anti-B1 to characterize a large number of malignancies thought to be of T, B, monocyte, myeloid, and Null cell origin. These studies demonstrate that anti-B1 reacts only with those B cell lymphomas that express either monoclonal κ or λ light chain. Of considerable interest is the demonstration that tumor cells from the majority of patients with the common acute lymphoblastic leukemia anti-

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gen (CALLA)¹ positive non-T cell form of acute lymphoblastic leukemia (ALL) and chronic myelocytic leukemia (CML) in blast crisis were also reactive with anti-B1 antibody. This study supports the notion that most B cell lymphomas and many CALLA⁺ ALL share a common B cell lineage.

METHODS

Patients and sample preparations. All patients in this study were evaluated at the Sidney Farber Cancer Institute, the Children's Hospital Medical Center, Peter Bent Brigham Hospital, Beth Israel Hospital, or the Massachusetts General Hospital. The diagnosis of lymphoma or leukemia was made using standard clinical, morphologic, and cytochemical criteria (25-27). Heparinized peripheral blood or bone marrow was collected from leukemic patients or from patients with circulating lymphomas (lymphosarcoma cell leukemias) before the administration of chemotherapeutic agents or blood products. Lymphocytes were separated from these specimens by Ficoll-Hypaque density gradient centrifugation, as previously described (28). Tumor masses and lymphoid tissue from patients with lymphomas were gently teased, minced into single cell suspensions, and passed through stainless steel mesh wire filters. Tumor cells were readily distinguishable from normal lymphocytes by Wright-Giemsa morphology, and all neoplastic preparations selected for this study had >75% abnormal cells. Isolated tumor cells were studied either fresh or cryopreserved in 10% dimethyl sulfoxide and 20% fetal calf serum at -196°C in the vapor phase of liquid nitrogen until the time of surface characterization.

Preparation of normal lymphocyte subpopulations. Human peripheral blood mononuclear cells were isolated from normal volunteer adult donors by Ficoll-Hypaque density gradient centrifugation. Normal lymphoid tissues from tonsil, lymph node, spleen, and thymus were prepared as described above. Unfractionated cells were then separated into B cell (surface immunoglobulin [sIg] positive), T cell (sheep erythrocyte rosette [E] positive), monocyte (adherent), and Null cell (sIg⁻, E⁻) by standard techniques (29). In particular, the B cell preparations were routinely >90% sIg⁺ and <5% E⁺, nonreactive with anti-T cell antibodies, and ~5% monocytes as judged by morphology, latex ingestion, and reactivity with the monocyte-reactive monoclonal antibody (M1) (30). The T cell populations obtained were <2% sIg⁺ and >95% E⁺, uniformly reactive with anti-T cell antibodies, and entirely negative with M1. Normal monocytes were obtained by adherence to plastic dishes as previously described (30), and were 95% M1⁺, but did not form erythrocyte rosettes, react with anti-T cell antisera, or express sIg. Null cells were sIg⁻, E⁻, and T cell antisera negative.

Cell surface markers. The cellular lineage of tumor cells was determined by a number of cell surface markers. The definition of T cell lineage was established by reactivity with a T cell-specific heteroantiserum (14) and monoclonal antibodies (15), and by reactivity with sheep erythrocytes as previously described (16). All the T cell leukemias and lymphomas were >75% reactive with the T cell-specific mono-

clonal antibodies and heteroantiserum, and these tumor cells were uniformly >20% erythrocyte rosette reactive (31, 32).

The B cell derivation of the tumor cell was demonstrated by the expression of either monoclonal κ or λ light chains on the tumor cell surface. Monoclonal antibodies specific for κ or λ light chain were used in all studies (provided by Dr. Victor Raso, Sidney Farber Cancer Institute, Boston, Mass.). In addition, a monoclonal antibody specific for the framework of the human HLA-D-related Ia-like antigen was used to analyze all normal and malignant cells for reactivity. The Ia-like antigens are gene products of the HLA-D region, which are present on the surface of normal peripheral blood B cells, a fraction of Null cells, monocytes, and activated T cells, but not on resting T cells (33). These Ia-like antigens have not been detected on the vast majority of T cell leukemias and lymphomas, but are expressed on most hematopoietic non-T cell malignancies. The anti-Ia antibody used in this study appears to be identical to the previously described heteroantiserum (19) and monoclonal antibodies (33), which identified a common framework expressed on all Ia-like antigens.

The non-T cell leukemias were characterized using a monoclonal antibody (J-5) (34), which has been shown to have the specificity of a previously described rabbit anti-CALLA antiserum prepared in this laboratory (35).

The preparation and characterization of the anti-B1 antibody was the subject of a previous report (24). In brief, anti-B1 was developed by somatic cell hybridization, was cloned by limiting dilution, and has been passaged in ascites form in BALB/c mice for over 1 yr. Ascites form anti-B1 was used for all experiments. This antibody has been shown to be of the IgG₂ subclass and can induce lysis of reactive cells with rabbit complement at dilutions up to 1:50,000. By indirect immunofluorescence, cytotoxicity, and quantitative absorption, the B1 antigen was present on >95% of B cells from blood and lymphoid organs in all individuals tested. Monocytes, resting and activated T cells, Null cells, myeloid cells, and T cell lines were B1 antigen negative. The B1 antigen was shown to be distinct from human immunoglobulin isotypes, Ia-like antigens, Fc receptor of immunoglobulin, and the C3 receptor. Functional studies demonstrated that removal of the B1 antigen positive population from peripheral blood by cell sorting or complement-mediated lysis eliminated the cell population that is induced to differentiate into immunoglobulin-secreting plasma cells by pokeweed mitogen.

Indirect immunofluorescence analysis of normal and malignant cells with monoclonal antibodies. Normal or malignant cells were used fresh or thawed and washed extensively at the time of study; their viability exceeded 85% in all cases. In brief, 1-2 × 10⁶ cells were treated with either 0.1 ml of a 1:500 dilution of the specific monoclonal antibody to be tested or 0.1 ml of a 1:500 dilution of an unreactive control antibody of a similar immunoglobulin isotype, incubated at 4°C for 30 min, and washed three times. These cells were reacted with 0.1 ml of a 1:40 dilution of fluorescein-conjugated goat anti-mouse IgG (G/M FITC) (Meloy Laboratories, Inc., Springfield, Va.), incubated at 4°C for 30 min, washed three times, and analyzed as previously described (36). Intensity of fluorescence was determined for 40,000 cells in each population on a fluorescence-activated cell sorter and compared with the fluorescence of a control nonreactive ascites. A displacement of the histogram of the test monoclonal antibody (Fig. 2A) was scored positive compared with the histogram of an unreactive isotype identical monoclonal antibody. In addition, for each test sample, a quantitative assessment of the number of positive cells was made (number of cells reactive with test monoclonal antibody minus number of cells reactive with the unreactive isotype identical monoclonal antibody divided by 40,000 total cells tested). Because the fluores-

¹ Abbreviations used in this paper: ALL, acute lymphoblastic leukemia; CALLA, common acute lymphoblastic leukemia antigen; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; E, sheep erythrocyte rosette; G/M FITC, fluorescein-conjugated goat anti-mouse IgG; sIg, surface immunoglobulin.

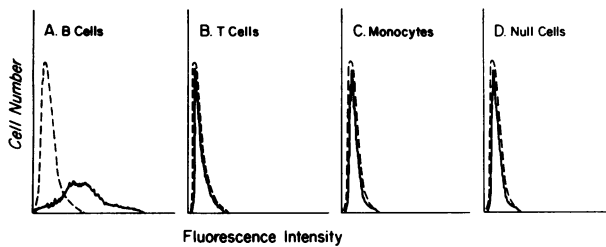


FIGURE 1 The fluorescence profile of fractionated B cells (A), T cells (B), monocytes (C), and Null cells (D) with anti-B1 antibody and G/M FITC (solid line) is depicted in this figure. It is seen that B cells react with the anti-B1 antibody. Background fluorescence staining (dotted line) was obtained by incubating cells with an unreactive monoclonal antibody and developing with G/M FITC.

cence intensity of these antibodies was not different in dilutions from 1:50 to 1:10,000 or greater, it appeared that the intensity of reactivity related to the number of specific antigen-reactive determinants on the cell surface.

RESULTS

Distribution of B1 antigen on normal hematopoietic tissues. As was previously shown (24), anti-B1 identified a surface antigen present on ~9% of unfractionated peripheral blood mononuclear cells. Mononuclear cells from several individuals were separated into T, B, Null, and monocyte fractions and analyzed for reactivity using anti-B1 and G/M FITC. As shown in Fig. 1, the B1 antigen was found uniquely on B cells (Fig. 1A), and was absent from T cells (Fig. 1B), monocytes (Fig. 1C), and Null cells (Fig. 1D). Moreover, the B1 antigen was present on the Ig⁺ cells from tonsil (64%; *n* = 3), lymph node (36%; *n* = 12), spleen (35%; *n* = 8), and a small population of normal bone marrow (5%; *n* = 5), but was not detected on thymocytes (*n* = 4). The intensity or amount of reactivity of these tissues with anti-B1 was similar to that found on peripheral blood B cells.

Reactivity of anti-B1 with malignant lymphomas. Anti-B1 was tested for reactivity with the tumor cells from patients with B cell lymphomas. These tumors were determined to be of B cell origin by the presence of cell surface monoclonal κ or λ light chains and by their failure to form erythrocyte rosettes or react with anti-T cell antisera. The tumors were classified according to the scheme of Rappaport (37) and included the following histologic types: (a) diffuse, poorly differentiated lymphocytic (*n* = 18); (b) diffuse histiocytic (*n* = 7); (c) nodular, poorly differentiated lymphocytic (*n* = 8); (d) Burkitt's lymphoma (*n* = 9); (e) nodular mixed (*n* = 3); (f) Waldenstrom's (*n* = 2); and (g) myeloma (*n* = 3). In contrast to the patterns obtained with normal B cells, the reactivity of anti-B1 with these B cell tumors varied, and is illustrated in Fig. 2. For ex-

ample, tumor cells from patients with chronic lymphocytic leukemia (CLL) were weakly but definitively reactive with anti-B1 (Fig. 2A). In contrast, the tumor cells from patients with nodular, poorly differentiated lymphocytic tumors (Fig. 2B) were moderately reactive, whereas the tumor cells from patients with Burkitt's lymphoma were strongly reactive (Fig. 2C). Of considerable interest was the finding that all plasma cell myelomas tested were unreactive (Fig. 2D). The results obtained with cells of 50 patients with B cell lymphoma are summarized in Table I. The tumor cells from all 47 patients with classical B cell lymphoma were reactive with anti-B1, anti-Ia, and either anti- κ or anti- λ light chain, but not both. The three plasma cell myelomas tested were unreactive with anti-B1. The tumor cells from two of these patients lacked both the Ia antigen and surface κ or λ ; however, the tumor cells from the third patient expressed both. In contrast, only one of three patients with Null cell lymphoma was B1⁺, although the tumor cells from all three of these patients were reactive with an anti-Ia antisera. Moreover, all 13

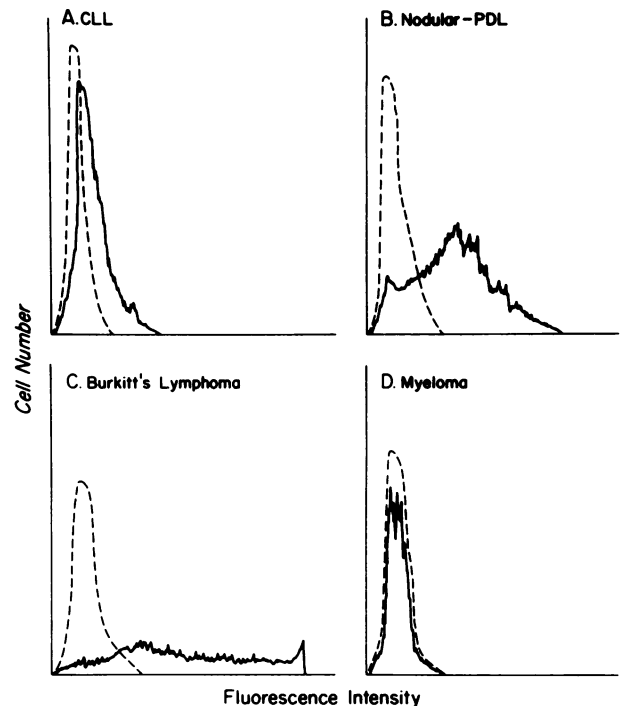


FIGURE 2 The fluorescence profile of tumor cells from patients with B cell CLL (A), nodular, poorly differentiated lymphocytic lymphoma (B), Burkitt's lymphoma (C), and plasma cell myeloma (D) with anti-B1 antibody and G/M FITC (solid line) is depicted in this figure. It can be seen that tumor cells from patients with CLL are weakly reactive, nodular, poorly differentiated lymphocytic cells are moderately reactive, Burkitt's lymphoma strongly reactive, and plasma cell myelomas are unreactive with anti-B1. Background fluorescence staining was performed as in Fig. 1.

TABLE I
Reactivity of Lymphomas and Leukemias with Anti-B1

Tumor	Number of patients	Number reactive with antisera			
		Ia	B1	κ or λ	Anti-T cell
Lymphomas					
B cell	50	48	47	48	0
Null cell	3	3	1	0	0
T cell	13	0	0	0	13
Leukemias					
CLL	18	18	18	18	0
ALL—non-T	41	41	21	0	0
ALL—T cell	17	0	0	0	17
CML—stable phase	6	4	0	0	0
CML—blast crisis	10	7	5	0	0
AML*	16	15	0	0	0

* AML, acute myeloblastic leukemia.

T cell lymphomas tested were unreactive with anti-Ia, anti-B1 and anti- κ or anti- λ monoclonal antibodies.

Reactivity of anti-B1 with leukemias. Since the vast majority of sIg-bearing (B cell) lymphomas were

reactive with anti-B1, we next evaluated the expression of the B1 antigen on leukemic cells. As can be seen in Table I, the tumor cells from patients with CLL expressed monoclonal surface κ or λ light chain, Ia antigen, and B1 antigen. An unexpected result was noted when the ALL cells were tested with anti-B1. It was found that the tumor cells from ~50% of the patients with non-T cell ALL were B1⁺. Leukemic cells from these individuals lacked surface κ or λ , but were B1⁺ and Ia⁺. T cell ALL were unreactive with anti-B1 and anti-Ia. In contrast, tumor cells from patients with acute myeloblastic leukemia were generally Ia⁺ and uniformly lacked the B1 antigen. These findings provided additional support for the view that the anti-B1 was unreactive with conventional sIg or Ia-like antigens. In addition, it was found that 5 of 10 patients with CML in blast crisis were B1⁺, whereas 0 of 6 patients with stable phase CML were unreactive with anti-B1.

Coexpression of B1 antigen and CALLA on non-T cell leukemias. Given the reactivity of anti-B1 with some, but not all, non-T cell ALL, the relationship of the B1 antigen to CALLA was then investigated. Previous studies have shown that CALLA is a tumor-associated antigen expressed on the leukemic cells from 80% of

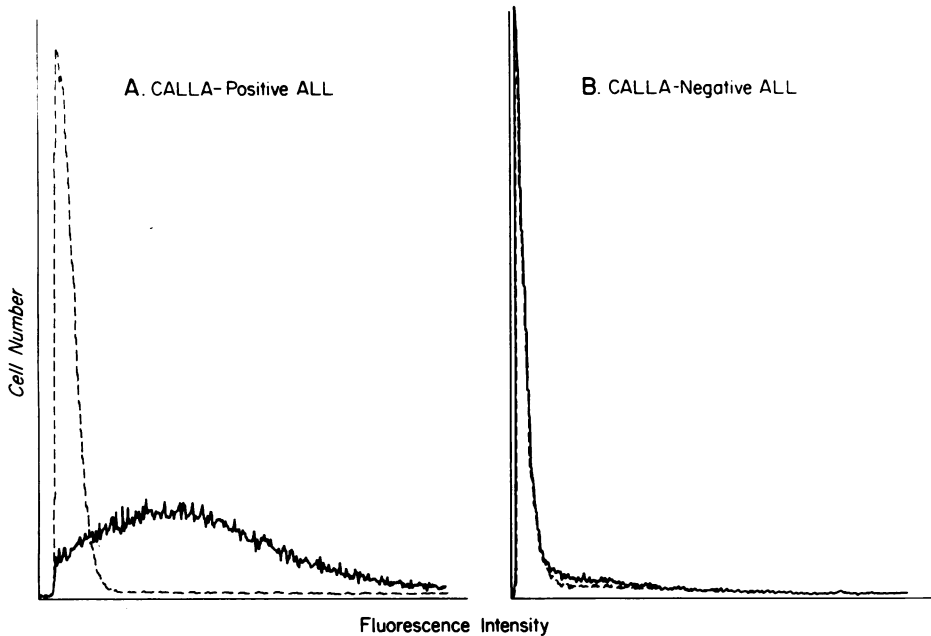


FIGURE 3 The fluorescence profile of the tumor cells from a patient with a CALLA⁺ ALL (A) and CALLA⁻ ALL (B) with anti-B1 antibody and G/M FITC (solid line) is depicted in this figure. It can be seen that the tumor cells from the CALLA⁺ ALL were uniformly reactive with anti-B1 (A), whereas the tumor cells from the CALLA⁻ patient were unreactive (B). Background fluorescence staining was performed as in Fig. 1. The tumor cells from approximately one-third of patients with CALLA⁺ ALL had an identical fluorescence pattern to the one depicted in Fig. 3A. The fluorescence intensity patterns of the two-thirds of patients with CALLA⁺ ALL were equally divided between weak expression (similar to Fig. 2A) and moderate expression (similar to Fig. 2B).

TABLE II
 Reactivity of Anti-B1 with CALLA Positive
 and Negative Leukemic Cells

Tumor	Number of patients	Number reactive with antisera	
		Ia	B1
Non-T cell ALL, CALLA ⁺	28	28	21
Non-T cell ALL, CALLA ⁻	13	13	0
CML—blast crisis, CALLA ⁺	7	6	5
CML—blast crisis, CALLA ⁻	3	1	0

patients with non-T cell ALL and ~30% of patients with CML in blast crisis (38). This antigen has been shown to be a glycoprotein with a molecular mass of 100,000 daltons. Recently, a monoclonal antibody (J-5), specific for CALLA, has been described. The tumor cells from 41 patients with non-T ALL and 10 patients with CML in blast crisis were then compared for their reactivity with anti-Ia, anti-CALLA, and anti-B1 monoclonal antibodies. The reactivity of anti-B1 with the tumor cells from a CALLA positive and a CALLA negative patient is depicted in Fig. 3. Fig. 3A shows that the tumor cells from a patient with CALLA⁺ ALL were reactive with the anti-B1 antibody, whereas Fig. 3B shows that the tumor cells from a patient with CALLA⁻ ALL were unreactive. Further heterogeneity of the CALLA⁺ ALL and CML in blast crisis could be demonstrated by their reactivity with anti-B1. As shown in Table II, the tumor cells from 21 of 28 patients with CALLA⁺, Ia⁺ ALL were reactive with anti-B1. In contrast, no tumor cells from the 13 patients with CALLA⁻, Ia⁺ ALL were reactive. Similarly, most of the CALLA⁺, Ia⁺ CML in blast crisis were anti-B1 reactive; and all of the CALLA⁻, Ia⁺ CML in blast crisis were unreactive.

DISCUSSION

In the present study, we have used a monoclonal antibody previously shown to be specific for a B cell surface-differentiation antigen to characterize malignant cells from patients with leukemias and lymphomas of various cellular origins. Examinations of the non-Hodgkin's lymphomas with classical cell surface markers demonstrated that ~80% of these tumors and >95% of CLL are of B cell lineage (3). Morphologically, the B cell lymphomas are heterogeneous, and the observed histologic diversity has led to the development of several classification schemes (37-40). These B cell tumors have also been shown to be variable in their amount of expression of surface or intracytoplasmic immunoglobulin, complement receptors, formation of monkey erythrocyte rosettes, Fc receptors, and Ia-like antigens. It has therefore been postulated

that the cell surface marker and histologic diversity seen in these tumors may reflect distinct stages of B cell differentiation in which the malignant cells are "frozen" (41). Unfortunately, the various cell surface markers presently used define neither unique histologic subtypes nor distinct clinical subgroups. Nevertheless, given the better prognosis of B cell neoplasms compared with T or Null cell tumors, a number of these markers have been widely used (42, 43).

In this study, the tumor cells from all 18 patients with B cell CLL and 47 of 50 patients with B lymphomas, all bearing κ or λ light chains, were reactive with the anti-B1 antibody. Moreover, anti-B1 was unreactive with acute T cell leukemias and lymphomas and with tumor cells from all patients with acute myeloblastic leukemia. These observations suggest that anti-B1 adds to the repertoire of cell surface determinants that define B cell tumors, and unlike Ia, Fc, and C3, it is restricted to this class of cells. More importantly, the presence of B1 antigen in conjunction with the expression of monoclonal κ or λ light chains provides additional criteria for the definition of a malignant B cell clone. In this regard, normal B cells and other cells capable of binding immunoglobulin via an Fc receptor are invariably heterogeneous with regard to their light chain phenotype, and as such, can be distinguished from B cell neoplasms.

Although anti-B1 was reactive with the vast majority of B cell lymphomas and all B cell CLL, the non-T cell ALL were divided into several distinct entities. These tumor cells have been shown to be unreactive with anti-T and anti-Ig reagents, but to be strongly reactive with anti-Ia and anti-CALLA. Previous studies have shown that 95% of non-T cell ALL are Ia⁺, whereas CALLA was coexpressed on ~80% of the non-T cell ALL. These studies indicated that the majority of non-T cell ALL were CALLA⁺, Ia⁺, and a small group were CALLA⁻, Ia⁺. Little is known about the small subset of patients (1-2%) who express cell surface immunoglobulin and are therefore thought to represent a more mature B cell ALL.

The present studies have shown that the tumor cells from ~50% of patients with non-T cell ALL were reactive with anti-B1. More importantly, most of the CALLA⁺, Ia⁺ ALL were anti-B1 reactive, whereas all of the CALLA⁻, Ia⁺ ALL were anti-B1 unreactive. Thus, the non-T cell ALL can now be divided into three major subclasses: (a) CALLA⁺, Ia⁺, B1⁺; (b) CALLA⁺, Ia⁺, B1⁻; and (c) CALLA⁻, Ia⁺, B1⁻. These studies provide additional support for the view that a significant fraction of CALLA⁺ ALL was B cell derived. Other investigators have demonstrated that ~20-30% of CALLA⁺ ALL had the characteristics of pre-B cells in that they contained intracytoplasmic μ chain and lacked both surface and cytoplasmic light chains (44-46). The present study would suggest that, in fact, the majority

of CALLA⁺, Ia⁺ ALL are B cell derived, since 75% were anti-B1⁺. Whether B1 is expressed earlier than cytoplasmic immunoglobulin in B cell differentiation, or is a more sensitive marker of pre-B cells, is yet to be resolved.

The demonstration that the B1 antigen is expressed on all normal B cells, B cell lymphomas, and a proportion of acute leukemias suggests that the B1 antigen is expressed on most stages of B cell differentiation. It was intriguing to find that the generally accepted end-stage cell in B cell ontogeny, the plasma cell, lacked B1. Thus, B1 appears to be a B cell differentiation antigen present throughout most stages of B cell maturation. Similarly, a number of anti-T cell antibodies have been described that are capable of dissecting normal intra- and extrathymic maturation (47), as well as defining distinct subsets of clinically relevant malignant T cell leukemias and lymphomas (31, 32, 38, 48, 49).

Because monoclonal antibodies are of extremely high titer and can be produced in unlimited quantities compared with heteroantisera, the utility of this marker can now be readily adopted by many laboratories studying B cell tumors. Additional B cell-specific antibodies will be required for the dissection of distinct stages of B cell differentiation and for the identification of clinically relevant subgroups of B cell lymphoproliferative malignancies.

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