Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 845–849, February 1983
Immunology

Monoclonal antibody that defines a unique human T-cell leukemia antigen

(cell membrane antigen/hybridoma/radioimmunoassay/immunodiagnosis)

BEN K. SEON*, SHIGERU NEGORO*, AND MAURICE P. BARCOS†

*Department of Molecular Immunology and †Department of Pathology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263

Communicated by David Harker, October 27, 1982

ABSTRACT **We have generated and characterized a hybridoma monoclonal antibody, termed SN1, that defines a unique human T-cell leukemia antigen. This antibody was generated by using a human leukemia antigen preparation isolated from cell membranes of MOLT-4, a leukemia T-cell line derived from a patient with T-cell-type acute lymphoblastic leukemia (T-ALL). SN1 was characterized by a sensitive microscale radioimmunoassay using a variety of cultured and uncultured human cells. In selected cases, the cell specimens were further tested by immunoperoxidase staining and an immunofluorescence staining test. The results of the radioimmunoassay were in agreement with those of the two other tests. Among the various cultured malignant and nonmalignant cell lines, SN1 reacted only with leukemia T-cell lines derived from patients with T-ALL; it reacted with all six T-ALL cell lines tested—i.e., JM, CCRF-CEM, CCRF-H-SB2, RPMI 8402, PEER, and MOLT-4. In the case of uncultured cell specimens derived from cancer patients, SN1 reacted with four of four cases of T-ALL but did not react with specimens derived from 41 patients with other types of cancer. SN1 did not react with any normal human cell specimens tested, both cultured and uncultured. These specimens include normal lymphoblastoid cell lines, thymocytes, bone marrow cells, spleen cells, lymph node cells, peripheral blood mononuclear cells, lymphocytes containing B and T cells, purified T cells, monocytes, granulocytes, erythrocytes, and platelets. Furthermore, SN1 did not react with phytohemagglutinin-activated T cells nor with concanavalin A-activated T cells. The results show that monoclonal antibody SN1 defines a type of human leukemia antigen that is expressed on the cell surface of T-cell-type ALL cells. The results further show the usefulness of SN1 in the diagnosis of cancer patients and suggest its therapeutic potential. We designate this antigen TALLA, a T-cell ALL antigen.**

Monoclonal antibodies (mAbs) have significant advantages over conventional antisera with respect to fine specificity and available amount. This is particularly so for mAbs directed to cell surface antigens (reviewed in ref. 1). However, it is difficult to generate mAbs directed to human leukemia-associated cell surface antigens by the conventional approach—i.e., by using intact human leukemia cells for immunizing mice to provide spleen cells for cell fusion. This is because the majority of mAbs so obtained are directed to the stronger and more abundant normal cell antigens such as the HLA-A,B,C antigens. We have recently generated several anti-human leukemia mAbs by using an isolated leukemia-associated cell membrane antigen preparation (2, 3). In this paper, we report the generation, characterization, and immunological specificity of a mAb termed SN1 that was obtained by using the same antigen preparation. SN1 defines a type of human leukemia-associated cell surface antigen that is specific for T-cell-type acute lymphoblastic leukemia (T-ALL) cells. Clinical use of this antibody will be important because T-ALL is associated with poor prognosis and the majority of T-ALL patients who have had remission suffer relapses (reviewed in ref. 4).

MATERIALS AND METHODS

Cells. Established human cell lines were cultured in RPMI 1640 medium/4–10% fetal calf serum as described (5). Specimens from cancer patients were provided by H. Ozer, D. J. Higby, T. Han, M. C. O'Leary, A. I. Freeman, and C. K. Tebbi of the clinics of the Roswell Park Memorial Institute. Mononuclear cells were isolated from these specimens by centrifugation on a Ficoll-Paque gradient (6, 7). Normal thymocytes and spleen cells were obtained as described (7). Various types of normal peripheral blood cells were isolated from buffy coat preparations from healthy volunteers as follows. Mononuclear cells containing both lymphocytes and monocytes were isolated by centrifugation on a Ficoll-Paque gradient (6). Lymphocytes were isolated from the mononuclear cells by treating them with iron carbonyl (8) followed by incubation in plastic tissue culture flasks (9). These nonphagocytic and nonadherent cells were composed of lymphocytes. T cells were purified from either the mononuclear cells or the lymphocytes by the procedure of Julius *et al.* (10). Monocytes were isolated from the mononuclear cells as described by Smith and Ault (9). Granulocytes and erythrocytes were purified from the buffy coat preparations by the procedure of Boyum (6). Platelets were isolated by modification of the procedure of Baenziger and Majerus (11).

Leukemia Antigen Preparation. This was prepared from cell-membranes of MOLT-4, a leukemia T-cell line, as described (3). This procedure is based on modification of our previous procedure (5). The present antigen preparation is not as pure as the previous well-characterized one (5) in that the steps of ethanol precipitation and rechromatography on serially connected immunoadsorbent columns were omitted. However, we found that the present preparation is more strongly immunogenic than the previous one because of the addition of iodoacetamide during solubilization of the cell membrane antigens and omission of the ethanol precipitation step. The present antigen preparation is more similar to another previously reported preparation (12).

Immunization. A mouse was immunized by subcutaneous injection of 40 μg of the isolated leukemia antigen preparation in Freund's complete adjuvant. In addition, 2×10^9 *Bordetella pertussis* bacteria were injected at different sites. Four booster immunizations were administered and the spleen was taken for the fusion 2 days after the last immunization.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: ALL, acute lymphoblastic leukemia; T-ALL, T-cell-type ALL; mAb, monoclonal antibody; $F(ab')_2$-GαMIgG, $F(ab')_2$ fragment of affinity-purified goat anti-mouse IgG antibodies.

Cell Fusion and Plating. Immune spleen cells were fused with the mouse myeloma cell line P3/NS1/1-Ag4-1 (abbreviated NS-1) by using polyethylene glycol, and the resulting hybrid cells were grown in hypoxanthine/aminopterin/thymidine medium according to the reported procedure (13, 14).

Screening and Cloning of Hybridomas. Monoclonal antileukemia antibodies in the culture supernatants of the hybridomas were screened by a radioimmunoassay. Cloning of hybridomas was carried out by propagating from a single cell by a limiting dilution procedure (14). To ensure the monoclonality of the clone, the hybridoma clone resulting from the first cloning was recloned.

Microscale Radioimmunoassay. To establish a radioimmunoassay, we first prepared the $F(ab')_2$ fragment of affinity-purified goat anti-mouse IgG antibodies and passed it through an immunoadsorbent column prepared with Sepharose CL-4B-conjugated human IgG. This treatment removes any $F(ab')_2$ components reacting with antigenic determinants common to mouse IgG and human IgG.

This $F(ab')_2$ fragment preparation of specific goat antibodies directed to mouse IgG [designated $F(ab')_2$-GαMIgG] was radiolabeled with carrier-free ^{125}I by the IODO-GEN method (5) and used in a radioimmunoassay. The present antibody reagent was found to react with mouse IgM almost as efficiently as with mouse IgG. Using this ^{125}I-labeled $F(ab')_2$-GαMIgG, we developed a microscale radioimmunoassay to determine the reactivity of mAbs with various cells. In a typical assay, triplicate 20-μl aliquots of various dilutions of culture fluids or ascites of hybridomas and 2–10 $\times$ 10^5 cells in 10 μl of Hepes buffer, pH 7.3/0.1% human IgG/IgM/IgA are incubated in individual wells of 96-well microtiter plates (Cooke Engineering, Alexandria, VA) for 60 min at 4°C with continuous shaking. The Hepes buffer consists of RPMI 1640 medium/25 mM Hepes/0.5% bovine serum albumin/Trasylol (50 kallikrein units/ml)/0.1% NaN_3. The human immunoglobulins are added to the Hepes buffer to minimize nonimmunospecific binding (both biospecific such as Fc receptor and nonbiospecific) of mouse antibodies and ^{125}I-labeled $F(ab')_2$-GαMIgG to the cells during the radioimmunoassay. In addition, the microtiter plate wells are treated before use with the Hepes buffer.

The mixtures are centrifuged at 500 $\times$ *g* and 4°C for 10 min and the pelleted cells are washed three times. Approximately 2 ng (3–5 $\times$ 10^4 cpm) of the ^{125}I-labeled $F(ab')_2$ in 10 μl of Hepes buffer is added to each washed pellet and the reaction mixtures are incubated with shaking and washed as described above. The radioactivity in the washed pellet is determined in a γ-ray spectrometer. When platelets are used as targets, the radioimmunoassay is carried out in conical polypropylene tubes (1.5-ml size) instead of microtiter plates because it is necessary to precipitate platelets at significantly higher centrifugal force (e.g., 2,500 $\times$ *g*).

Immunoperoxidase Staining and Immunofluorescence Staining Tests. The immunoperoxidase staining using fixed tissues was carried out by a published procedure (15). For the immunofluorescence staining of cells, a standard indirect procedure was used.

Activation of T Cells with Phytohemagglutinin or Concanavalin A. Purified T cells isolated from normal human peripheral blood lymphocytes were activated with phytohemagglutinin or concanavalin A as described by Boto and Humphreys (16).

RESULTS

Initial Characterization of SN1. We have primarily used a radioimmunoassay for screening and characterizing our hybridomas. Hybridoma SN1 was obtained by repeated cloning of a primary hybrid whose culture supernatant showed reactivity against three human leukemia T-cell lines (MOLT-4, JM, and CCRF-H-SB2) but no significant reactivity against two nonmalignant B-cell lines (RPMI 1788 and CCRF-SB) and a leukemia non-T-/non-B-cell line (NALM-1). mAb SN1 was found to be IgG1κ in a double-diffusion agarose plate test by using a 20-fold concentrated culture fluid of the hybridoma. Ascites tumors were prepared by injecting pristane-primed (14, 17) BALB/c mice with viable cells of hybridoma SN1. Serial dilutions of both culture fluid and ascites were tested against various cultured and uncultured cells by a radioimmunoassay; some of the results are shown in Fig. 1. Both culture fluid and ascites showed significant reactivity against JM and MOLT-4, both leukemia T-cell lines, but no reactivity against K562 (a leukemia myeloerythroid cell line), CCRF-SB (a nonmalignant B-cell line), normal peripheral blood lymphocytes, and normal thymocytes. The absence of reactivity of SN1 against thymocytes was confirmed by an immunofluorescence staining test, an immunoperoxidase staining test, and a quantitative absorption test. With respect to the antibody titer, a 1:100,000 dilution of the SN1 ascites showed significant reactivity against leukemia T-cell lines.

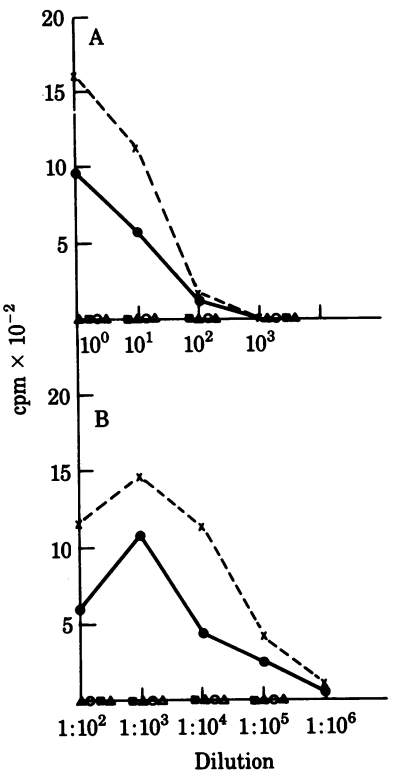

FIG. 1. Reactivity of culture fluid and ascites of hybridoma clone SN1. A radioimmunoassay was used to test serial dilutions of culture fluid (*A*) or ascites (*B*) against the leukemia T-cell lines MOLT-4 (●) and JM (x) (2 $\times$ 10^5 cells, respectively), a leukemia myeloerythroid cell line, K562 (○; 2 $\times$ 10^5 cells), a normal B-cell line, CCRF-SB (■; 2 $\times$ 10^5 cells), normal peripheral blood lymphocytes (△; 1 $\times$ 10^6 cells) and normal thymocytes (▲; 1 $\times$ 10^6 cells). Dilutions of both the culture fluid and the ascites were made with RPMI 1640 medium/15% fetal calf serum. Cell culture medium (i.e., RPMI 1640/15% fetal calf serum) and medium containing mouse IgG1 (10 μg/ml) were included in the assays as controls against SN1 culture fluid for individual cell specimens (see Table 1). Serial dilutions of a murine ascites containing monoclonal hybridoma antibody directed to the hapten phthalate were included as controls against the corresponding dilutions of SN1 ascites for individual cell specimens. Both the antiphthalate and the SN1 mAbs are of the IgG1 isotype. The appropriate control radioactivity was subtracted from individual values.

The reactivity of SN1 against various cultured leukemia/lymphoma and nonmalignant cell lines is summarized in Table 1. SN1 reacted with the six leukemia T-cell lines derived from individual patients with ALL—MOLT-4, JM, CCRF-H-SB2, CCRF-CEM, RPMI 8402, and PEER—but did not react with either a Sézary T-cell line, HUT 78, or a Japanese adult lymphoma T-cell line, HPB-MLT. It did not react with any of the other leukemia/lymphoma and normal cell lines tested, including ALL non-T/non-B-cell lines and ALL B-cell lines.

Reactivity of SN1 with Uncultured Cell Specimens from Cancer Patients. Unlike a cultured established cell line, an uncultured cell specimen from cancer patients consists of heterogeneous groups of cells and only a portion of these cells may represent malignant cells. In view of this problem in testing uncultured cell specimens, we investigated the sensitivity of our radioimmunoassay by testing SN1 against mixtures of leukemia T cells and normal cells. The test results (Fig. 2) suggest that we may be able to detect as little as 2.5% leukemia T cells in a heterogeneous cell mixture and less than 1% leukemia T cells by increasing the total number of cells in the assays (e.g., from 2×10^5 to 1×10^6 cells).

Table 1. Reactivity of hybridoma SN1 culture fluid with human leukemia/lymphoma (HLL) and normal cell lines

Cell line	Origin of cell line*	Reactivity, cpm: SN1	Control: Medium†	Control: IgG‡
HLL T				
MOLT-4	ALL	1,205 ± 119	204 ± 54	264 ± 7
JM	ALL	1,864 ± 137	139 ± 18	216 ± 19
CCRF-H-SB2§	ALL	704 ± 64	164 ± 20	220 ± 46
CCRF-CEM	ALL	2,757 ± 80	141 ± 26	188 ± 14
RPMI 8402	ALL	689 ± 18	107 ± 9	105 ± 25
PEER	ALL	680 ± 106	132 ± 30	207 ± 21
HUT 78	SS	252 ± 41	196 ± 14	208 ± 23
HPB-MLT	JAL	144 ± 13	77 ± 16	168 ± 28
HLL non-T/non-B				
NALM-1	CML-BC	136 ± 34	137 ± 18	141 ± 14
NALM-6	ALL	121 ± 8	97 ± 12	105 ± 7
KM-3	ALL	134 ± 11	113 ± 42	121 ± 6
NALM-16	ALL	157 ± 20	103 ± 19	130 ± 21
HLL B				
BALM-2	ALL	173 ± 9	125 ± 16	165 ± 26
BALL-1	ALL	109 ± 28	83 ± 11	167 ± 28
Raji	BL	224 ± 19	139 ± 8	166 ± 25
Ramos	BL	133 ± 32	116 ± 20	117 ± 12
HLL myeloerythroid				
K562	CML-BC	267 ± 11	194 ± 32	241 ± 22
HLL myelomonocytic				
HL-60	APL	294 ± 16	247 ± 11	280 ± 40
U-937	HL	202 ± 21	164 ± 9	211 ± 28
HLL undefined				
SU-DHL-1	HL	180 ± 34	121 ± 21	237 ± 43
Normal B				
CCRF-SB§		146 ± 26	103 ± 14	164 ± 18
RPMI 1788		175 ± 36	108 ± 4	225 ± 30
RPMI 8057		114 ± 18	111 ± 18	188 ± 30

The properties of the HLL cell lines used are reviewed in refs. 18 and 19. HLL pre-B-cell lines (NALM-1 and NALM-6) are included in the group of HLL non-T/non-B-cell lines. Reactivity was determined by a radioimmunoassay using 20 μl of culture fluid of hybridoma SN1 and 2×10^5 cells per test. Values given are mean ± SD of triplicate tests.

* ALL, acute lymphoblastic leukemia; SS, Sézary syndrome; JAL, Japanese adult lymphoma; CML-BC, chronic myelocytic leukemia in blast crisis; BL, Burkitt lymphoma; APL, acute promyelocytic leukemia; HL, histiocytic lymphoma.

† Culture medium of hybridoma—i.e., RPMI 1640/15% fetal calf serum/2 mM glutamine containing penicillin (100 units/ml) and streptomycin (100 μg/ml).

‡ Normal mouse IgG (10 μg/ml) or mouse myeloma IgG1 (10 μg/ml) in hybridoma culture medium.

§ These cell lines were derived from the same individual.

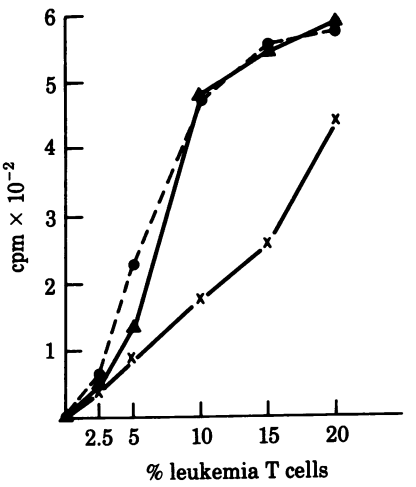

FIG. 2. Sensitivity of a radioimmunoassay using SN1 as determined by using mixtures of human leukemia T cells and normal cells. JM and MOLT-4, both leukemia T-cell lines, were mixed with RPMI 1788, a normal B-cell line, to obtain mixtures containing 2.5%, 5%, 10%, 15%, and 20% leukemia T cells, keeping total cells in the mixtures constant at 2×10^7/ml. Pure RPMI 1788 cells (i.e., 0% leukemia T cells) were also included in the test. The radioimmunoassay was carried out in triplicate using 2×10^5 cells in 10 μl. Assays of the JM-containing cell mixtures were carried out using 20 μl of neat hybridoma culture fluid (●) or a 1:5 dilution (▲). Assays of the MOLT-4-containing cell mixtures (x) were carried out using 20 μl of a 1:5 dilution of the hybridoma culture fluid.

The usefulness of SN1 and our radioimmunoassay in diagnosis was shown by tests with uncultured cells obtained from cancer patients. To avoid knowledge of the associated diseases, blind tests were carried out using coded cell specimens. When the test results were checked against the clinical and laboratory diagnosis of each patient's disease, SN1 was found to react only with T-ALL cells. The results of the radioimmunoassays with these patients' uncultured cells are summarized in Table 2. Of the 45 specimens obtained from patients with different neoplasms, SN1 reacted only with the four cell specimens obtained from patients with T-cell ALL. The test results of these four T-ALL cell specimens along with those for two control cell specimens (nos. 5 and 6) obtained from individual T-ALL patients in remission are shown in Table 3. It is important to note that SN1 did not react with these remission specimens. The results of the radioimmunoassays were confirmed by testing two T-cell ALL specimens (nos. 2 and 3) and one B-cell chronic lymphocytic leukemia specimen by the conventional indirect immunofluorescence staining test using a 1:100 dilution of SN1 ascites. Sixty-two and 50%, respectively, of the cells of the two T-cell ALL specimens showed strong staining but none of the cells of the B-cell chronic lymphocytic leukemia specimen showed staining. Thus, the results of the immunofluorescence staining test agree with those of the radioimmunoassay.

Test of SN1 Against Uncultured Normal Cells. SN1 was further tested by the radioimmunoassay against different types of normal cells to obtain further information regarding its origin, biological function, and potential usefulness in therapy. The results are summarized in Table 4. SN1 did not react with any

Table 2. Reactivity of SN1 with uncultured human malignant cells

Disease of patient	Reactivity*
T-cell acute lymphoblastic leukemia	4/4
Non-T/non-B-cell acute lymphoblastic leukemia	0/6
B-cell acute lymphoblastic leukemia	0/1
Cutaneous T-cell lymphoma†	0/2
T-cell lymphoma	0/4
B-cell lymphoma	0/9
B-cell chronic lymphocytic leukemia	0/8
Chronic myelocytic leukemia	0/2
Acute myelocytic leukemia	0/3
Acute promyelocytic leukemia	0/1
Acute myelomonocytic leukemia	0/1
Hairy cell leukemia	0/1
Erythroleukemia	0/1
Thymoma	0/1
Hodgkin disease	0/1

Individual cell specimens were obtained from peripheral blood of different patients except for some specimens that were derived from bone marrow, lymph node, or thymus of different patients. Reactivity of SN1 with the different cell specimens was determined by radioimmunoassay. In the assay, four or three different controls were included with each cell specimen. These were (*i*) mouse IgG1 (10 μg/ml) in the hybridoma culture medium and (*ii*), in many cases, hybridoma culture medium in place of hybridoma SN1 culture fluid (see Table 1). The other controls were (*iii*) MOLT-4 cells (a positive control) and (*iv*) CCRF-SB cells (a negative control) in place of the target cell specimen. An immunofluorescence test was also carried out for specimens of T-cell ALL, T-cell lymphoma, B-cell lymphoma, and B-cell chronic lymphocytic leukemia.

* Number of reactive specimens per total number of specimens tested.

† The two cutaneous T-cell lymphoma cell specimens tested were derived from individual patients with Sézary syndrome.

of the normal cells, including thymocytes, spleen cells, bone marrow cells, and various peripheral blood cells—i.e., mononuclear cells containing lymphocytes and monocytes, purified lymphocytes containing B and T cells, purified T cells, monocytes, granulocytes, erythrocytes, and platelets. When tested by an immunoperoxidase method on fixed tissue sections, SN1 was negative with two childhood thymus specimens and two lymph node specimens. Two specimens of normal bone marrow cells were also negative for SN1 by an immunofluorescence method.

Reactivity Against Activated T Cells. SN1 did not react with either phytohemagglutinin- or concanavalin A-activated T cells in a radioimmunoassay and in a quantitative absorption test.

Table 3. Radioimmunoassay of uncultured leukemia cells derived from patients with T-cell acute lymphoblastic leukemia

		Reactivity, cpm		
			Control	
Specimen	Source	SN1	Medium*	IgG*
1	Peripheral blood	600 ± 20	NT	214 ± 25
2	Peripheral blood	790 ± 124	NT	253 ± 2
3	Bone marrow	432 ± 42	NT	199 ± 11
4	Bone marrow	592 ± 4	128 ± 75	211 ± 15
5 (remission)	Bone marrow	116 ± 15	103 ± 56	275 ± 50
6 (remission)	Bone marrow	134 ± 18	114 ± 22	293 ± 66

Reactivity was determined by a radioimmunoassay using approximately 2×10^5 (nos. 1 and 2), 4×10^4 (no. 3), or 1×10^6 (nos. 4–6) cells in each test. Values given are mean ± SD of triplicate tests. NT, not tested.

* See Table 1.

Table 4. Reactivity of uncultured normal human cells with SN1

Cells	Reactivity*
Thymocytes	0/8
Spleen cells	0/5
Bone marrow cells	0/7
Lymph nodes†	0/2
Peripheral blood	
Mononuclear cells	0/3
Lymphocytes	0/2
T cells	0/8
Monocytes	0/4
Granulocytes	0/6
Erythrocytes	0/4
Platelets	0/6

Individual cell specimens were obtained from different donors and tested separately. Typical cell numbers used per test were 1×10^6 cells for thymocytes, spleen cells, bone marrow cells, mononuclear cells, and T cells; 2×10^5 cells for lymphocytes and erythrocytes; 3×10^5 cells for monocytes and granulocytes; and 2×10^7 cells for platelets. Each test of an individual cell specimen was carried out in triplicate by radioimmunoassay. In the assay, four different controls were included with each cell specimen (see Table 2).

* Number of reactive specimens per total number of specimens tested.

† Determined by an immunoperoxidase method.

DISCUSSION

Previous studies have shown the presence of a human leukemia-associated cell surface antigen termed common ALL antigen or CALLA (18, 20–24). CALLA has been found mainly on non-T ALL cells and on leukemia cells of patients with chronic myelocytic leukemia in lymphoid blast crisis. It was also found on a small proportion of normal bone marrow cells, as well as on some T-ALL cells, Burkitt lymphoma cells, and other tissues. Despite the lack of absolute specificity for leukemia and the unresolved issue of its origin, CALLA and its antisera have been useful for diagnosis as well as for developing a clinically valuable subclassification of ALL.

There is another type of ALL-associated cell surface antigens—i.e., human thymus-leukemia-associated antigens that have been detected on normal thymocytes and on some T-ALL cells but not on normal T and B cells of the peripheral blood (7, 25–30). These antigens appear to be normal differentiation antigens associated with thymocytes and immature T cells.

Another interesting ALL-associated cell membrane marker is asialo-GM_1, a glycosphingolipid. This was initially reported as a marker for normal T cells and thymocytes of mice (reviewed in ref. 31). More recently, Nakahara *et al.* (32) reported that asialo-GM_1 is a useful marker for human ALL cells with both T and non-T phenotypes based on a study using rabbit anti-asialo-GM_1 antiserum.

In contrast to the three types of leukemia-associated antigen discussed above, the leukemia antigen detected by mAb SN1 was found neither on non-T-ALL cells nor on normal thymocytes.

Recently, Deng *et al.* (33) reported a mAb termed CALL2 directed to T-ALL cells. The specificity of CALL2 appears to be similar to that of SN1. However, there are significant differences in the specificity between these mAbs. For instance, CALL2 strongly reacted with HPB-MLT, a Japanese adult lymphoma T-cell line (34) but did not react with CCRF-H-SB2, an ALL T-cell line. In contrast, SN1 did not react with HPB-MLT but reacted with CCRF-H-SB2 (Table 1).

With respect to the molecular nature of the antigen defined by SN1, our initial study involving immunoprecipitation of the ^{125}I-labeled antigen preparation and $NaDodSO_4$/polyacrylamide gel electrophoresis was not successful in revealing an

^{125}I-labeled component corresponding to this antigen. Several groups of investigators have also experienced difficulty in immunoprecipitating antigens by using mAbs because of the monospecificity of the mAbs (1).

The results described in this paper show the usefulness of SN1 for the diagnosis of ALL (Tables 1–4). Another important use of SN1 will be for therapy of T-cell ALL patients. In this regard, SN1 IgG conjugated with the A chain of ricin, a plant toxin, showed specific killing of T-ALL cells *in vitro* in our recent studies. Therapeutic use of SN1 will be important in view of the fact that T-cell ALL is associated with poor prognosis (reviewed in ref. 4).

Our present approach using an isolated antigen preparation rather than intact cells will be useful for obtaining mAbs directed to other cell membrane antigens. This will be particularly so for the weakly immunogenic cell surface antigens for which generation of mAbs by immunizing mice with whole cells is difficult. In addition, our microscale radioimmunoassay will be useful for screening and characterizing both noncytolytic and cytolytic mAbs directed to various cell surface antigens.

We thank Drs. H. Ozer, D. J. Higby, T. Han, M. C. O'Leary, A. I. Freeman, J. Minowada, and C. K. Tebbi for providing us with specimens from patients. We also thank Mrs. H. Tsai and Mrs. J. Prendergast for their skillful technical assistance and Dr. O. A. Roholt, Mrs. D. Ovak, and Miss J. Stevens for their help in the preparation of the manuscript. This was supported by National Cancer Institute Grant CA19304.

1. Milstein, C. & Lennox, E. (1980) *Curr. Top. Dev. Biol.* **14**, 1–32.
2. Seon, B. K. & Negoro, S. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 310 (abstr.).
3. Negoro, S. & Seon, B. K. (1982) *Cancer Res.* **42**, 4259–4262.
4. Greaves, M. F. (1981) *Cancer Res.* **41**, 4752–4766.
5. Seon, B. K., Negoro, S., Minowada, J. & Yoshizaki, K. (1981) *J. Immunol.* **127**, 2580–2588.
6. Boyum, A. (1974) *Tissue Antigens* **4**, 269–274.
7. Yoshizaki, K., Seon, B. K., Barcos, M. P., Minowada, J. & Pressman, D. (1979) *J. Immunol.* **122**, 1943–1950.
8. Han, T. & Takita, H. (1979) *Cancer* **44**, 2090–2098.
9. Smith, B. R. & Ault, K. A. (1981) *J. Immunol.* **127**, 2020–2027.
10. Julius, M. H., Simpson, E. & Herzenberg, L. A. (1973) *Eur. J. Immunol.* **3**, 645–649.
11. Baenziger, N. L. & Majerus, P. W. (1974) *Methods Enzymol.* **31**, 149–155.
12. Negoro, S. & Seon, B. K. (1981) *Cancer Res.* **41**, 2973–2976.
13. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497.
14. Oi, V. T. & Herzenberg, L. A. (1980) in *Selected Methods in Cellular Immunology*, eds. Mishell, B. B. & Shiigi, S. M. (Freeman, San Francisco), pp. 351–372.
15. Stein, H., Bonk, A., Tolksdorf, G., Lennert, K., Rodt, H. & Gerdes, J. (1980) *J. Histochem. Cytochem.* **28**, 746–760.
16. Boto, W. O. & Humphreys, R. E. (1981) *J. Immunol.* **126**, 759–765.
17. Potter, M. (1972) *Physiol. Rev.* **52**, 631–719.
18. Minowada, J., Janossy, G., Greaves, M. F., Tsubota, T., Srivastava, B. I. S., Morikawa, S. & Tatsumi, E. (1978) *J. Natl. Cancer Inst.* **60**, 1269–1277.
19. Minowada, J., Koshiba, H., Sagawa, K., Kubonishi, I., Lok, M. S., Tatsumi, E., Han, T., Srivastava, B. I. S. & Ohnuma, T. (1981) *J. Cancer Res. Clin. Oncol.* **101**, 91–100.
20. Greaves, M. F., Brown, G., Rapson, N. T. & Lister, T. A. (1975) *Clin. Immunol. Immunopathol.* **4**, 67–84.
21. Billing, R., Minowada, J., Cline, M., Clark, B. & Lee, K. (1978) *J. Natl. Cancer Inst.* **61**, 423–429.
22. LeBien, T. W., Hurwitz, R. L. & Kersey, J. H. (1979) *J. Immunol.* **122**, 82–88.
23. Ritz, J., Pesando, J. M., Notis-McConarty, J., Lazarus, H. & Schlossman, S. F. (1980) *Nature (London)* **283**, 583–585.
24. Veit, B. C., Melvin, S. L. & Bowman, W. P. (1980) *J. Natl. Cancer Inst.* **64**, 1321–1328.
25. Schlossman, S. F., Chess, L., Humphreys, R. E. & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1288–1292.
26. McMichael, A. J., Pilch, J. R., Galfre, G., Mason, D. Y., Fabre, J. W. & Milstein, C. (1979) *Eur. J. Immunol.* **9**, 205–210.
27. Tanigaki, N., Tokuyama, H., Fukunishi, T., Minowada, J. & Pressman, D. (1979) *J. Immunol.* **123**, 2906–2914.
28. Reinherz, E. L., Kung, P. C., Goldstein, G., Levey, R. H. & Schlossman, S. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1588–1592.
29. Dowell, B. L., Falletta, J. M., Moore, J. O. & Metzgar, R. S. (1980) *J. Natl. Cancer Inst.* **65**, 691–701.
30. Gross, N. & Bron, C. (1980) *Eur. J. Immunol.* **10**, 417–422.
31. Hakomori, S. (1981) *Annu. Rev. Biochem.* **50**, 733–764.
32. Nakahara, K., Ohashi, T., Oda, T., Hirano, T., Kasai, M., Okumura, K. & Tada, T. (1980) *N. Engl. J. Med.* **302**, 674–677.
33. Deng, C. T., Chia, J., Terasaki, P. & Billing, R. (1982) *Lancet* **i**, 10–11.
34. Morikawa, S., Tatsumi, E., Baba, M., Harada, T. & Yasuhira, K. (1978) *Int. J. Cancer* **21**, 166–170.