HUMAN B-LYMPHOCYTE ANTIGENS EXPRESSED BY LYMPHOCYTIC AND MYELOCYTIC LEUKEMIA CELLS

I. Detection by Rabbit Antisera*

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The presence of surface membrane immunoglobulin (SmIg)[1] detected by immunofluorescent staining has been the most frequently used method for distinguishing B lymphocytes from T lymphocytes (1). However, a substantial amount of SmIg found on B cells is not an integral part of the membrane but represents IgG bound through Fc receptors (2-4). Thus far, nonlabile, nonimmunoglobulin human B-lymphocyte antigens have not been well characterized. Recently we reported on rabbit antisera raised to papain digests of human malignant spleen cell membranes which appeared to detect leukemia-associated antigens (5). By complement (C)-dependent cytotoxicity both chronic and acute leukemia cells were positive, although the majority of unfractionated normal lymphocytes appeared negative. This report concerns further testing of the antisera using purified subpopulations of lymphocytes as targets and immunofluorescent-staining techniques to detect minor populations of positive cells. From these tests it appears that the antisera are detecting a nonimmunoglobulin B-lymphocyte antigen that is expressed by the majority of leukemia cells, including both lymphocytic and myelocytic subclasses.

Materials and Methods

Antisera. Antisera with B-cell specificity were produced by immunization with papain digests of cell membranes of spleens from patients with hairy cell leukemia, histiocytic and lymphocytic lymphoma and Hodgkin's disease, and a normal donor. The method of antigen solubilization has been described before (5). Briefly, fresh or frozen spleen tissue was cut into small pieces with scissors and then homogenized with a vortex homogenizer. Large cellular debris was removed by a low centrifugation step (1,500 g). Membrane fragments were recovered from the supernate by ultracentrifugation at 80,000 g for 90 min (no. 40 rotor; Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The crude membrane pellet was washed with 0.15 M NaCl-10 mM Tris, pH 7.2 (Tris-NaCl buffer) and then resuspended in water at 30 mg protein/ml. This suspension was then digested with an equal vol of crude papain (Sigma Chemical Co., St. Louis, Mo.) at 0.5 U/mg protein in 0.28 M NaCl-20 mM Tris, pH 8.6, containing 0.1 M cysteine. After incubation at 37°C for 1 h, a 0.5 M iodoacetic acid solution (neutralized to pH 7.6) was added to a final concentration of 0.1

M. The undigested membrane was removed by ultracentrifugation at 80,000 g for 60 min. The supernate was dialyzed against Tris-NaCl buffer and stored at $-90°C$. Sodium deoxycholate (0.5%)-solubilized membranes were also used to produce antiserum which had B-cell specificity. The soluble membrane extract was emulsified with complete Freund's adjuvant and injected intradermally into the back and subcutaneously into the foot pads of New Zealand white rabbits. A booster injection was given 4 wk later and after 2 more wk the animal was sacrificed.

Cells. Lymphocytes and leukemia cells were isolated from heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation. B-cell-enriched and T-cell-enriched lymphocyte populations were isolated from 115 normal unrelated Caucasians. The B-cells were isolated from total lymphocytes by rosette formation of the T-cells with neuraminidase-treated sheep erythrocytes, and relayering on Ficoll-Hypaque. After centrifugation the rosetted T-cells sedimented to the bottom of the tube and the B-cells remained at the interface. The T-cells were recovered by lysis of the sheep erythrocytes with hypotonic Hanks' balanced salt solution. The proportion of the B lymphocytes in the B-cell-enriched population of some samples was measured by fluorescent staining of surface immunoglobulin using polyvalent rabbit antihuman immunoglobulin. T-cell contamination was less then 5%. The B-cell-enriched and T-cell-enriched lymphocyte suspensions were adjusted to 2.0×10^6/ml in McCoy's medium.

The type of leukemia and the number of leukemia cells present were determined by morphological examination. Relapse patients had high abnormal peripheral leukocyte counts; those patients in remission had less than 5% abnormal cells in the bone marrow aspirates. Bone marrow specimens were obtained by aspiration of the posterior superior iliac spine and leukocytes were freed of erythrocyte contamination by hemagglutination and hypotonic shock.

B-Lymphoblastoid Cell Lines. Daudi, Raji, P3 HR1K, BJAB, and Ramos were derived from patients with Burkitt's lymphoma; IM101 and Laim were derived from peripheral blood of patients with infectious mononucleosis; 6410, 8866, 4265, LA256, LA190, CCRM-SB, and 8392 from leukemia patients; and F265 and NC37 from normal donors. T-lymphoblastoid cell lines MOLT 4, MOLT 3, CCRF-HSB, CCRF-CEM, and 8402 were derived from acute lymphocytic leukemia (ALL) patients. CCRM-SB and CCRF-HSB, 8402, and 8392 are paired B and T lines derived from the same donors.

Cytotoxicity Test. Antisera were judged positive when 1 μl lysed more than 80% of 2,000 target cells after 60 min in the presence of 5 μl of rabbit C. Less than 10% dead cells was considered a negative reaction. Cell lysis was measured by the eosin-dye exclusion technique (6). Normal human sera or preimmunized rabbit sera were used as negative controls.

Soluble antigens in sera or membrane extracts were detected by inhibition of cytotoxicity. 1 μl of inhibitor was added to 1 μl of antisera at its end point; after 30 min, target cells were added and then C as described above.

Immunofluorescence. 2×10^6 target cells were incubated at 22°C for 30 min with antisera diluted 1:200 in phosphate-buffered saline. After washing three times in McCoy's medium supplemented with 0.5% fetal calf serum the cells were reincubated for 30 min with 100 μl fluorescein-conjugated goat antirabbit IgG (Meloy Laboratories Inc., Springfield, Va.) at a dilution of 1:100. After washing three times to remove the excess fluorescein reagent, the cells were examined with a Leitz orthoplan fluorescein microscope (E. Leitz, Inc., Rockleigh, N. J.).

Under ultraviolet light, positive cells showed bright membrane immunofluorescent staining (Fig. 1), whereas negative cells appeared completely dark. Controls of the goat conjugate alone and preimmunized rabbit serum were negative.

Results

Of 27 rabbits immunized with papain digests of malignant spleen membranes from lymphoma patients (and 1 normal spleen) 25 produced antisera which had considerably higher C-dependent cytotoxicity titers against B lymphocytes than against T lymphocytes (Table I). Without prior absorption the antisera had titers ranging from 1:64 to 1:32,000 against B cells and most leukemia cells, whereas in most cases the undiluted sera were completely negative against T cells. Five sera had low titers (1:8 or lower) against T cells. One rabbit (no. 112) immunized with a spleen extract solubilized with 0.5% sodium deoxycholate also

TABLE I

C-Dependent Cytotoxicity Titers of Various Rabbit Antisera against Normal T
Lymphocytes and Normal B Lymphocytes or Leukemia Target Cells

Rabbit	Source of Immunogen		Cytotoxicity titers*	
	Patient	Diagnosis	T lymphocyte	B lymphocyte/ leukocyte cell
63	AC	Histiocytic lymphoma	0	128
64	AC	Histiocytic lymphoma	4	1,024
66	AC	Histiocytic lymphoma	0	512
68	AC	Histiocytic lymphoma	0	1,024
69	AC	Histiocytic lymphoma	8	512
70	AC	Histiocytic lymphoma	8	2,048
71	AC	Histiocytic lymphoma	0	10,000
74	AC	Histiocytic lymphoma	0	512
75	AC	Histiocytic lymphoma	0	512
77	AC	Histiocytic lymphoma	8	4,000
78	AC	Histiocytic lymphoma	0	1,500
79	AC	Histiocytic lymphoma	0	400
80	AC	Histiocytic lymphoma	0	256
85	DK	Lymphocytic lymphoma	0	64
86	DK	Lymphocytic lymphoma	0	64
90	MO	Hodgkins disease	0	256
91	MO	Hodgkins disease	0	256
93	SB	Hairy cell leukemia	0	256
94	SB	Hairy cell leukemia	0	256
97	UK	Normal	0	256
98	PD	Histiocytic lymphoma	0	32,000
99	IK	Histiocytic lymphoma	0	0
105	PD	Histiocytic lymphoma	4	512
107	PV	Hodgkins disease	0	0
111	AC	Histiocytic lymphoma	0	256
112	AC	Histiocytic lymphoma‡	32	4,000
120	AC	Histiocytic lymphoma	0	256

* Average titer against minimum of four cells.
‡ Sodium deoxycholate preparation.

produced antisera with anti-B-cell activity. This serum, however, had more activity against T cells than those produced by papain-solubilized antigens.

Table II shows the cytotoxic reactivity of the antisera from rabbits 63, 68, and 98, tested separately against various normal and malignant lymphoid cells. All three antisera gave identical results showing selectivity for B cells and certain leukemia cells. T and B lymphocytes were isolated from 115 different normal healthy donors. In all cases these undiluted sera were completely unreactive against the T cells, although the same sera showed high titers against the B-cell-enriched populations from the same donors. Other normal peripheral blood cells tested, 56 granulocytes, 5 erythrocytes, and 3 PHA blasts, were negative. Leukemia cells from the majority of patients with active disease were positive. Of 40 cases of acute myelocytic leukemia (AML), blast cells from 30 of the patients reacted with the antisera, whereas 10 did not. To summarize the reactivity against leukemia cells: about 70% of AML, chronic myelocytic leukemia (CML), and ALL cells and seven out of eight chronic lymphocytic leukemia

TABLE II

C-Dependent Cytotoxicity of Rabbit Antisera 63, 68, and 98 against Normal Blood Cells, Leukemia Cells, and Cultured Lymphoblastoid Cell Lines

Cell type	No. of cells tested	No. positive	Positive
			%
T lymphocytes	115	0	0
B lymphocytes	115	115	100
Granulocytes	56	0	0
Erythrocytes	5	0	0
AML	40	30	75
ALL	41	28	68
CML	13	9	69
CLL	10	9	90
Phytohemagglutinin blasts	3	0	0
T-cell lines*	5	0	0
B-cell lines‡	16	16	100

* Molt 4, Molt 3, CCRF-HSB, CCRF-CEM, and 8402.

‡ Daudi, Raji, P3HR1K, BJAB, Ramos, IM101, Laim, 6410, 8866, 4265, LA256, LA190, CCRM-SB, 8392, F265, and NC37.

(CLL) cells reacted with the rabbit antisera. Therefore, the antigen concerned is expressed by a majority but not all leukemia cells. However, those which were positive did include both the lymphocytic and myelocytic subclasses of leukemia.

The reactivity of the sera against cultured lymphoblastoid lines (Table II) supports the conclusion obtained using uncultured cells, namely that the antisera are reacting with an antigen found on B cells but not T cells. All 16 lines tested with B-cell characteristics were positive irrespective of whether they were derived from normal or malignant sources, whereas 5 out of 5 T-cell lines and a myeloid line, K562, were negative. In two cases B and T lines were derived from the same patient; the B lines reacted, whereas the T lines did not. The titers against cultured B lines were approximately the same as those against fresh B lymphocytes and cultured leukemia cells.

The specificity of the antispleen antisera was also determined by immunofluorescent staining using a double antibody technique with goat antirabbit gamma globulin fluorescein conjugate as the second layer. Fig. 1 shows the type of strong membrane staining obtained with positive cells; unreactive cells showed no fluorescent staining. The percentages of immunofluorescent-positive cells present in various preparations of cells from blood and bone marrow are given in Table III. The numbers of positive cells found in total peripheral blood lymphocyte preparations from 50 healthy donors ranged from 6 to 15%, which is within the range generally accepted for the percentage of B cells. As expected, the percentage of positive cells in B-enriched lymphocyte preparations increased significantly (to 48–85%) while T-lymphocyte preparations, granulocytes, and platelets were completely negative. The immunofluorescent-staining technique was again able to distinguish between two groups of leukemia patients: those with blast cells positive to the antisera and those with negative blast cells. Peripheral blood and bone marrow preparations from positive patients with active leukemia had 30–95% reactive cells. A later table (Table VII) will show that these figures vary according to the number of leukemia blast cells present
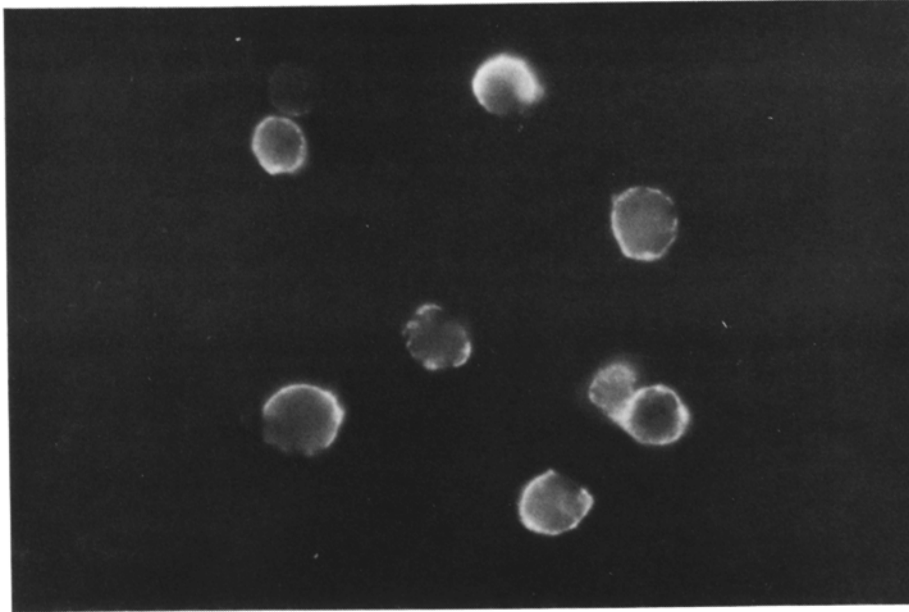
Fig. 1.   Immunofluorescent staining of leukemia cells using rabbit anti-B-cell antisera followed by goat antirabbit gamma globulin conjugate.

TABLE III

Immunofluorescent Staining of Various Normal and Malignant Blood Cells with Antispleen Sera

Target cell	No. Cases	Positive cells
		%
Normal peripheral lymphocytes	50	6-15
B-rich normal lymphocytes*	9	48-85
T-rich normal lymphocytes	6	0
Normal granulocytes	4	0
Platelets	4	0
Active‡ leukemic peripheral leukocytes	9	40-95
Active‡ leukemic bone marrow	15	30-95
Remission leukemia bone marrow	61	0-4
Normal donor bone marrow	7	0-4

* Less than 5% erythrocyte-rosetting cells.

‡ Positive cases with high blast cell counts.

in blood and bone marrow from these patients. A group of approximately 25% of the patients had leukemia blast cells which did not fluoresce.

Bone marrow preparations from 61 leukemia patients in remission and from normal donors had low numbers of positive cells (0-4%). Whether these positive cells are mature B cells or immature precursor cells has not been determined.

It has been observed that nonspecific binding of antibody molecules to B cells can occur through Fc receptors (3). In order to eliminate the possibility that this kind of binding is involved, immunofluorescent-staining studies using $F(ab')_2$ fragments of the antisera were performed. The results (Table IV) show that the

TABLE IV

Immunofluorescent Staining of Normal Peripheral Blood Lymphocytes with Rabbit
Antispleen Cell Antiserum (98), F(ab')₂ Fragments of Serum 98 and Goat Antihuman
Gamma Globulin Antisera

Antisera	Target cells	Positive
		%
98	Peripheral blood lymphocytes	11, 8, 14, 12, 6, 13 *
98	B-enriched lymphocytes	85, 48, 76, 76, 67, 49
98 F(ab')₂	B-enriched lymphocytes	81, 71, 52
Antihuman γG	B-enriched lymphocytes	32, 45, 35, 51, 56, 43

percentage of positive cells using F(ab')₂ fragments was similar to that obtained with whole antisera indicating that binding through the Fc receptor is not involved. The finding that the antibody could not be removed by washing at 37°C, which removes antibody molecules bound through the Fc region, also supports this conclusion.

It is clear from the above results that the antisera are not reacting with T lymphocytes but it is not clear which of the subpopulations of the non-T cells are reacting. Table V shows that the numbers of B-rich cells that were positive to antihuman gamma globulin sera (SmIg-positive cells) range from 32 to 56%, whereas those that are positive with the rabbit antispleen antisera (98) range from 48 to 85%. It appears that in any one B-cell preparation the number of cells positive for antiserum 98 always exceeded the number of SmIg-positive cells. In addition, experiments in which both antisera were used simultaneously indicated that the SmIg-positive cells were part of the population positive for antiserum 98. Therefore, it appears that the rabbit antispleen sera are reacting not only with a population of non-T, SmIg-positive cells (true B cells) but also a population of non-T, SmIg-negative cells.

The presence of soluble B-cell antigen in serum and in spleen extracts could be detected by inhibition of the cytotoxicity of the antispleen antisera against leukemia cells. Significant inhibition was obtained with 5 out of 17 sera from leukemia patients who had peripheral blast cells which were positive for the rabbit antisera (Table V). 4 of 17 sera from lymphoma patients also showed significant inhibition. The inhibiting titers of the positive sera were less than 1:8. No inhibition was seen with serum from normal individuals, nonlymphoproliferative cancer patients, and negative leukemia patients.

The clinical course of leukemia could be followed by examining bone marrow samples for immunofluorescent-positive cells using the antispleen antisera. Bone marrow samples from children with leukemia were examined before and after chemotherapy. The numbers of fluorescein-positive cells were found to correspond to the stage of the disease as determined by morphological examination (Table VI). In newly diagnosed cases, the number of fluorescein-positive cells in the bone marrow was high (over 80%). After inducing remission by chemotherapy the numbers of positive cells decreased to 0-2% which was in general agreement with the number of blast cells found at this time by morphological examination. After relapse the increase of fluorescein-positive cells was again commensurate with the increased numbers of blast cells.

Absorption experiments (Table VII) seem to rule out the possibility that the

TABLE V

Detection of Soluble B-Cell Antigen in the Serum of Patients with Leukemia and Lymphoma

Serum	No. positive/no. tested
Leukemia (+)*	5/17
Leukemia (−)‡	0/12
Lymphoma	4/17
Other cancers	0/6
Normals	0/12

* Serum from leukemia patients with peripheral blasts that were positive to the rabbit antispleen antisera.

‡ Serum from leukemia patients with peripheral blasts that were negative to the rabbit antispleen antisera.

TABLE VI

A Comparison of the Number of Blasts and Immunofluorescent (IF)-Positive Cells in Bone Marrow Aspirates from ALL Patients before and after Treatment

Date	Patient	Treatment	Diagnosis	Blast	IF
				%	%
2/18	GE	Untreated	ALL new case	87	95
3/19		Chemotherapy	ALL remission	1	Neg.
2/19	PR	Untreated	ALL new case	93	70
3/25		Chemotherapy	ALL remission	3	2
3/5	SJ	Untreated	ALL new case	97	80
4/5		Chemotherapy	ALL remission	0	1
5/30		Chemotherapy	ALL remission	0	2
4/11	CH	Untreated	ALL new case	66	60
7/12		Chemotherapy	ALL remission	0	Neg.
4/11	JW	Untreated	ALL new case	46	46
6/24		Chemotherapy	ALL partial relapse	17.5	19
7/1		Chemotherapy	ALL partial relapse	17.0	15

rabbit antisera contain two specificities, one directed against normal B cells and the other against leukemia cells. B lymphocytes and T lymphocytes isolated from the same normal healthy donor (no. 238) and AML cells 812 were used to absorb rabbit serum 68. The absorbed sera were retested against five different B-cell preparations, three AML, and one ALL cell. In general, 10^6 B-lymphocyte 238 cells were able to remove the activity of serum 68 against both B cells and leukemia cells. 10^6 AML 812 cells were also able to remove both anti-B and antileukemia activity. T-cell preparation 812 was not able to remove either the anti-B or antileukemia activity of serum 68. 10^6 AML 694 cells removed the activity of antiserum 63 against both AML 694 and CLL 227 suggesting that they express common antigens.

Discussion

Rabbit antisera raised to papain digests of human spleen cell membrane appear to recognize a previously uncharacterized B-cell antigen. The sera were

TABLE VII

Cytotoxicity of Rabbit Anti-B-Cell Sera 68 and 63 Absorbed with B Lymphocytes and Leukemia Cells

Serum	Absorbing cells	Target cells								
		B lymphocytes					Leukemia cells			
		463*	547	636	712	840	AML 553	ALL 640	AML 112	AML 812
68	Unabsorbed	+	+	+	+	+	+	+	+	+
68	10^5 B lymphocytes 238*	+	+	+	+	+	+	+	+	+
68	10^6 B lymphocytes 238	–	–	±	–	–	–	–	–	–
68	10^5 T lymphocytes 238	+	+	+	+	+	+	+	+	+
68	10^6 T lymphocytes 238	+	+	+	+	+	+	+	+	±
68	10^5 AML 812	+	+	+	+	+	+	+	+	+
68	10^6 AML 812	–	–	–	–	–	–	–	–	–

Serum	Absorbing cells	AML 694	CLL 227
63	Unabsorbed	+	+
63	10^6 AML 694	–	–
63	10^6 CLL 227	–	–

+ >80% kill
– <20% kill
± 30-70% kill

* These numbers refer to the cell donor.

strongly positive by both cytotoxicity and immunofluorescence tests against peripheral blood B cells and cultured B-cell lines but completely negative against normal and cultured T cells, granulocytes, erythrocytes, and platelets. Most of the T and B lymphocytes and two of the T- and B-cell lines were paired, i.e., derived from the same donor. There were large differences in the titers between the T and B cells which would tend to rule out reactivity against antigens such as HLA that are present on T cells.

Leukemia cells from approximately 70% of patients with ALL, AML, and CML, and seven of eight patients with CLL were also positive. Although it is possible that the antisera contain antibodies against leukemia-associated antigens that are distinct from antibodies against normal B lymphocytes, most of the evidence appears to be against this: (a) absorption experiments with B cells and leukemia cells indicate that there is a common antigen involved; (b) antisera with leukemia activity can be raised to normal spleen extracts; and (c) the antisera have the same titers against B cells and against leukemia cells. Therefore, it appears that the antisera made to papain digests of spleen membranes are recognizing a normal B-lymphocyte antigen which is also expressed by both lymphocytic and myelocytic leukemia cells.

Several previous studies have shown that leukemia cells from the majority of cases of ALL, AML, and CML are null cells lacking known B- and T-cell markers such as Fc and C3 receptors, surface immunoglobulins, and receptors for sheep erthyrocytes (7, 8). From these studies it appears that the "null" leukemia cells express the B-cell antigen defined by our rabbit antisera. This group rather surprisingly includes the myeloid leukemias. It is not clear why

cells derived from the myeloid series should express an antigen that is found on mature B lymphocytes but that is not found on mature granulocytes. However, although the antigen is expressed by B lymphocytes, it should not necessarily be considered to indicate that myelocytic leukemias are of B-cell origin. Most CLL cells, however, are of B-cell origin (9) which makes it less surprising that seven out of eight CLL cells reacted with the antisera (the negative CLL appeared to be one of the rare CLL's expressing T-cell characteristics). Whether or not the negative ALL, AML, and CML cells have T-cell characteristics is not presently clear. However, in the case of childhood ALL, this appears to be the case. The proportion of patients with unreactive ALL blasts (30%) is approximately the same as the proportion of T-cell ALL's characterized by erythrocyte rosette formation (7). In addition, we have found that all those leukemia cells positive for the rabbit anti-B-cell serum are also positive for human anti-B-cell alloanti-sera.[2] A study by Fu et al. has shown that the non-B, non-T ALL appear to be those that react with the human anti-B-cell sera, whereas the T-cell ALL's do not (10).

The reactivity of cultured lymphoblastoid cell lines with the antispleen antisera confirms its specificity for a B-cell antigen. All 14 B-cell lines tested were positive, irrespective of whether the cells were derived from normal or malignant sources; 5 T-cell lines and one myeloid cell were negative. One of the positive cell lines was Daudi, a cell on which we and others (11) have been unable to detect HLA antigens or beta$_2$ microglobulin. The reactivity of this cell would suggest that beta$_2$ microglobulin is not associated with the B antigen in the manner that it is associated with HLA antigens (12).

All of the B-cell lines that react with the rabbit anti-B-cell sera also react with human anti-B-cell sera directed against Ia-like antigens. Therefore it is possible that the B antigen is associated with part of these Ia-like molecules. Because the rabbit sera reacted with all normal and cultured B cells thus far tested, they are probably not reacting with the Ia antigenic site itself which is polymorphic but perhaps a common region of the same molecule. The fact that human anti-B-cell sera can be specifically blocked with rabbit anti-B F(ab')$_2$ fragments would support this conclusion which is considered in detail in another publication.[2]

From immunofluorescent-staining studies on peripheral blood lymphocytes the antisera appeared to be reacting with a larger population of cells than those expressing SmIg. However, the number of positive cells seen when the anti-B-cell antisera were used alone was the same as that when both anti-B-cell and antihuman gamma globulin were used together. Therefore it appears that SmIg cells are positive and also, in addition, a population of non-T, non-SmIg cells are positive. Whether or not these positive non-T, non-SmIg cells are null cells (SmIg negative, Fc receptor positive) is not yet clear. However, the anti-B antisera are cytotoxic for the effector cell in the LDA test which suggests that they may react with null cells because these cells have been implicated as lymphocyte-dependent antibody effector cells (13).

Several authors have described both rabbit (14, 15) and human (16) antisera

that were cytolytic for leukemia cells but not normal cells. However, in most cases these sera were not tested for reactivity against normal B-lymphocyte preparations. From our studies it would seem that most leukemia cells express B-cell antigens and therefore antisera that appear to be leukemia-specific might in fact be reacting against B-cell antigens. It appears that papain extracts of both fresh or cultured B-lymphoid cells (5) produce antisera that react with B lymphocytes and most leukemia cells. Recently Cresswell and Geier (17) and Thieme and Colombe (18) produced rabbit antiserum to papain extracts of the human B-cell line RPMI 4265 which reacted against peripheral B lymphocytes (17) and to B-lymphoid cell lines (18). Although a direct comparison between sera has not been made, it would seem most likely that both these sera have similar specificities to the antisera described here. It is not clear whether or not rabbit anti-B-cell sera produced by immunization with whole cultured B-cell lines (19) are reacting against the same determinant. Nonhuman primate antileukemia antisera (15) appear to be specific for individual subclasses of leukemia and therefore different from the rabbit serum described here.

Although the method used to solubilize spleen membrane antigens is the one generally used to solubilize HLA antigens, the antisera were not very reactive to HLA antigens. Cresswell and Geier (16) showed by immunoprecipitation that similar antisera raised to cultured B-cell lines did appear to contain some antibodies directed against HLA antigens and beta$_2$ microglobulin in addition to antibodies against B-cell antigens. The reason for the lack of HLA activity in our sera is not yet clear; however, it is possible that the malignant spleens which we used as a source of antigen have more B antigen than HLA. It is also possible that the B antigen is more immunogenic than HLA.

Because the anti-B-cell antisera described here do not require absorption, sufficient quantities could become available for general use as an immunological diagnostic tool which might expand our capabilities for studying subpopulations of human lymphoid cells. The fact that soluble B-cell antigen was detected in the serum of leukemia and lymphoma patients might provide the basis of more sensitive diagnostic aids for these lymphoproliferative diseases.

Perphaps the most significant finding here is that AML, ALL, and CML appear to be divisible into the B-cell antigen-positive type (about 70%) and the non-B-cell antigen-negative type on the basis of the rabbit antiserum. The fact that the myeloid leukemias are indistinguishable from the lymphoid leukemias in this respect is surprising. The B-cell antigen is apparently present on blast cells of both the lymphoid and myeloid series, but, significantly, not in the cultured T lymphoblasts. The clinical significance is not yet clear, although both with respect to etiology and treatment, a difference should emerge.

Summary

A previously uncharacterized human B-lymphocyte antigen has been detected by rabbit antisera raised to papain digests of spleen cell membranes. The unabsorbed sera reacted in both cytotoxicity and immunofluorescent tests with normal B lymphocytes and cultured B-cell lines but not with normal T lympho- cytes or cultured T-cell lines. The cytotoxicity titers against B cells were as high as 1:32,000, whereas the same sera undiluted were negative against T cells. By

immunofluorescent staining 6–14% of unfractionated normal lymphocytes and 48–85% of B-rich lymphocyte preparations were positive. Normal peripheral blood granulocytes, platelets, erythrocytes, and phytohemagglutinin blasts were negative. The antisera reacted with the same high titers against leukemia cells from approximately 70% of the patients with acute lymphocytic leukemia, acute myelocytic leukemia, chronic myelocytic leukemia, and seven of eight cases of chronic lymphocytic leukemia. From absorption studies it appeared that the same antigen was being expressed by leukemia cells and normal B lymphocytes. Using immunofluorescent staining the anti-B-cell antisera were able to detect positive leukemia cells in the bone marrow of patients with advanced leukemia and to monitor the elimination of these cells after chemotherapy. Soluble B-cell antigen was found in the serum of some leukemia and lymphoma patients but not in normal serum.

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