Membrane-Bound Immunoglobulins on Human Leukemic Cells

EVIDENCE FOR HUMORAL IMMUNE RESPONSES OF PATIENTS TO LEUKEMIA-ASSOCIATED ANTIGENS

RICHARD S. METZGAR, T. MOHANAKUMAR, and DONALD S. MILLER

From the Department of Microbiology and Immunology and the Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT Immunoglobulins were detected on the membranes of human leukemic cells by a microcytotoxicity technique. A significant percentage of lymphocytes from normal donors failed to react with goat antisera to human heavy chain determinants or to λ-light chains. Lymphocytes from some normal donors, however, did react with antisera to κ-light chains. A high percentage (50–90) of cells from some leukemic patients were killed by antisera to light chains and by one or more antisera to heavy chain determinants. Trypsin treatment of leukemic cells resulted in a loss of cytotoxic activity with all immunoglobulin antisera. Reactivity with the κ-light chain antiserum was detectable 2 h after trypsinization of chronic myeloid leukemic (CML) cells and 8 h after treatment of acute lymphocytic leukemic (ALL) cells. Reactivity with the antisera to heavy chain determinants and λ-light chains could not be detected 8 and 48 h after trypsinization of CML and ALL cells, respectively. The cytotoxic activity of the immunoglobulin antisera to heavy chains was abolished by absorption with the specific immunoglobulin used to define the antisera by precipitation.

Eluates (pH 3.2) prepared from leukemic cells which reacted by cytotoxicity with the immunoglobulin antisera were shown to contain immunoglobulins of different heavy chain classes. In addition, some of the eluates had cytotoxic antibody activity to human leukemia cells. The specificity of the eluted antibodies is similar to the specificity previously described for cytophilic antibodies from leukemic patients and nonhuman primate antisera to human leukemia cells. The possible in vitro detection and in vivo significance of the eluted non-complement-fixing antibodies is considered.

INTRODUCTION

Cell surface markers, especially immunoglobulin determinants, have been extensively employed as indicators of specific lymphocyte populations, and on the basis of these membrane markers, a number of lymphoproliferative disorders have been classified as "B" or "T" cell derived (1, 2). By employing immunofluorescence and radiolabeling techniques, peripheral blood cells from human chronic lymphocytic leukemia (CLL)[1] and cells from other patients with different morphological types of leukemia have been studied for their immunoglobulin surface receptors (3–10). Although some reports indicated that the immunoglobulins detected on the cell surface were monoclonal with respect to their light and heavy chain classes (3, 4), others have supported a polyclonal origin (1, 8). Studies by Rowe, Hug, Forni, and Pernis (11) demonstrated that most normal lymphocytes bearing IgM also have IgD on their surface. Other investigators found that cells from some CLL patients may have both IgD and IgM on their membrane (9). Fu, Winchester, Feizi, Walzer, and Kunkel (12) have also noted that IgM and IgD on the CLL cells could have the same idiotypic specificity. However, lymphocytes from several CLL patients have been shown to bear simultaneously κ- and λ-light chain determinants as well as IgM and IgD heavy chain specificities, indicating a polyclonal origin of the immunoglobulins. Most of the studies, therefore, were primarily concerned with use of the surface immunoglobulins of leukemic cells as an indicator of the origin and nature of the leukemic cell types. However, Gutterman et al. (10), using immunofluorescent techniques, proposed that the immunoglobulins on the surface of acute myelogenous leukemia (AML) cells might represent antibodies absorbed to the cells rather than the immunoglobulins being a product of the cell. The above observation suggested that these polyclonal cell surface immunoglobulins could be antibodies to leukemia-associated antigens on the mem-

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[1] *Abbreviations used in this paper:* ALL, acute lymphocytic leukemia; AML acute myelogenous leukemia; AMML, acute myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HBSS, Hanks' balanced salt solution.

branes of these cells. Moreover, Mitchell, Mokyr, Aspnes, and McIntosh (13) had recently presented convincing evidence that cytophilic antibodies specific for leukemia-associated antigens common to a morphologic class of leukemia could be found in the serum of leukemic patients. We therefore decided to investigate whether any of the immunoglobulins detectable on leukemic cells had specific antibody activity.

This report describes a cytotoxicity technique which can detect immunoglobulin determinants on the surface of cells of some patients with myeloproliferative and lymphoproliferative disorders. In addition, low pH elution experiments demonstrate that antibodies to leukemia-associated antigens can be detected in eluates of immunoglobulin-bearing cells from some of these patients.

METHODS

Test cells Leukemic patients in this study were considered to be in remission if there were no blast cells present in the peripheral blood or bone marrow by morphological criteria and the peripheral blood cells showed no reactivity for rabbit and nonhuman primate antisera to human leukemia-associated antigens (14). Leukemic patients studied were in the relapse or untreated stage of their disease and had cells in the peripheral blood which reacted with cytotoxic antisera to human leukemia antigens.

Lymphocytes or leukemic cell suspensions were prepared as follows: 10 ml of heparinized venous blood was mixed with 2.5 ml of Plasmagel (Laboratoire Roger Bellon, Neuilly-Paris, France) and the erythrocytes allowed to sediment at 37°C for approximately 20 min. The supernate containing leukocytes, platelets, and some erythrocytes was centrifuged at 1,000 *g* for 10 min at room temperature and the plasma removed. The buffy coat cells were resuspended in 1 ml of autologous plasma, incubated on washed nylon fiber in a Pasteur pipette at 37°C for 20 min, and eluted with 4 ml of warm Hanks' balanced salt solution (HBSS). This procedure did not selectively remove normal B lymphocytes or immature cells from the blood of lymphocytic or myelocytic leukemia patients. Residual erythrocytes were lysed with 0.17 M Tris buffer (pH 7.2). The cells were washed and resuspended in HBSS at 4×10^6 cells/ml.

Cytotoxicity testing. Goat or sheep antisera to class-specific human heavy chains of IgM, IgA, IgG, IgD, and IgE and to κ- and λ-light chains were obtained from Meloy Laboratories, Inc., Springfield, Va. The antisera were checked by immunodiffusion in our laboratory with known heavy chain-specific paraproteins or with Bence Jones κ- and λ-light chains. Only those antisera that gave a specific precipitin line with the appropriate immunoglobulins were used. The cytotoxicity assay was the same as that used for the testing of simian and rabbit antisera to human leukemia-associated antigens (14). The immunoglobulin antisera were inactivated at 56°C for 30 min and absorbed with an equal volume of human group AB erythrocytes until they no longer agglutinated these cells. Antisera dilutions (1 μl) were added under oil to Falcon microtest plates (no. 3034, Falcon Plastics, Division of BioQuest, Oxnard, Calif.) and then stored at −70°C. The plates were thawed, and 0.5 μl of the test cell suspension (4×10^6/ml) was added to each well. The antisera-cell mixtures were incubated at room temperature for 35 min; 5 μl of rabbit complement was added and the mixture incubated for 35 min at 37°C. 5% eosin (5 μl) and 30% buffered formalin, pH 7.0, (2 μl) were added. The test was read by inverted phase microscopy. The percentage of leukemia cells killed by complement alone varied with the individual test cells. When this percentage exceeded 20, the complement was absorbed twice with ½ vol of packed, washed leukemic cells at 0°C in the presence of EDTA.

When eluates were tested for cytotoxic antibody activity, 1 μl of eluate (pH 7.0–7.2) was used instead of an antiserum to human immunoglobulin. The rest of the test was the same as that just described for the goat and sheep antisera. Each of the eluates was initially screened for toxicity for normal and leukemic cells and found to be nontoxic in the cytotoxic assay. Moreover, the eluates at the concentrations tested were not anticomplementary in the test.

Elution procedure. Plasmagel was added to heparinized blood from leukemic patients and the erythrocytes were sedimented at 37°C. The supernatant plasma containing leukocytes, a few erythrocytes, and platelets was centrifuged at 1,000 *g* for 15 min and the cell pellet thrice washed with at least 10 vol of isotonic saline. 1 vol of packed washed cells was then mixed with 9 vol of 0.02 M citrate buffer, pH 3.0, and the mixture incubated with constant stirring at 37°C for 2 h. The suspension was then centrifuged at 2,000 *g* for 30 min. The supernate was drawn off, adjusted to pH 7, and dialyzed against phosphate-buffered saline (pH 7.2) for 48 h. The retentate was centrifuged at 10,000 *g* for 30 min and concentrated 20–50-fold by ultrafiltration (B-15, Amicon Corp., Scientific Sys. Div., Lexington, Mass.).

Quantitative immunoglobulin determinations. These assays were performed on the eluates by Dr. Rebecca Buckley, Duke University Medical Center, Durham, N. C. IgA, IgD, IgG, and IgM were measured by single radial immunodiffusion (15). IgE concentrations were determined by a double antibody radioimmunoassay (16).

Absorption of immunoglobulin antisera. In order to check the specificity of the anti-heavy chain sera used for cytotoxicity testing, these antisera were absorbed with certain purified immunoglobulin paraproteins. The purity of the absorbing immunoglobulin preparations was confirmed by agar precipitation with known heavy chain-specific antisera and were found to be specific for the designated heavy chain class by this criterion. 10 vol of antisera was mixed with 1 vol of an immunoglobulin preparation (1–2 mg/ml). The mixtures were incubated overnight at 4°C, centrifuged at 100,000 *g* for 1 h, and the procedure repeated. The twice absorbed antisera did not react by agar precipitation with the absorbing purified immunoglobulins.

RESULTS

Cytotoxicity reactions. Serial dilutions of the commercial antisera to human heavy and light chain immunoglobulin determinants which had been absorbed with human erythrocytes were tested by cytotoxicity against peripheral blood cells from normal donors and donors with leukemia, lymphoma, or myelofibrosis. The results are shown in Table I. None of the antisera to the heavy chain or λ-light chain determinants gave greater than 20% increase in cytotoxicity over normal serum controls with cells from normal donors, but reactivity was noted with antisera to κ-light chains. The percentage of lymphocytes from some normal donors killed at a 1:2

TABLE I

Cytotoxic Reactivity of Goat or Sheep Antisera to Human Immunoglobulins with Peripheral Blood Cells of Patients with Leukemia, Lymphoma, or Myelofibrosis

Diagnosis	Disease state	Number of patients tested	Number of samples reacting with goat or sheep antisera to: κ	λ	IgG	IgA	IgM	IgD	IgE	One or more heavy chain classes
Normals		38	26/38*	0/38	0/38	0/38	0/38	0/38	0/38	0
CLL	Remission	8	2/9‡	0/9	0/9	0/9	0/9	0/9	0/9	0
	Relapse	32	33/57	4/57	4/57	6/57	6/57	5/48	10/57	15
CML	Remission	16	10/39	0/39	0/39	0/39	0/39	0/34	0/39	0
	Relapse	23	16/33	13/33	11/33	8/33	10/33	7/26	10/33	15
ALL	Remission	22	12/33	0/33	0/33	0/33	0/33	0/33	0/33	0
	Relapse	7	9/13	1/13	1/13	3/13	5/13	4/13	5/13	7
AML	Remission	12	9/40	0/40	0/40	0/40	0/40	0/35	0/40	0
	Relapse	13	8/15	1/15	1/15	1/15	1/15	0/13	2/15	2
AMML	Remission	4	7/17	0/17	0/17	0/17	0/17	0/15	0/17	0
	Relapse	8	10/21	2/21	1/21	3/21	2/21	1/19	3/21	4
Myelofibrosis	Remission	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0
	Relapse	10	15/23	6/23	3/23	7/23	5/23	8/17	11/23	12
Polycythemia vera	Relapse	3	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0
Hodgkins disease and lymphomas		30	11/32	0/32	0/32	0/32	0/32	0/24	0/32	0

* Number of peripheral blood samples from normal donors with 10–40% dead cells at a 1:2 or 1:4 dilution of antiserum/number of samples tested.

‡ Number of samples with greater than 30% dead cells at a 1:2 or 1:4 dilution of antiserum/number of samples tested. When more than one sample from each patient was tested, bleeding dates were at least 1 wk apart.

dilution of κ antiserum ranged from 20 to 40% above normal serum controls. When serial dilutions (1:2, 1:4, 1:8) of the various antisera were tested by cytotoxicity with cells from patients with leukemia, lymphoma, or myeloproliferative disease, a variety of positive reaction patterns was observed. Cells from some patients failed to react with any of the antisera or like the cells from some normal donors, reacted only with the κ antiserum. However, cells from some leukemic patients reacted with one or more antisera to heavy chain determinants as well as with antisera to κ- and/or λ-light chain determinants. The percentage of cells killed varied from 20% increase over normal serum controls to 90% total lysis, and the titers ranged from 1:2 to 1:8 or greater. Since the erythrocyte-absorbed antisera were often slightly anticomplementary at a 1:1 dilution, the lowest dilution routinely tested was 1:2.

Table II shows the cytotoxicity titers and percentage of cells lysed from four of the reactive CLL and acute myelomonocytic leukemia (AMML) patients. These examples were chosen to show two patterns of reactivity. In one type, cells from patients C.L.E. and C.L.A. reacted only with the antisera to κ-light chain and one heavy chain determinant, suggesting a possible monoclonal origin of the surface immunoglobulin. In the other, cells from patients B. U. and H. A. reacted with several antisera to light and heavy chain determinants, suggesting a polyclonal distribution of immunoglobulins on the membrane. Cells from all reactive leukemic patients diagnosed as AML, acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), and myelofibrosis gave reaction patterns which suggested a polyclonal origin of the immunoglobulins. Reactions of selected patients in these categories are shown in Table III. Cells from remission patients in all leukemia categories and patients with Hodgkins' disease, lymphoma, or polycythemia vera failed to react with the antiserum to λ-light chains and antisera to all heavy chain determinants.

Absorption studies. The goat antisera to human immunoglobulin heavy chain determinants were absorbed with purified immunoglobulins in order to determine whether the cytotoxic activity of the antisera could be correlated with the immunoglobulin class specificity. The antisera were absorbed as described in Methods and then tested by cytotoxicity with cells from patients who were reactive with the unabsorbed antisera. The absorbed antisera were also tested by immunodiffusion with the purified immunoglobulin preparations. In all instances absorption of a heavy chain antiserum with

TABLE II

Cytotoxicity Reactions of Selected CLL and AMML Patients with Goat Antisera to Human Immunoglobulins

Patient	Diagnosis	Antiserum dilution	Cytotoxicity reactions of patients cells with goat antisera to: κ	λ	IgG	IgM	IgA	IgD	IgE
C. L. E.	CLL	1:2	90*	—‡	—	50	—	—	—
		1:4	90	—	—	90	—	—	—
		1:8	90	—	—	80	—	—	—
B. U.	CLL	1:2	90	50	30	—	80	—	60
		1:4	80	50	30	—	90	—	40
		1:8	50	70	30	—	80	—	30
C. L. A.	AMML	1:2	90	—	—	—	40	—	—
		1:4	90	—	—	—	50	—	—
		1:8	80	—	—	—	50	—	—
H. A.	AMML	1:2	50	50	—	60	90	NT§	80
		1:4	80	70	—	90	90		90
		1:8	80	80	—	90	90		90

* Percent dead cells.
‡ —, >80% viable cells.
§ NT, not tested.

a specific immunoglobulin class removed the precipitin line for that class whereas absorption with other immunoglobulin classes, as expected, did not alter the agar precipitation reactivity of the antiserum. The cytotoxic activity of the immunoglobulin antisera to heavy chain class determinants was abolished by absorption with the specific immunoglobulin used to define the antisera by precipitation (Table IV).

Effect of trypsin. Previous studies from our laboratory (14, 17) demonstrated that treatment of human leukemic cells with trypsin resulted in a loss followed by a regeneration of leukemia-associated antigens de-

TABLE III

Cytotoxicity Reactions of Selected ALL, CML, and Myelofibrosis Patients with Goat Antisera to Human Immunoglobulins

Patient	Diagnosis	Antiserum dilution	Cytotoxicity reactions of patients cells with goat antisera to: κ	λ	IgG	IgM	IgA	IgD	IgE
D. E.	Myelofibrosis	1:2	70*	60	—‡	50	30	60	60
		1:4	80	60	—	60	—	50	60
		1:8	80	60	—	70	—	30	40
B. U. C.	CML	1:2	80	80	30	50	—	60	—
		1:4	90	90	—	40	—	40	—
		1:8	90	90	—	—	—	—	—
W. I.	Myelofibrosis	1:2	90	—	—	70	50	—	80
		1:4	80	—	—	70	60	—	70
		1:8	40	—	—	—	—	—	—
C. A.	CML	1:2	60	40	60	60	—	NT§	—
		1:4	30	30	30	30	—		—
		1:8	—	—	—	—	—		—
S. E.	ALL	1:2	90	—	—	30	90	50	70
		1:4	90	80	—	40	90	40	50
		1:8	80	90	—	—	90	30	—

* Percent dead cells.
‡ —, >80% viable cells.
§ NT, not tested.

tected by nonhuman primate antisera. Moreover, other investigators have shown that trypsin treatment of leukemic cells resulted in a loss of Fab regions from the cell surface (18). We, therefore, studied the effect of trypsin on the reactivity of leukemic cells with the antisera to human immunoglobulins. The leukemic cells were treated with 0.25% trypsin for 20 min at 37°C. Normal human serum was added to inactivate the trypsin and the cells were washed three times with HBSS. A sample of the trypsinized cells was tested immediately, the remaining cells were incubated at 37°C, and additional samples were tested at various intervals with the reactive immunoglobulin antisera. Cell viability was consistently greater than 90% after trypsin treatment. Representative results of studies with cells from an ALL, an AMML, and CML patients are given in Tables V and VI. Immediately after trypsin treatment, reactivity with all immunoglobulin antisera was completely lost for all leukemic cells. With cells from ALL donors this nonreactivity persisted for 8 h at which time activity with the anti-κ reagent began to reappear. Although after 48 h, pretreatment levels of reactivity with the anti-κ serum had returned, no reactivity with the anti-λ or anti-heavy chain sera could be detected. Identical results were seen with CML and AMML cells except that the reactivity with the anti-κ serum returned earlier and had reached pretreatment levels within 8 h. 12 h after trypsin treatment the viability of the CML and AMML cells was too low to allow continuation of the study.

TABLE IV

Cytotoxicity Reactions of Antisera to Human Immunoglobulins after Absorption with Purified Immunoglobulins

Leukemic cell donor	Goat antiserum to:	Treatment	Cytotoxicity reactions with antiserum dilutions 1:2	1:4	1:8
R. G.	IgA	Unabsorbed	70*	60	40
		Abs c IgG	60	40	30
		Abs c IgA	—‡	—	—
C. C.	IgM	Unabsorbed	—	50	90
		Abs c IgG	30	50	50
		Abs c IgM + G	—	—	—
J. B.	IgG	Unabsorbed	80	30	—
		Abs c IgA	80	30	—
		Abs c IgG	—	—	—
W. B.	IgD	Unabsorbed	80	40	—
		Abs c IgA	80	40	—
		Abs c IgD	30	—	—
H. L.	IgE	Unabsorbed	50	60	NT§
		Abs c IgA	50	50	NT
		Abs c IgE	—	—	NT

* Percent dead cells.
‡ NT, not tested.
§ —, >80% viable cells.

TABLE V

Effect of Trypsin on Cytotoxic Reactivity of ALL Cells with Immunoglobulin Antisera

Cell treatment	Hours after trypsinization	Goat antisera to:	Cytotoxicity reactions of cells from an ALL patient with antiserum dilutions 1:1	1:2	1:4	1:8
Untreated		κ	90*	80	70	40
		D	90	70	50	—‡
		E	90	70	40	—
Trypsin (0.25%)	0, 2, 4	κ	—	—	—	—
		D	—	—	—	—
		E	—	—	—	—
	8	κ	30	30	—	—
		D	—	—	—	—
		E	—	—	—	—
	25	κ	80	80	50	—
		D	—	—	—	—
		E	—	—	—	—
	48	κ	80	70	50	—
		D	—	—	—	—
		E	—	—	—	—

* Percent dead cells.
‡—, >80% viable cells.

TABLE VI

Effect of Trypsin on Cytotoxic Reactivity of CML and AMML Cells with Immunoglobulin Antisera

Cell treatment	Hours after trypsinization	Goat antisera to:	Cytotoxicity reactions of cells from patients with 1:2 antisera W. I. (CML)	G. A. (CML)	K. H. (AMML)*
Untreated		κ	80‡	80	70
		λ	70	70	60
		E	60	—§	90
		D	60	50	80
		A	60	70	80
		M	—	—	80
Trypsin (0.25%)	0	κ	—	—	—
		λ	—	—	—
		E	—	—	—
		D	—	—	—
		A	—	—	—
		M	—	—	—
	2, 4	κ	40	40	50
		λ	—	—	—
		E	—	—	—
		D	—	—	—
		A	—	—	—
		M	—	—	—
	6, 8	κ	60–70	60–70	70
		λ	—	—	—
		E	—	—	—
		D	—	—	—
		A	—	—	—
		M	—	—	—

* Cells treated with 2.5% trypsin.
‡ Percent dead cells.
§ —, >80% viable cells.

TABLE VII
Information on Eluates Studied

Patient	Diagnosis	Eluate prepared from:*	Immunoglobulin antisera reacting with cells by cytotoxicity	Concentration of immunoglobulins in eluates				
				IgA	IgD	IgE	IgG	IgM
				mg/100 ml		*U/ml*	*mg/100 ml*	
B. U.	CLL	PBL	A, E, G	5.6	<1	96	25.6	5.7
B. U.	CLL	Spleen cells	A, E, G	4.3	<1	39	12.8	1.2
W. I. L. M.	CML	PBL	A, D, E, G, M	5.0	24	164	14.4	104
B. O.	CML	PBL	D, E, G, M	0	38.5	44	8	165
B. U. C.	CML	PBL	D, E, G, M	0	31.5	86	6.3	940

PBL, peripheral blood leukocytes.
* From 5×10^9 cells, 1 ml of eluate was prepared and the protein concentration ranged from 1–10 mg per ml.

Elution studies. Since we could not demonstrate regeneration of the heavy chain sites and since these sites could not be detected on significant numbers of normal cells or cells from remission patients, we felt that they might represent antibodies absorbed in vivo to leukemic cell surface antigens. Therefore, low pH (3.2) eluates were prepared from some patients' cells which had been reactive with antisera to heavy chain determinants. Serum from these patients at the time the cells were studied for elution did not have detectable antibodies to human leukemia-associated antigens. Information on the cell donors and the eluates studied is given in Table VII. All of the eluates studied had immunoglobulins detectable by immunodiffusion or radioimmunoassay. Eluates from the cells of three CML patients studied had particularly high levels (>100 mg/100 ml) of IgM.

The eluates were then tested by cytotoxicity for antibodies to leukemia and normal cell-associated antigens. The results of these studies are given in Table VIII. The leukemic cell data in Table VIII reflects only the reactions of cells from patients who were in the relapse or untreated stage of their disease. Three of the eluates reacted with cells from 2 normal donors out of the 26 tested. The eluates from the peripheral blood and spleen cells of the one CLL patient thus far studied reacted with cells from many of the CLL and AMML patients studied. Although the spleen eluate reacted with cells from a patient with CML and a patient with myelofibrosis, the eluate from the peripheral blood leukocytes from this same patient reacted only with cells from CLL and AMML patients. Peripheral blood cells at a later date from the CLL eluate donor were also available for testing with her own eluate. Both the spleen and peripheral blood cell eluates were able to lyse 80% of her own CLL cells. The peripheral blood cells at the time of testing with eluates could not be lysed by human or rabbit complement alone.

Eluates from the peripheral blood cells of the three CML patients studied had good cytotoxic activity especially for cells from patients with myeloid leukemia or myelofibrosis. Occasional reactions with cells from normal donors or patients with CLL were also noted; however, the reactions were low titered (1:1 or 1:2), and less than 50% of the cells were lysed. The reactive eluates always gave a greater percentage of lysis and were higher titered when tested with leukemic cells of

TABLE VIII
Cytotoxic Reactivity of Eluates from Leukemic Cells

Patient	Diagnosis	Eluate prepared from:	Cytotoxic reactivity with cells from:					
			Normal donors	CLL patients	CML patients	AML patients	AMML patients	Myelofibrosis patients
B. U.	CLL	PBL	0/26*	30/40	0/14	0/8	5/9	0/16
B. U.	CLL	Spleen	1/26	27/39	1/14	0/5	5/9	1/15
W. I. L. M.	CML	PBL	0/25	0/36	6/11	2/5	2/8	8/16
B. O.	CML	PBL	2/26	2/37	11/11	4/5	4/9	11/15
B. U. C.	CML	PBL	1/26	1/37	8/11	3/5	2/9	9/15

* Number of patients whose cells reacted (≥ 40% dead cells) with the eluates/number of patients tested.
PBL, peripheral blood leukocytes.

the same morphological type as the donor of the cells used for elution. The highest titers were 1:4 or 1:8 and in some instances 100% of the target cells were killed by the eluate.

Two of the eluates prepared from cells from CML donors (B. O. and B. U. C.) were absorbed with peripheral blood cells from leukemic patients. The eluates were absorbed for 30 min at room temperature with 2×10^8 leukemic cells/ml of eluate. Absorption of these eluates with cells from two different CLL donors had no effect on the cytotoxic reactivity of the eluates whereas absorption with seropositive cells from two different CML donors removed the reactivity for all reacting CML and AML cells.

DISCUSSION

Wernet, Feizi, and Kunkel (19) had earlier used a complement-dependent cytotoxic assay to detect μ and idiotypic determinants on lymphocytes of normal and CLL donors. More recently Gmelig Meyling, Kooy-Blok, and Ballieux (20) reported complement-dependent cytolysis of human B lymphocytes by an antisera to human immunoglobulin light chains. The data of these workers on the percentage of normal blood lymphocytes reacting with light chain antisera is similar to the percentage of cells lysed by our anti-κ serum (10–30%). There are, however, numerous technical problems with using the microcytotoxicity assay to reliably detect specific cell cytolysis in the 5–20% range. This is because of the variability in the background lysis with rabbit complement and normal goat or rabbit serum controls and the method used to prepare the lymphocytes. Nevertheless, when the percentage of cells bearing detectable immunoglobulin determinants are above these ranges (e. g., tonsillar lymphocytes or cells from leukemic patients) the microcytotoxicity test provides a relatively simple and rapid technique for detecting cell surface immunoglobulin determinants.

The reactivity of several heavy chain antisera with leukemia cells from the same donor indicated a polyclonal distribution and suggested that if surface immunoglobulins were being detected they may be present on the cell membrane as a result of in vivo sensitization or that the immunoglobulin antisera were detecting other antigens by cytotoxicity. The data presented in Table IV show that the cytotoxicity reactions are related to the immunoglobulin specificity of the commercial antisera as defined by agar precipitation. The loss of reactivity of the immunoglobulin antisera with trypsinized target cells and the failure of the cells to regenerate antigenic reactivity with the antisera to λ-light chain- and heavy chain-specific determinants (Table V and VI) support the concept that some of the membrane-bound immunoglobulins are adsorbed antibodies rather than products of leukemic cells. We have not as yet had the opportunity to study the effect of trypsin on cells from CLL patients with a monoclonal immunoglobulin pattern. In those instances we would expect regeneration of the heavy chain sites if the cells could be maintained long enough in vitro in a functional, viable state. The reactivity of the myeloid leukemic cells with the immunoglobulin antisera also indicates that the immunoglobulins on these cells were not being synthesized *de novo*.

The elution data provide more definitive proof that the immunoglobulins on the surface of cells from some leukemic patients represent specific antibodies. The cytotoxic specificity of the eluates (Table VIII) is similar to that described for nonhuman primate antisera to leukemia cells (14, 17). The eluted antibodies react strongest with leukemic cells from patients with the same morphological type of leukemia as the eluate cell donor and the antibody activity could be removed only by absorption with cells from the same morphological type of leukemia as that used for the preparation of the eluates. Reactivity of cells of AMML patients with eluates from both CLL and CML cells is similar to that noted with the cytotoxic nonhuman primate antisera to CLL and CML cells. In this instance CLL and CML antisera are capable of reacting with the same target AMML cells (unpublished data).

Additional eluates from leukemic cells of other patients need to be studied for controls. Eluates from relapse or untreated patients whose cells are negative with the heavy chain antisera and eluates from CLL patients with a monoclonal reaction pattern should not have cytotoxic activity for leukemic cells.

The clinical significance of the detection of antibodies on cells from certain leukemic patients cannot be resolved from the studies to date. We have not yet determined whether the patients whose cells react by cytotoxicity with the heavy chain antisera have a better or worse prognosis than those whose cells are nonreactive. The eluates have been tested thus far only for cytotoxic antibody activity but are known to contain immunoglobulins (e. g., IgE and IgA) which are not capable of fixing complement by the conventional pathway. These immunoglobulins might have significant in vivo function. IgE type antibodies have been detected in animal allograft rejection studies (21). Some of the noncytotoxic antibodies may be capable of interfering with cytotoxic antibody or lymphocyte-mediated cytolysis, or they may facilitate target cell lysis by normal lymphocytes.

Cells from these eluate donors at the time the eluates were prepared were not able to be lysed in vitro in the presence of human or absorbed rabbit complement alone. However, some of the eluates were capable of lysing cells from other patients and, in the case of the eluates from the CLL patient, autologous cells at a later date with

both human and rabbit complement. If the in vitro cytotoxic activity of the eluates were due only to IgG, then one could argue that in vivo antibodies in the serum of patients in the relapse or untreated stage are in such extreme cell excess and that there would be only a remote chance of two IgG molecules being situated close enough on the cell membrane to trigger the complement sequence. If the cytotoxic antibody activity of the eluates from the CML patients is shown by column chromatography or gradient centrifugation to be related to IgM then this argument would not be valid and interference or blocking by non-complement-fixing antibodies on the target cell would be a more logical explanation for the failure to get in vivo lysis.

Eluates from leukemic cells could represent valuable diagnostic and prognostic reagents for detecting and studying human leukemia-associated antigens. Large numbers of cells from patients subjected to plasmapheresis could provide reasonable quantities of eluates for this purpose. The eluates have a distinct advantage over heteroantisera for the detection of leukemia-associated antigens (14, 17) in that they do not require extensive absorption with normal cells. The few reactions noted between the eluates and cells from normal donors or patients with different histological types of leukemia may be nonspecific and might be eliminated by Sephadex fractionation of the eluates.

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