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Immunology

Detection of antibody to autologous human leukemia cells by immune adherence assays

(cell surface/serology/human cancer)

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ABSTRACT The sera of 21 adult patients with acute leukemia were studied for the presence of antibody reacting with surface antigens of autologous leukemia cells. Sequential serum samples were obtained from patients and were tested on cryopreserved leukemia cells in immune adherence assays. Three patients showed autologous serum reactivity and the serum of one of them was analyzed in detail. This antibody reacted with autologous acute lymphocytic leukemia cells but not with autologous cells obtained from peripheral blood, bone marrow, or spleen during clinical remission. In absorption tests, the antigen could not be detected on normal autologous or allogeneic blood lymphocytes, lymphoblastoid lines of T- or B-cell origin, or cells infected with simian sarcoma virus, baboon C-type virus, or Mason–Pfizer virus. Leukemia cells from two other patients with acute lymphocytic leukemia and one patient with acute nonlymphocytic leukemia absorbed specific reactivity. These studies indicate that certain acute leukemia cells express a common antigen that elicits a humoral immune response in the autologous host.

Immunological approaches to the study of cancer in man have been hampered by current inability to define tumor-specific antigens in human cancer with the precision possible in experimental animals. To develop as unambiguous a serological test system as possible, we have stressed autologous reactions (i.e., serological tests with sera and cancer cells derived from the same patient) in our search for human antibodies with specificity for cancer cells (1–3). In this way, it has been possible to define several systems of surface antigens on cultured lines of malignant melanoma by mixed hemadsorption and immune adherence assays. Three of these antigens are strictly tumor-specific in the sense that they show an absolute restriction to autologous melanoma cells. Another represents a class of shared melanoma antigens, having been found on 5 of 12 melanoma cell lines but not on any other type of cultured human cancer cell (2). The remaining antigens detected by autologous serum reactions are expressed by an extensive range of normal and malignant cells of human and animal origin (3). Because these broadly reacting antibodies can introduce confusion into the serological analysis, their identification is important.

Acute leukemias of humans have been the subject of considerable serological study, much of it directed to the search for antigens related to mammalian leukemia viruses (4). Although some suggestive data have been presented, there is no solid evidence that such viral antigens exist in human leukemia. Heteroimmune sera have been useful in distinguishing classes of human leukemia by characteristic surface markers, particularly those related to the differentiation history of the leukemia (5). With regard to reports of human sera with specificity for surface antigens of leukemia cells, the analysis of these reactions has been too limited to permit any firm conclusions (5, 6).

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In the present study of acute leukemia, we have made use of the approaches that have been valuable in defining cell surface antigens in malignant melanoma (1–3). Because cultured lines of leukemia cells cannot be established with any degree of regularity, tests were carried out with cryopreserved leukemia cells as the source of target cells.

MATERIALS AND METHODS

Peripheral blood obtained from leukemia patients at the time of admission to the Adult Hematology Service, Memorial Hospital, was diluted with an equal volume of Hanks' balanced salt solution and then layered over Ficoll-Paque (Pharmacia, Piscataway, NJ). Following centrifugation at $400 \times g$ for 30 min, the interphase layer of cells was removed, washed three times in Hanks' balanced salt solution, and resuspended in RPMI 1640 with 20% fetal calf serum at a concentration of 12 to 40×10^6 cells per ml. An equal volume of cold RPMI 1640 with 20% fetal calf serum and 20% dimethyl sulfoxide was mixed in slowly, and the resulting cell suspension was distributed as 1.0-ml samples into plastic vials (Nunc, Roskilde, Denmark). Freezing was carried out at a controlled rate in either an automatic liquid-freezing apparatus (Planer-Ether, Sunbury-on-Thames, England) or a biological freezer (Type BF-5, Union Carbide Corp., NY). The vials were stored in liquid nitrogen at −176°. Samples of peripheral blood and bone marrow taken during remission were processed in a similar manner. The surgically removed spleen from patient 2 was finely minced and the resulting single cell suspension was washed in Hanks' balanced salt solution, layered over Ficoll-Paque, and processed as outlined above. Samples of serum collected at 2- to 3-month intervals during the patient's clinical course were stored at −70°.

For serological studies, ampoules of cells were thawed rapidly in a 37° water bath. A 10-ml volume of RPMI 1640 with 20% fetal calf serum was added slowly to each vial and the cells were washed three times in barbital buffer medium with 5% bovine serum albumin. Cell viability by trypan blue exclusion was usually 80–95%. The immune adherence (IA) tests, as performed in our laboratory with monolayer cells (2), was modified for cell suspensions. A 0.05-ml suspension containing 3.0×10^6 cells per ml in barbital buffer medium was added to 0.05 ml of serially diluted serum. In initial screening tests, serum was diluted 1:4, 1:16, and 1:64. Duplicate tubes were prepared with one set incubated at 37° and the other at 4°, both for 30 min. Following three washes in barbital buffer medium, the cells were resuspended in 0.025 ml of preselected guinea pig serum diluted to 1:40 (complement source) and 0.025 ml of a 1.0% suspension of human group O, Rh^+ indicator erythrocytes. Following a 60-min incubation at 37°, the cells were gently

Abbreviations: IA, immune adherence; ALL, acute lymphocytic leukemia; ANL, acute nonlymphocytic leukemia.

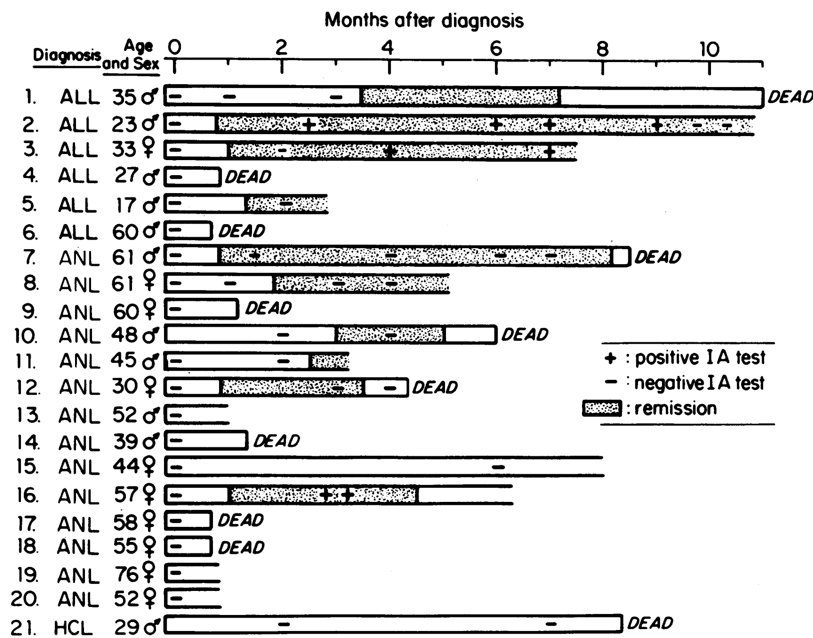

FIG. 1. IA assays for antibody to autologous leukemia cells in patients with acute leukemia. HCL, hairy cell leukemia.

resuspended and a drop was placed on the counting chamber of a hemocytometer. The number of rosettes (defined as a target cell with a minimum of three adherent erythrocytes) was determined per 200 target cells. Because of the absence of background rosetting, a test could easily be scored as positive if as few as 1% of the target cells demonstrated rosette formation. The endpoint (titer) was taken as the last serum dilution showing a positive reaction. Controls included target cells with indicator erythrocytes and complement.

Absorption tests were based on techniques described previously from this laboratory (1, 2). An aliquot of serum (0.06 ml) two doubling dilutions below the endpoint was mixed with 30×10^6 cells at 4° for 1 hr with occasional shaking; 15×10^6 cells were used in absorption tests with tissue culture cell lines because of the larger size of the cultured cells. Virus-infected monolayer cells were removed from the flask by scraping, washed in Hanks' balanced salt solution, and used for absorption tests. After centrifugation, the serum was removed and the residual activity was tested in IA tests.

The A204 human rhabdomyosarcoma cell line infected with simian sarcoma virus or with baboon C-type virus and the NC-37 human lymphoblastoid cell line infected with Mason-Pfizer virus were provided by S. Mayyasi at Pfizer, Inc. (Maywood, NJ). The MOLT-4F human T-cell line (7) came from J. Minowada at Roswell Park Memorial Institute (Buffalo, NY) and the CCRF-CEM and CCRF-HSB2 human T-cell lines, originally derived by Kaplan et al. (8), were provided by J. Hansen of this Institute. The P3HR-1 and Raji cell lines derived from Burkitt's lymphoma have been maintained in our laboratory for 10 years (9).

Brief Clinical History of Patient 2. This patient, a 23-year-old white man, developed cervical lymphadenopathy in early March 1976 and about 2 weeks later began to have left upper quadrant pain. On the day of admission, Mar. 29, 1976, he had enlarged bilateral cervical, inguinal, and axillary nodes, liver palpable 2 cm below the right costal margin, and spleen palpable 5 cm below the left costal margin. The leukocyte count was $141,000/mm^3$ with 95% lymphoblasts, the hemoglobin level was 14.5 g/100 ml, and the platelet count was $27,000/mm^3$. Bone marrow examination confirmed the diagnosis of

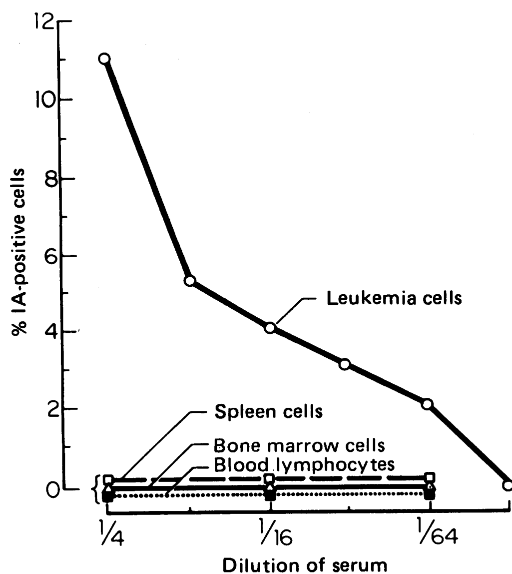

FIG. 2. IA assays of serum from patient 2 on autologous leukemia cells. No reactions were observed with cells obtained from peripheral blood, bone marrow, or spleen following clinical remission.

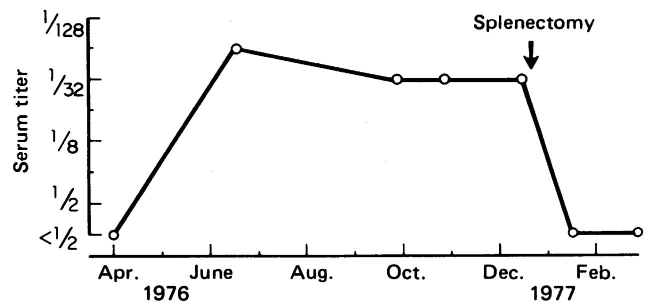

FIG. 3. Titer of antibody to autologous leukemia cells determined by IA assays in sequential serum specimens from patient 2.

Table 1. Absorption of IA reactivity from serum of patient 2 tested against autologous cryopreserved leukemia cells

Positive absorptions	Negative absorptions	
Autologous leukemia cells:	Autologous normal cells:	Cultured lymphoid cell lines:
ALL (patient 2)	Blood lymphocytes	MOLT-4F
	Spleen cells	CCRF-CEM
		CCRF-HSB2
Allogeneic leukemia cells:		P3HR-1
ALL (patient 1)	Allogeneic normal cells:	Raji
ALL (patient 5)	Blood lymphocytes from two individuals	
ANL (patient 7)		Oncornavirus-infected cells:
		Simian sarcoma virus-infected A204
		Baboon C-type virus-infected A204
	Allogeneic leukemia cells:	Mason-Pfizer virus-infected NC-37
	ALL (patient 3)	
	ALL (patient 4)	Xenogeneic cells and serum:
	ANL (patient 14)	Sheep erythrocytes
	ANL (patient 15)	Fetal calf serum
	ANL (patient 17)	

acute lymphocytic leukemia (ALL). The leukemia was classified as a T-cell leukemia on the basis of surface markers (B. Koziner of this Institute, personal communication). The patient was started on a combination chemotherapeutic regimen incorporating vincristine, prednisone, adriamycin, and intrathecal methotrexate. By mid-April, he was in full hematological remission but continued to exhibit persistent splenomegaly. During the following 6 months of maintenance therapy, there was no change in spleen size. Because of concern that the spleen contained residual leukemia cells, a splenectomy was performed on Dec. 16, 1976, 8½ months after admission. However, the spleen was shown to be histologically normal with no leukemia infiltrates. The patient continues in remission.

RESULTS

Twenty-one patients were studied: 6 with ALL, 14 with acute nonlymphocytic leukemia (ANL), and 1 with hairy cell leukemia. Three patients (no. 2, 3, and 16) had evidence of antibody in their serum at some point during their illness (Fig. 1). In patients 3 and 16, antibody reactivity was optimal at 4°. Sera from these two patients were also positive in direct IA tests with autologous remission leukocytes; absorption tests to determine whether remission leukocytes remove reactivity for autologous leukemia cells have not been performed because of the patients' clinical condition.

In patient 2, serum drawn after chemotherapy-induced remission was reactive with autologous leukemia cells (Figs. 1 and 2); no reaction was observed with autologous cells from peripheral blood, bone marrow, or spleen obtained after clinical remission. Fig. 3 shows antibody titers in sequential serum specimens from this patient. No antibody was detected at the time of diagnosis but was demonstrable in serum collected during clinical remission. In contrast to the findings in patients 3 and 16, this antibody reacted optimally at 37°. Following splenectomy, there was an abrupt drop in serum reactivity, suggesting that the spleen was the major source of antibody in this patient. The absorption tests shown in Table 1 further defined the specificity of this antibody. Autologous leukemia cells removed IA activity, whereas autologous remission blood lymphocytes and spleen cells failed to do so. Absorption with allogeneic leukemia cells indicated that two of four patients with ALL and one of four with ANL shared this antigen. The antigen was not present on three T-cell lines, two B-cell lines, or in cell lines infected with simian sarcoma virus, baboon C-type virus, or Mason–Pfizer virus.

DISCUSSION

One patient in this series was found to have antibody that showed specific reactivity with autologous leukemia cells. Although the analysis of this antibody was necessarily limited by the number of leukemia cells originally obtained from this patient and preserved by freezing, direct IA and absorption tests revealed that autologous cells obtained from peripheral blood, spleen, or bone marrow after chemotherapy-induced remission lacked the antigen. The fact that leukemia cells from two patients with ALL and one patient with ANL expressed the antigen makes it feasible to consider allogeneic typing systems to extend the serological analysis of antibody in this patient's serum.

From this study, it is not possible to say how frequently antibody with specific leukemia reactivity will be found in patients with acute leukemia. Many patients in our series have not been studied over a sufficiently long period or died before achieving remission. Because antibody was not demonstrated in patient 2 at the time of diagnosis, presumably due to *in vivo* absorption by the leukemia cell population, the time to look for serum reactivity is apparently after remission, and thus far this has been possible in only a limited number of patients. Two technical aspects of this study may be significant in relation to the question of frequency of antileukemia antibody. The IA technique detects primarily the IgM class of antibody (10) and because some patients may form IgG antibody to leukemia cells, we are now including techniques for the detection of other classes of immunoglobulins. The use of target cells that are cryopreserved presupposes that the antigen is stable to such manipulations. The AH and BD antigens of malignant melanoma are greatly decreased on cells that are recovered after controlled freezing (2) and the same may also be true for leukemia antigens. This could account for the low percentage of positive cells (1–15%) reacting with antibody in the serum of patient 2. Antigenic modulation [e.g., antibody-induced antigen suppression (11)] and cell cycle-dependent antigenic expression may also explain this observation. Tissue culture lines of leukemia cells that express these antigens would greatly facilitate further analysis. Because ALL cells can be cultured under certain conditions (7, 8), it may be possible to find one that carries the antigen detected in this study. At that point, ques-

tions relating to the nature of the antigen and its genetic origin can be more directly addressed.

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