Evaluation of a Complement-Dependent Human Cytotoxic Antibody Reactive with Burkitt's Lymphoma Biopsy Cells[1] (34457)

LEROY FASS AND RONALD B. HERBERMAN
(Introduced by J. L. Fahey)

Lymphoma Treatment Centre, Makerere University Medical School, Kampala, Uganda; and the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Extensive work has been done in search of serological evidence of host immunity in Burkitt's lymphoma (1–7). Mainly by immunofluorescent techniques (1, 2, 5, 6), antibodies have been detected in the sera of patients with Burkitt's lymphoma, patients with other diseases, and in normal people. The possible relationship of these antibodies to Burkitt's lymphoma has been difficult to evaluate, since they have not been proved to be tumor-specific.

A complement-dependent cytotoxic antibody has been found to react with established cultures derived from Burkitt's lymphoma (7). Quantitative absorption studies indicated that the antigen detected by the cytotoxic reaction was not tumor-specific and was present in small quantities in normal tissues (8). The failure to detect tumor-specific antigens on these tissue culture cell lines by the cytotoxic antibody, and other experimental data, raised some doubt as to whether the cells in culture were representative of the original tumor cell population (9). In the present study, the complement-dependent cytotoxic antibody reaction was performed with fresh Burkitt's lymphoma biopsy cells to determine whether these cells had antigens similar to those on the tissue culture cells.

Materials and Methods. Cells were obtained from the tumor biopsies of patients with untreated Burkitt's lymphoma admitted to the Lymphoma Treatment Centre, Makerere University Medical School, Kampala, Uganda. A single cell suspension was obtained by gently teasing the tumor tissue into Eagle's minimum essential medium with 10% fetal bovine serum (MEM/10% FBS). The ease with which high concentrations of single cell suspensions were produced by merely shaking the biopsy specimen in the media was a characteristic of this tumor. The viability of the cell suspension was determined by adding 0.2% trypan blue in isotonic saline. No biopsy was used for investigations unless viability was greater than 85%. In most cases viability was greater than 90%. The cell concentration was adjusted to 10^6 cells/ml.

Peripheral blood lymphocytes from patients with Burkitt's lymphoma, and the bone marrow lymphoblasts and the normal peripheral lymphocytes from a patient with acute lymphocytic leukemia were also used as target cells. Heparinized blood and bone marrow were collected and lymphocytes and lymphoblasts were isolated by the method of Greenwalt *et al.* (10). Residual erythrocytes were removed by brief hypotonic lysis. Cell concentration was adjusted to 10^6 cells/ml. Differential counts of the final cell suspensions revealed greater than 90% morphologically normal lymphocytes from the blood and greater than 85% lymphoblasts from the bone marrow.

Sera were obtained from patients with Burkitt's lymphoma before treatment, in remission, and in relapse; and from patients with Hodgkin's disease and acute leukemia. Sera from normal African children and adults, and normal American adults served as

[1] Supported by Contract No. PH-43-67-1343 and Contract No. PH-43-67-47 from the National Cancer Institute, National Institutes of Health, United States Public Health Service. Send reprint requests to Dr. Herberman, National Cancer Institute, Bethesda, Md. 20014.

controls. Serum was separated and stored at −70°. The sera were not heat inactivated. There was no history of previous blood transfusions in any serum donor.

Sera were tested for cytotoxic activity as previously described for tissue culture cells (7). Serial twofold dilutions of the serum to be tested were made in fresh MEM/10% FBS. One-tenth ml of the cell suspension was added to 0.1 ml of each of the serum dilutions and incubated for 30 min at 37°. One-tenth ml of undiluted normal rabbit serum as a source of complement, was then added and incubated for an additional 30 min at 37°. Then 0.3 ml of 0.2% trypan blue in isotonic saline was added to each tube and the percentage of stained cells was determined by counting 100 cells. The serum titer was defined as the reciprocal of the serum dilution producing a level of cytotoxicity twice that of the complement control. A relatively nontoxic pool of rabbit serum was used as the source of complement, and this produced 10–20% cytotoxicity with the biopsy cells. A control with cells alone was also routinely performed; this varied from 5 to 15% cell lysis.

Absorption of cytotoxic antibody was performed with one serum sample from an untreated patient with Burkitt's lymphoma. The cytotoxic antibody titer was first determined against both fresh Burkitt's lymphoma biopsy cells and Raji, a tissue culture cell line derived from a patient with Burkitt's lymphoma (11). The serum was then absorbed with peripheral blood leukocytes (5×10^8 cells/0.1 ml serum) from a normal adult blood donor. The mixture was incubated for 30 min at 37° and the cells then sedimented at 500*g* for 10 min. The supernatant serum was carefully removed and tested for residual cytotoxic antibody against Raji cells and against Burkitt's lymphoma biopsy cells. This procedure was previously shown to remove cytotoxic antibody reactive with cultures of human lymphoid cells [see (8) for details of controls for nonspecific absorption].

Results. Seventeen sera from 13 Burkitt's lymphoma patients were tested against fresh Burkitt's lymphoma cells and all had positive antibody titers (Table I). Six sera were tested with cells from at least 2 different patients' biopsies. The pretreatment and remission sera from 3 patients were tested with essentially no significant difference in titers. Three sera were tested against autologous tumor and against other Burkitt's lymphoma biopsy cells; approximately the same titers were obtained against both target cells. The mean titer of sera from Burkitt's lymphoma patients was 11.4. None of these sera produced cytotoxicity when tested against the patients' peripheral lymphocytes. Two sera from patients with generalized Hodgkin's disease were tested against several Burkitt's tumor biopsies as were 2 sera from children with acute leukemia (1 patient with untreated lymphocytic leukemia and 1 with myelocytic leukemia on chemotherapy). The mean titers were 9.3 and 15.6, respectively.

TABLE I. Cytotoxic Reactivity of Human Sera with Burkitt's Lymphoma Biopsy Cells.

Diagnosis	No. of sera	Mean titer of sera[a]
Burkitt's lymphoma	17	11.4
Hodgkin's disease	2	11.0
Acute leukemia (child)	2	15.6
African controls (5 children, 2 adults)	7	13.9
American controls (3 adults)	3	11.0[b]

[a] Obtained by computing the arithmetic mean of each serum against multiple biopsy cells and then obtaining the mean of all the sera.

[b] One control negative.

The titers of African control sera averaged 13.9. One American control serum was negative and the two other controls had an average of 11.0.

A panel of the same sera were also tested against the bone marrow lymphoblasts from the patient with acute lymphocytic leukemia. All of the sera tested gave positive antibody titers similar to those obtained with the Burkitt's lymphoma cells. These sera did not cause cytotoxicity when the normal peripheral lymphocytes of this patient with leukemia were used as target cells. The leukmia patient's own serum was cytotoxic for her bone

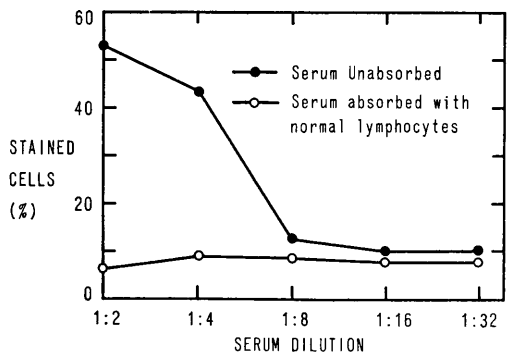

FIG. 1. Cytotoxic antibody reactive with Burkitt's lymphoma biopsy cells:cytotoxic activity of serum from untreated patient with Burkitt's lymphoma when tested against Burkitt's lymphoma biopsy cells. Unabsorbed serum (●); serum absorbed with nomal peripheral lymphocytes, 5×10^8 cells/0.1 ml of serum (○).

marrow lymphoblasts but not for her own peripheral lymphocytes.

Leukocyte alloantibodies were not the cause of these cytotoxic reactions. Selected sera were tested against peripheral blood lymphocytes and lymphocytes from normal lymph nodes, and all results were negative.

The one Burkitt's lymphoma serum tested against biopsy cells and against Raji tissue culture cells showed identical titers (1:4). After absorption was performed using large numbers of normal peripheral lymphocytes (5×10^8 cells/0.1 ml of serum), the serum showed no residual cytotoxicity when tested against fresh biopsy cells (Fig. 1) or against Raji cells.

*Discussion.*This study using fresh Burkitt's lymphoma biopsy cells extends the information reported by Herberman and Fahey (7) using tissue culture cells derived from patients with Burkitt's lymphoma and from other individuals. They tested large numbers of sera from children with Burkitt's lymphoma, other diseases, and normal children, and demonstrated cytotoxic activity against the lymphoid tissue culture cells. Adults with lymphoid and nonlymphoid malignancies, nonmalignant diseases, and normal subjects all had a high percentage of positive titers. A natural antibody was thought to be responsible for the cytotoxic activity. The activity in positive sera was precipitated by 33% saturated ammonium sulfate. Further fractionation by Sephadex G-200 gel filtration indicated the active material to be approximately 7*S* in size. Only IgG was detected in the active fraction by immunoelectrophoresis. The cytotoxic activity was complement dependent, being blocked by a variety of complement inhibitors (Herberman, unpublished data). The cytotoxic activity was heat labile; incubation of serum at 56° for 30 min resulted in complete loss of toxicity. [Similar lability has been noted for other natural antibodies (12)] Absorption studies indicated that the antigen detected by these cytotoxic reactions was not tumor-specific and that normal peripheral leukocytes contained small quantities of the necessary antigenic determinants (8).

The cytotoxicity of the human sera for the biospy cells from Burkitt's lymphoma paralleled the results obtained with tissue culture cells. The mean titers obtained in the previous study (7) and in the present study were similar. Since only cells with relatively large amounts of antigen were sensitive to the cytotoxic effects of the antibody (8), the antigenic density on the biopsy cells was probably similar to that on the lymphoid cell lines. The absorption experiment demonstrated that the cytotoxic reaction with neither the biopsy cells nor the Raji tissue culture cells was tumor-specific, with activity removed by normal peripheral leukocytes. The cytotoxic tests with biopsy cells and with tissue culture cells are likely measuring the same antigen–antibody reaction.

The relationship of this cytoxic antibody system to the membrane immunofluorescence test described by Klein *et al.* (1, 5, 6) remains to be determined. The serum antibody detected by the cytotoxicity technique appears to differ from that detected by immunofluorescence. The cytotoxic antibody is heat labile and is found in most human sera; the immunofluorescent antibody is not heat labile and is found more often in the sera of Burkitt's tumor patients than in control groups. The antigens detected by the two techniques, however, may be similar. Both tests have shown positive direct reactions with Burkitt's tumor biopsy cells and nega-

tive reactions with peripheral leukocytes. The lack of tumor specificity for the antigen detected by cytotoxicity was demonstrated mainly by absoption experiments (8). Comparable absorption experiments with normal control cells have not been reported for the immunofluorescence technique. It should be stressed that the failure to detect tumor-specific antigens on Burkitt's tumor cells by the present technique does not imply that such antigens do not exist. Other immunologic techniques may be better able to distinguish between tumor-specific antigens and the quantitative variations of normal antigens on the tumor cells.

The finding of increased concentration of antigenic sites on the lymphoid cell lines compared with peripheral leukocytes was noted previously and several explanations were considered (9): (a) The cell lines have a primitive lymphoid morphology and the quantitative expression of the antigen could be related to the state of differentiation of the cells. (b) The tissue culture conditions could induce somatic changes in the cell membranes or result in the selection of somatic cell mutants. (c) Some of the antigenic sites of freshly harvested cells could be masked by antibody bound *in vivo*. The sensitivity of freshly harvested Burkitt's lymphoma biopsy cells and bone marrow lymphoblasts to the cytotoxic antibody makes the last two possibilities unlikely and indicates that the quantitative expression of this antigen on cells may be closely related to the state of differentiation of the cells. Normal well-differentiated lymphocytes from the same patients were not sensitive to the cytotoxic antibody.

The autoimmune aspects of this cytotoxic reaction were previously noted (8, 9) and were again observed in the present study. It remains to be determined whether these antibodies, which are cytotoxic *in vitro* for Burkitt's lymphoma cells and lymphoblasts, play a significant host defense role by limiting the proliferation of these abnormal cells *in vivo*.

Summary. An antibody in human sera that reacts with Burkitt's lymphoma biopsy cells and with leukemic lymphoblasts was detected by use of a complement-dependent cytotoxicity test. This antibody was detected in the sera of patients with Burkitt's lymphoma and also in the sera of other indivdiuals. The studies indicate that the antibody reacted with an antigen that is not tumor-specific but is also present on lymphoid cell lines and on normal peripheral lymphocytes.

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Received Sept. 17, 1969. P.S.E.B.M., 1970, Vol. 133.