Cytotoxic Antibody in Burkitt's Tumor and Normal Human Serum Reactive with Cultures of Lymphoid Cells (32839)

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Tests detecting antigens on tumor cells are an important aspect of tumor research, especially tests that can be used quantitatively. Several investigators (1–5) have reported the presence of antibodies in the sera of Burkitt's tumor patients, detected mainly by immunofluorescent techniques, which react with biopsy material or with tissue culture cells derived from Burkitt's lymphoma. The present study has employed a complement-dependent cytotoxicity method, which has proven quit useful in quantitative studies of the H-2 histocompatibility antigens (6), to look for antibodies reactive with established cultures of human lymphoid cells, particularly those derived from Burkitt's tumor patients. Preliminary data indicate that antibody is responsible for the cytotoxic activity. The serum activity is complement-dependent, is precipitated by 33% ammonium sulfate and separates with the immunoglobulins on Sephadex G-200.

Materials and Methods. The tissue culture cell lines, obtained from several laboratories (7), were maintained in Eagle's minimum essential medium (MEM) with 10% fetal bovine serum. The cells were washed twice with fresh medium and finally suspended in a concentration of 1000 cells/mm^3. To prepare suspensions from the monolayer cultures, the cells were first incubated with 0.2% trypsin for 15 min at 37°C. Serial twofold dilutions of the serum to be tested were made in fresh MEM with 10% FBS. One-tenth ml of the cell suspension was added to 0.1 ml of each serum dilution, and the mixture was incubated for 30 min in a 37°C water bath. Then 0.1 ml of undiluted normal rabbit serum, as a source of complement, was added to each tube and incubation was continued for an additional 30 min. Then 0.3 ml of 0.2% trypan blue in saline was added to each tube and samples from each tube were examined microscopically. The percentage of stained, i.e., nonviable, cells was determined by a count of 100–200. The titer of the serum was defined as the reciprocal of the serum dilution producing 50% stained cells.

Results. Sera from 296 individuals were tested for cytotoxicity against the Raji cell line (8), derived from a Burkitt's lymphoma. A summary of the results is given in Table I. Sera from Burkitt's lymphoma patients, both African and American, showed a high incidence of positive reactions (93%) with a mean titer of 15.4. The American and African pediatric control groups showed a lower incidence of positive reactions, with lower titers. All of the adult groups, except the leukemic patients, showed a high incidence of positive reactions. The adult leukemic patients' sera, mainly chronic myelogenous and chronic lymphocytic leukemia, had a considerably lower incidence of positive reactions, but the mean titer of the positive sera was quite high.

Two sera, from a normal adult and from a patient with Burkitt's lymphoma, were tested against other cell lines derived from Burkitt's tumor, and also against nine other human tissue culture cell lines. Representative results are shown in Table II. Both sera gave positive reactions with five cell lines derived from Burkitt's lymphoma [Raji (8), EB1 (13), EB2 (14), EB3 (9), AL2 (15)], with four cell lines derived from patients with leukemia or lymphoma [RPMI 4265 (10), LK1D (11), YH1, IM1] and with two cell lines derived from the peripheral leukocytes of normal adults (RPMI 2177 and 2367). No positive results were obtained with cell lines derived from nonlymphoid tissues [HeLa (12), KB (16), WI-38 (17)].

Leukocyte isoantibodies were ruled out as a cause for these cytotoxic reactions. Five positive sera, two from Burkitt's lymphoma patients and three from normal adults, were tested for cytotoxicity against a panel of peripheral blood lymphocytes from six individuals. The lymphocytes of these individuals, which had been previously typed for leukocyte antigens were selected so that the major leukocyte antigens would be tested for. All tests against

TABLE I. Survey of Human Sera for Cytotoxic Activity Against Burkitt Tissue Culture Cells (Raji).

Age group	Diagnosis	No. of sera tested	Positive (%)	Mean titer of positive sera[a]
Children	Burkitt's lymphoma	54	93	15.4
	Other diseases	73	59	7.2
	Normal	30	50	9.7
Adults	Leukemias	46	59	24.5
	Other malignant diseases	23	96	6.4
	Nonmalignant diseases	20	80	14.3
	Normal	50	87	9.6

[a] The titer of the serum is the reciprocal of the serum dilution producing 50% stained cells.

TABLE II. Cytotoxic Reactivity of Human Serum with Established Human Tissue Culture Cell Lines.

Cell line	Source	Cytotoxic titer of serum	
		Normal adult	Burkitt's lymphoma
Raji	Burkitt (8)	8	16
EB3	Burkitt (9)	6	18
RPMI4265	Chronic myelogenous leukemia (10)	4	12
LK1D	Acute lymphocytic leukemia (11)	6	10
IM1	Lymphoma (Finegold)	8	14
RPMI2177	Normal adult (Moore)	8	16
RPMI2367	Normal adult (Moore)	6	14
HeLa	Carcinoma of cervix (12)	0	0

this panel of cells were entirely negative. Furthermore, the cell lines Raji and RPMI-4265 have different leukocyte isoantigens (19), but they give similar results when tested in the present system with sera from Burkitt's tumor patients and from normal individuals.

The possibility that bovine serum in the culture fluid contributed to the positive cytotoxicity test was investigated by growing the cultures in media with human serum substituted for bovine serum. Raji cells were maintained in culture for 4 weeks, using MEM with 10% human serum (cytotoxic positive or cytotoxic negative) in place of fetal bovine serum. The cells grown in the presence of the cytotoxic negative serum grew as well as the cells grown in the presence of fetal bovine serum. They also gave the same pattern of cytotoxic activity as the cells grown in fetal bovine serum. The cells grown in the presence of the cytotoxic positive serum did less well, but did retain viability and divide. Addition of complement alone to a suspension of these washed cells caused cytotoxicity. These cells were presumed to be coated with the cytotoxic antibody and only the addition of complement was needed for the cytotoxic reaction.

Discussion. The present report contributes an investigation of human serum antibody detected by a complement-dependent cytotoxicity test. Available evidence indicates that the antibody differs from that detected in previous studies using other methodologies, that the antibody activity may be altered in disease, and that the antigen detected may not be specifically related to tumors.

Cytotoxicity tests introduce specific requirements on the antibody molecule (complement fixation) and, possibly, on specific characteristics of the antigenic material. Thus complement-dependent cytotoxicity tests may reveal information not discernable by immunofluorescent or precipitin tests. Compari-

son of the present studies with those employing other techniques, but similar reagents, therefore, is relevant.

The serum antibody detected in the present system appears to differ from that reported previously. Klein and associates (1, 2), using an immunofluorescent test, and an index of reaction which would help discriminate high from low reactive sera, found higher positive activity in sera from Burkitt's tumor patients than in control groups. The activity detected by Klein *et al.* (1, 2) is present in heated serum (56° for 30 min). Since this procedure inactivates the activity detected in our cytotoxic system, it seems likely that they are measuring a different serum antibody. Henle and Henle (3), using a different immunofluorescent test, report an association with the presence or absence of herpes-like particles. No such correlation was found in our series.

Gerber and Birch (20) used a complement fixation test to detect antibodies to partially purified viral antigens. More than 90% of human subjects contained antibodies to the antigens, and the authors present evidence that the reaction was viral specific. The results differ from the present study in the evidence for viral specificity and failure to react with Raji material, whereas the latter cells reacted well in our system.

Old *et al.* (5) found an increased incidence in Burkitt's tumor sera (54%, vs control 11%) of precipitation reaction detected by Ouchterlony tests against an extract of one (Jiyoye) of several tissue culture lines. Information is insufficient to permit comparison of this report with others.

In the present study, the findings with Burkitt's tumor, with adult leukemia, and with different age groups indicate that the cytotoxic antibody can be altered by environment and disease. Investigation of temporal changes in the cytotoxic activity in the sera of Burkitt's tumor patients as well as in patients with other neoplasms and nonneoplastic diseases is underway.

Summary. Human serum antibodies that react with a variety of human lymphoid cultures were detected by use of a complement-dependent cytotoxicity test. Cytotoxic antibody was detected in the sera of patients with Burkitt's lymphoma and also in sera from normal individuals. Positive reactions occurred with cell lines derived from the leukocytes of normal adults and with cell lines that lack detectable herpes-like virus. It seems likely, therefore, that this serum antibody is not tumor-specific or herpes virus-specific. The cell antigen is not associated with known leukocyte isoantigens and the available evidence indicates that this antibody reflects a separate antigenic identity in human cells.

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