*PRECIPITATING ANTIBODY IN HUMAN SERUM TO AN ANTIGEN PRESENT IN CULTURED BURKITT'S LYMPHOMA CELLS**

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Burkitt's lymphoma[1] has been the object of intense interest in recent years, particularly in view of epidemiological evidence suggesting that it may be caused by a virus.[2] Herpes simplex virus,[3, 4] reovirus,[5, 6] and unidentified infectious agents[7] have been isolated from the tissues of patients with Burkitt's lymphoma, but it is not known that any of them is etiologically related to the disease.

Burkitt's lymphoma cells were established in continuous cultures first by Epstein and Barr[8] and by Pulvertaft,[9] and more recently by Stewart *et al.*,[10] Rabson *et al.*,[11] and Osunkoya.[12] Epstein, Achong, and Barr[13] observed particles resembling herpes virus in cultured Burkitt's lymphoma cells; similar particles have been seen by Stewart *et al.*[10] and Rabson *et al.*[11] in the lines they established. In an extensive study, Epstein *et al.*[14] found no evidence of infectivity in preparations containing these particles. Stewart *et al.*,[15] however, described a transmissible encephalitis in hamsters which was originally initiated by inoculating cultured lymphoma cells or extracts of these cells into thymectomized hamsters.

These culture lines have been used to detect antibody in human serum by immunofluorescence tests on fixed cells[16] and by complement-fixation tests.[17] Positive reactions were observed in high frequency with the sera of patients with Burkitt's lymphoma and with sera from adult residents of the United States. The two tests did not give uniformly parallel results and so may be detecting different antigens. Using fresh Burkitt's lymphoma cells obtained by biopsy, Klein *et al.*[18] observed positive reactions in the indirect immunofluorescence test on viable cells with sera of patients with Burkitt's lymphoma, including in some instances the serum of the patient whose cells were used for the test. Positive reactions were observed also with sera from African patients with other diseases and infrequently with sera from healthy relatives of patients with Burkitt's lymphoma. We have examined approximately 400 sera from African and American donors for cytotoxic antibodies reactive with cultured Burkitt's lymphoma cells[19] by the same methods that led to the demonstration and description of several leukemia-specific antigens in the mouse (see review[20]). Positive reactions were found, but on further analysis these were attributed to isoantibodies or to naturally occurring antibody against a cell-attached component of the calf serum in which the cells were grown, the latter reactions disappearing when the cells were cultured in human serum.

The purpose of this communication is to report the occurrence in human sera of precipitating antibody that reacts with antigen prepared from a culture line of Burkitt's lymphoma cells (*Jiyoye*).

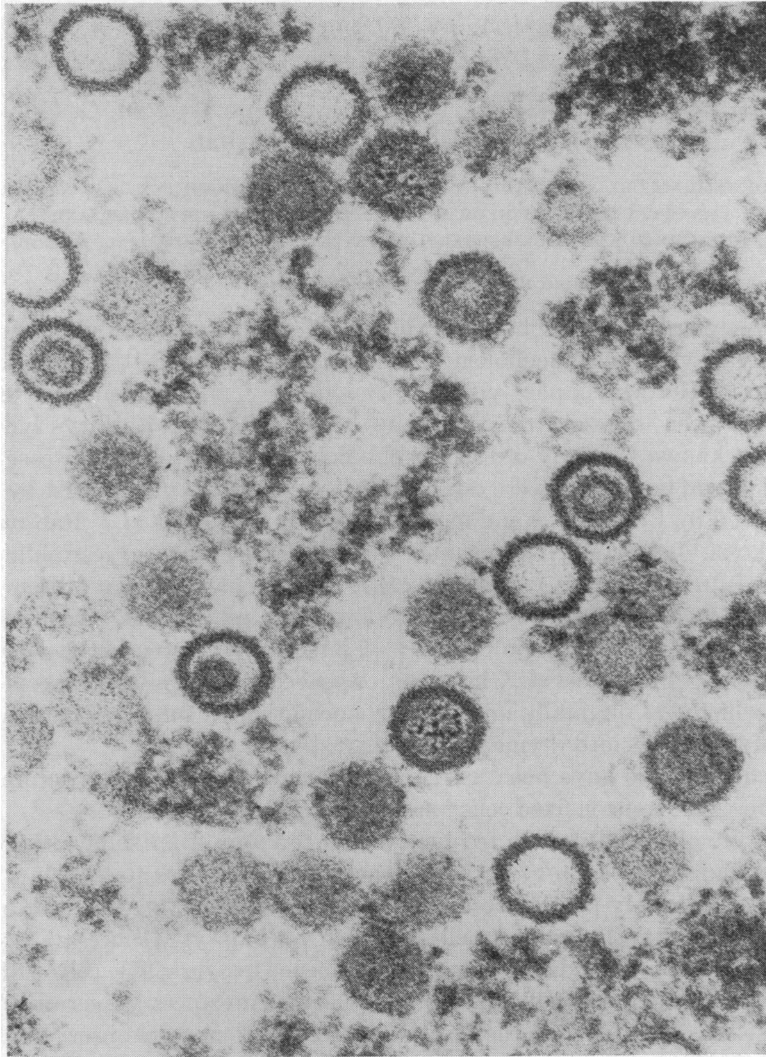

FIG. 1.—Thin section of a cell from the Burkitt's lymphoma culture line *Jiyoye* showing the type of particles observed in the nuclei of degenerating cells (×106,000).

Materials and Methods.—Immunodiffusion (Ouchterlony) tests were performed in 2 per cent Noble agar (Immunoplate pattern C, Hyland Laboratories; center-to-center distance between central and peripheral wells: 5 mm). The plates were incubated at room temperature in a humidified chamber.

The Burkitt's lymphoma cell line *Jiyoye* used as the source of antigen was established in 1965 by Professor R. J. V. Pulvertaft with cells from ascitic fluid. The line was kindly made available to us by Dr. R. A. Manaker of the National Cancer Institute, Bethesda, Md. This line is known to have the herpes-like particles described by Epstein *et al.*[13,21] and these are particularly numerous in the disintegrating cells of aging cultures (Fig. 1). In our preliminary survey of the three Epstein

TABLE 1

SURVEY OF HUMAN SERA FOR PRECIPITATING ANTIBODY TO ANTIGEN PREPARED FROM CULTURED BURKITT'S LYMPHOMA CELLS

	Diagnosis	No. of sera tested	Positive Sera No.	Positive Sera Per cent
Sera from Africa	Burkitt's lymphoma	55	31	56
	Carcinoma of the postnasal space	19	14	74
	Malignancy other than Burkitt's lymphoma and carcinoma of the postnasal space	12	1	12
	Nonmalignant disease	40	5	
Sera from U. S.	Carcinoma of the postnasal space	20	19	95
	Healthy adult donors	82	8	
	Infectious mononucleosis	17	1	
	Thalassemia (multiple transfusions)	10	2	
	Acute leukemia	41	6	11
	Mammary carcinoma	33	4	
	Bronchogenic carcinoma	23	3	

lines (EB1, EB2, EB3), the four Pulvertaft and Osunkoya lines (*Raji*, *Kudi*, *Jiyoye*, *Ogun*), and the Stewart line (SL1), the *Jiyoye* line was found to contain exceptionally large numbers of these virus particles. These particles were scattered either within nuclei as polygonal naked nucleocapsids or within the cytoplasm as more complex enveloped virions.

The *Jiyoye* cells were grown in Eagle's minimum essential medium (MEM) with 15 per cent fetal bovine serum and were passed every three or four days on reaching their optimal density of $8\text{–}10 \times 10^5$ cells/ml. Like all lines of Burkitt's lymphoma the cells grow free in suspension as isolated cells or clumps of cells that do not attach to glass. Cultures of our subline of *Jiyoye* cells have been examined for mycoplasma by Dr. J. Fogh (method of Fogh and Fogh[22]) and by Dr. D. Armstrong (method of Chanock, Hayflick, and Barile[23]). No mycoplasma was detected.

For preparing antigen, cultures were "aged" by leaving the cells for five to seven days after the usual time for passage (by which time up to 80% were dead) with the object of securing a maximal yield of the herpes-like particles. The cells were first separated by low-speed centrifugation and resuspended in 6 vol of isotonic saline. This suspension was frozen and thawed three times, homogenized in a Virtis "45" high-speed homogenizer, and cleared of gross debris by centrifugation at $2000 \times g$ for 40 min. The supernate was concentrated approximately fivefold in dialysis tubing surrounded by Sephadex G100 and was dialyzed against saline. The entire procedure was carried out in the cold. One ml of the final preparation of antigen represents the yield from approximately 1.5×10^9 cells.

Results.—Sera from 352 individuals were tested by the double-diffusion (Ouchterlony) method for reaction with antigen prepared from the *Jiyoye* line of cultured Burkitt's lymphoma cells. Table 1 summarizes the results. The positive reaction consisted of a sharply defined precipitation band, usually in the mid-zone between antigen and serum wells, becoming visible within 12–48 hours (Fig. 2). With some strongly positive sera one or two additional bands formed. The test was regarded as negative if no bands appeared within seven days. (Plates have been observed for as long as 3–5 weeks but no positive reaction has taken longer than 2 days to appear.)

The incidence of positive reactions was high in two groups of patients—those with Burkitt's lymphoma, 56 per cent of 55 African cases, and those with epidermoid carcinoma of the postnasal space, 85 per cent of 39 African and American cases. Positive reactions were obtained with sera from untreated patients with Burkitt's lym-

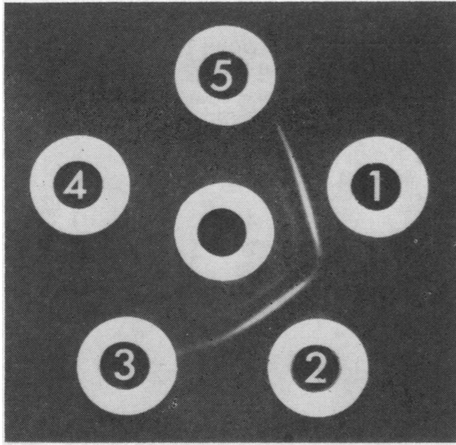

FIG. 2.—Immunodiffusion (Ouchterlony) tests with antigen prepared from a culture line of Burkitt's lymphoma cells (center well). Peripheral wells contain sera from African patients with (*1*) Burkitt's lymphoma, (*2*) carcinoma of the postnasal space, (*3–5*) nonmalignant conditions. The two positive sera show a reaction of identity.

phoma and from patients who had been treated with chemotherapy. Similarly with carcinoma of the postnasal space there was no correlation between positive reactions and stage of disease; positive sera were obtained from treated patients who were clinically free of disease.

In all other groups, representing normal individuals and patients with other diseases, malignant and nonmalignant, from both the U.S. and Africa, the incidence of positive reactions was approximately 11 per cent (Table 1). These included sera from donors of both sexes and from both children and adults. Positive sera from these various groups gave reactions of identity with the *Jiyoye* antigen and so are detecting the same component.

The reactions observed with sera from normal individuals and patients with nonmalignant diseases were weak and late in developing. As a rule the strong reactions were limited to cases of Burkitt's lymphoma and of carcinoma of the postnasal space, although strong reactions have been observed rarely with other malignancies.

A number of strongly positive sera have been tested with antigen prepared from the following sources according to the method used for *Jiyoye* antigen:

(1) fetal bovine serum (concentrated ×5);

(2) human cell lines grown in bovine serum—HeLa, Detroit-6, Chang, J-111, FL amnion (five preparations; cultures not aged), KB (one preparation; culture aged);

(3) leukemia cells from the blood of patients with acute or chronic leukemia (seven preparations from seven cases);

(4) human tonsils (four preparations from four different individuals; two from fresh cells and two from cells kept for 8 days in MEM with 15% fetal bovine serum, when about 75% of the cells were dead);

(5) human leukemia cell lines grown in fetal bovine serum—myeloblastic: R.P.M.I. 64-10 (ref. 24); lymphoblastic: SKL-2 (ref. 25) and SKL-6 (recently established in culture); Burkitt's lymphoma: SL1 (ref. 15) and EB3 (ref. 26) (five preparations, cultures aged).

Without exception, these tests were negative.

Discussion.—Precipitating antibody to antigen prepared from cultured Burkitt's lymphoma cells of the *Jiyoye* line was found with particularly high frequency in the serum of African patients with Burkitt's lymphoma and of African and American patients with carcinoma of the postnasal space. Apart from these two conditions, the incidence of positive reactions was low (approximately 11%) with sera from both African and American populations.

One of the first things to consider is whether the precipitating antibody is directed against a component of the herpes-like particles that are found in Burkitt's lym-

phoma cells, but this is a question that cannot be answered at the moment. Nevertheless, the high frequency of positive sera among patients with carcinoma of the postnasal space indicates the desirability of searching for similar particles in cultures of this class of tumor also. The particles in the *Jiyoye* line of Burkitt's lymphoma cells do not have the infective properties of herpes simplex virus; cells from aged cultures of the sort used to prepare antigen did not induce encephalitis on intracerebral inoculation into newborn mice. Many of the sera we have tested gave precipitation reactions with concentrated herpes simplex antigen (prepared from the chorioallantoic membrane of infected eggs) in agreement with Tokumaru's finding of precipitating herpes antibody in the majority of adults.[27] However, there was no relation between the antigen prepared from Burkitt's lymphoma cells and the two antigens demonstrable in our herpes preparation in tests with human serum. Some sera were strongly positive with herpes antigen and negative with antigen from Burkitt's lymphoma cells; other sera were positive in both systems, but these gave reactions of nonidentity. As herpes simplex virus contains several distinct antigens demonstrable by precipitation with human sera,[27] and as there are a number of viruses morphologically similar to herpes simplex, the antigen detected in Burkitt's lymphoma cells may nonetheless be related to a known virus of the herpes group.

In the series of 41 American patients with acute leukemia, there were six positive sera—5 per 32 cases of acute leukemia in children and 1 per 9 cases of acute leukemia in adults. This suggests that the antigen is not present in leukemias of this type; otherwise the incidence of positive sera would presumably be as high as it is in patients with Burkitt's lymphoma. This question was approached more directly by testing for antigen in preparations of either fresh or cultured cells from patients with acute leukemia. It was not demonstrable in the seven preparations from fresh leukemia cells from patients with acute or chronic leukemia or in the three preparations from aged cultures of established lines of leukemia cells. However, the antigen was also not demonstrable in aged cultures of the SL1 and EB3 lines of Burkitt's lymphoma cells, which obscures any interpretation of its absence in leukemias of American origin. The *Jiyoye* cell line was chosen for the preparation of antigen because it contains considerably more virus particles than the other lines of Burkitt's lymphoma cells. This factor may be the basis of quantitative differences in the amount of antigen extractable from cells of different lines.

Further surveys will indicate whether the high incidence of positive sera characteristic of patients with Burkitt's lymphoma and patients with carcinoma of the postnasal space is confined to these two malignant diseases or whether a similarly high incidence may be found in association with other malignant or nonmalignant conditions.

Summary.—Sera from 352 individuals were tested for precipitating antibody to antigen prepared from an established line of cultured Burkitt's lymphoma cells (*Jiyoye*). Two groups of patients showed a high incidence of positive reactions, those with Burkitt's lymphoma, 31 per 55 African cases, and those with carcinoma of the postnasal space, 33 per 39 cases (19 African and 20 American). An incidence of approximately 11 per cent positive reactions was observed among 258 sera (52 African and 206 American) obtained from normal donors, patients with malignant neoplastic diseases of other types, and patients with nonneoplastic diseases.

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