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Complement-Fixation Test for Detection of Herpes-like Viruses in Cell Cultures of Burkitt's Lymphoma

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Although biopsies of Burkitt's tumors contained no detectable complement-fixing (CF) antigen or antigens, tumor cell lines contained CF antigen or antigens related to the presence of a herpes-like virus particle.

Complement-fixation (CF) and fluorescent antibody procedures have been applied to cell lines derived from Burkitt's lymphoma and other normal or neoplastic lymphoid tissues (1, 4; J. L. Ambrus and H. V. Strandstrom, Proc. Amer. Ass. Cancer Res. **8**:2, 1967). Purified viral antigen from the P3 line of Burkitt's lymphoma has also been used in the CF test (2). These techniques were used in surveys of human and animal sera for antibodies to viral or cellular antigens. This report describes the correlation of CF reactivity with the presence of herpes-like viruses in newly established lines of Burkitt lymphoma cells.

The following cell lines from patients with Burkitt's lymphoma were employed: Jijoye (P3) and Ogun (R. J. V. Pulvertaft, *unpublished data*), Silfere (NK9c), Margret (NK11a), Esther (NK8b), and Issac IV (NK15d1; 9). Cells were grown in Eagle's minimal essential medium supplemented with 20% fetal calf serum. For the growth of the Jijoye (P3) and Ogun lines, the calf serum was inactivated at 56 C for 30 min. CF antigens were prepared according to Armstrong et al. (1) by one cycle of freezing and thawing, with 1.0 to 1.2 $\times$ 10^7 viable cells per ml. The preparation of antigens from tumor biopsies and the performance of the CF test were previously described (8). Antigens were stored at -70 C.

Human sera, obtained from African patients and controls, were inactivated at 56 C for 30 min. Antigens from cell lines were used undiluted and usually represented 1 to 2 units of antigen. Antigens from tumor biopsies were diluted 1:4. Initial dilutions of sera were 1:8. All tests were incubated at 4 C overnight with 2 exact units of guinea-pig complement.

Approximately 50% of African sera fixed complement in the presence of antigens from the Jijoye (P3), Silfere (NK9c), and Esther (NK8b) cell lines (Table 1). Of the sera, 33% were positive with Issac IV (NK15d) antigen. Serum titers ranged from 1:8 to greater than 1:64. Highest titers were obtained with the Jijoye (P3), Silfere (NK9c), and Esther (NK8b) lines. Higher titers and a higher percentage of positive reactions were obtained with Esther (NK8b) antigen following

TABLE 1. *Numbers of African sera that react in the CF test with Burkitt and other cell antigens*

African sera	Jijoye and P3	Ogun	Silfere NK9c	Margret NK11a	Esther NK8b	Issac IV NK15d	Burkitt biopsies[a]	African lympho-sarcoma biopsies[b]	Human spleen (Amer-ican)
Burkitt	12/15[c]	.0/15	8/15	0/17	6/11	2/10	0/15	0/15	0/15
Other neoplasms[d]	4/8	0/8	5/8	0/8	4/6	2/5	0/7	0/7	0/7
Control	1/5	0/5	1/5	0/6	2/4	2/4	0/5	0/5	0/5
Total	17/28	0/28	14/28	0/31	12/21	6/19	0/27	0/27	0/27

[a] Determined on four tumors.
[b] Determined on two tumors.
[c] Values indicate the ratios of number of sera positive to number tested.
[d] The five positive sera were from patients with multiple lymphoma, osteogenic carcinoma of the skull (?), Hodgkins disease, mixed salivary tumors, and osteoclastoma. The three negative sera were from lymphoblastic lymphoma, thymic tumor, and glomus jugulare.

30 min of centrifugation at 35,000 × g. Some sera reacted with only one, two, or three of the positive cell lines; however, 44% of the sera tested against all cell-culture antigens were consistently positive or negative for all four "positive" cell lines. Although our data and that of Armstrong et al. indicate that perhaps more than one antigen is responsible for CF reactivity, differing amounts of viral (or virus-induced) antigen in the cell lines could explain our results.

Accordingly, electron-microscopic studies were undertaken to determine if herpes-like viruses were present in these cell lines. Cell pellets were fixed in 3% glutaraldehyde, postfixed in 1% osmium, and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate for examination under a Siemens Elmiskop 1A electron microscope (Table 2). Only cell lines that were positive in the CF test contained the herpes-like virus particles (Fig. 1), and the degree of CF reactivity correlated with ease of virus detection. The Issac IV (NK15d) cell line, which exhibited low CF reactivity, contained very sparse virus particles. Thus, the CF test appears to be useful for detection of virus-containing Burkitt cell lines. Tests of these lines for membrane antigenicity and Henle's antigen are in general agreement with our CF results (7).

Of the Burkitt serum donors of Table 1, the only three long term survivors without disease had negative sera (two cases) or a low titer (1:8 versus Jijoye). A fourth long term survivor with a 1:64 titer against Jijoye relapsed. Of the nine others with titers of 1:32 or higher, all but one are dead.

TABLE 2. *Correlation of CF activity of African sera and the presence of herpes-like virus particles detected by electron microscopy*

Cell line	CF activity	Presence of virus
Jijoye (P3)	+	+
Ogun	−	−
Silfere (NK9c)	+	+
Margret (NK11a)	−	−
Esther (NK8b)	+	+
Issac IV (NK15d)	+	+

Lack of CF reactivity in biopsies of Burkitt's lymphoma (Table 1) suggests a lack of detectable viral (or virus-induced) antigen. However, virus particles have been reported in some tumor biopsies (3). Perhaps the surface antigen revealed by immunofluorescence of Burkitt tumor cells and of cell lines containing virus particles (5, 6) is virus-induced, exists in the presence or absence of intact virus particles, and does not depend on the presence of the CF antigen for its integrity. Further clarification of tumor-specific and viral antigens in the Burkitt system must be attempted.

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FIG. 1. *Part of the nucleus of a degenerating cell showing numerous herpes-like virus particles in various stages of maturation, typical of cell cultures exhibiting strong CF reactivity. Inset shows the extra-cellular form of particle which has acquired an external envelope.* × *75,000.*