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Antibody Coating and Agglutination of Virus Particles Separated from the EB3 Line of Burkitt Lymphoma Cells

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Several lines of lymphoblasts, derived from Burkitt tumors and maintained in continuous culture, harbor as yet unidentified, herpeslike virus particles (Epstein et al., Lancet **1**:702, 1964; Wistar Inst. Symp. Monograph **4**:69, 1965; Stewart et al., J. Natl. Cancer Inst. **34**:319, 1965; Rabson et al., Intern. J. Cancer **1**:89, 1966). It was shown that a small percentage of the cells in such lines can be stained with the aid of appropriate human sera by the indirect immunofluorescence technique (G. Henle and W. Henle, J. Bacteriol. **91**: 1248, 1966). All of 30 sera from patients with Burkitt tumors thus far tested induced positive and, as a rule, intensely brilliant reactions. Of 140 sera from healthy African control children, 54% were positive, but the fluorescence observed was mostly of low intensity (J. A. Levy and G. Henle, J. Bacteriol. **92**:275, 1966). Similar results were obtained with a considerable number of sera collected from American children (35%) and adults (85%), regardless of the histories of the donors. Because of the frequency of positive results among sera of American adults, fluorescein isothiocyanate-conjugated pooled human γ globulin was found suitable for direct staining.

Several observations denoted that the immunofluorescence technique detects those cells which harbor herpeslike virus particles (G. Henle and W. Henle, J. Bacteriol. **91**:1248, 1966): (i) Burkitt cell lines free of detectable virus particles failed to reveal stainable cells; (ii) the percentage of fluorescent cells in lines carrying the indigenous virus corresponded roughly to the estimate of infected cells based upon electron microscopy; (iii) cell lines in which after earlier positive results virus particles were no longer detectable (Rabson et al., Intern. J. Cancer **1**:89, 1966; Epstein, *personal communication*) contained no more than about 1 in 1,000 or 1 in 10,000 stainable cells; (iv) the number of fluorescent cells was reduced by >95% in response to treatment of cultures with 5-methylamino-2′-deoxyuridine, a compound effective against herpes simplex virus (Nemes and Hilleman, Proc. Soc. Exptl. Biol. Med. **119**:515, 1965); and (v) stained cells often exhibited varying degrees of degeneration as did infected cells, especially when virus particles were present in the cytoplasm (Epstein et al., J. Exptl. Med. **121**:761, 1965). Various virus-specific immunofluorescence tests failed to relate the reactive antigen(s) to herpes simplex, varicella, cytomegalo, and a number of animal herpes viruses. Since all points listed above provide only indirect support, further proof was sought for identification of the stainable cells with those harboring virus particles.

When the EB3 line of Burkitt lymphoma cells is maintained under less than optimal conditions for cellular growth, the percentage of fluorescent cells may increase as much as eightfold (G. Henle and W. Henle, *to be published*). Cells were pelleted from cultures containing either about 20% or 2 to 3% stainable cells, and thin sections were prepared for electron microscopy. In the first type of specimen, cells with virus particles were readily found and appeared at a frequency which seemed to correspond roughly to the percentage of fluorescent cells. In the second type of preparation, virus-containing cells were observed only after long search.

In further experiments, virus particles were separated from EB3 cells, partially purified, and concentrated for use in electron microscopic agglutination tests (Hummeler et al., Virology **16**:84, 1962). The starting cultures contained between 12 and 20% cells which stained by the immunofluorescence technique. From 4×10^8 to 1×10^9 cells were sedimented at $600 \times g$ for 20 min, resuspended in 20 to 30 ml of phosphate-buffered saline solution at *p*H 7.2 (PBS) and disrupted by exposure to sonic oscillation for 3 to 10 min (Disintegrator-Forty, Ultrasonic Industries, Inc.,

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Plainview, N.Y.). After clarification at 600 × *g* for 20 min, the supernatant fluids were successively centrifuged at 1,700 and 41,000 × *g* for 30 min each. The last sediments were suspended in 20 ml of PBS, dispersed by brief sonic treatment (20 to 30 sec), and subjected once more to the same sequence of centrifugations. The final sediments were taken up in 0.5 ml of PBS, dispersed by brief sonic treatment, and clarified at 600 × *g* for 10 min. Although these preparations contained only limited numbers of virus particles and still much cellular debris, they proved workable for preliminary agglutination tests. Human sera or γ globulin in 10-fold dilution was mixed with an equal volume (0.1 ml) of virus suspension, and samples were withdrawn for negative-contrast staining after incubation for 1 hr at 37 C followed by incubation at room temperature for 30 to 120 min.

Table 1 lists the antibody preparations employed, together with their pretreatments and other pertinent data. The first three sera were from Burkitt tumor patients, and these, as well as the American γ globulin, titered to 1:160 to 1:1,280 in indirect immunofluorescence tests with EB3 cells. They were used after adsorption with Raji cells (Pulvertaft, Lancet **1**:238, 1964), a Burkitt cell line recently shown to be free from indigenous virus (Epstein et al., J. Natl. Cancer Inst., *in press*). One of these sera in addition had been adsorbed exhaustively with herpes simplex virus (HSV). The sera had been adsorbed also with sheep red blood cells for earlier complement-fixation tests. The remaining five sera, all from American patients, failed to elicit immunofluorescence in EB3 cells. Two of these contained known high levels of antibody to HSV, and one each to varicella or cytomegalovirus. These five sera caused neither electron microscopically detectable aggregation of virus particles nor their coating with antibody (Fig. 1). In contrast, virus particles which had been exposed to the Burkitt lymphoma sera or the American γ globulin were found to be surrounded by a fringe of structures of the length and width of antibody modecules (Fig. 2). The virus concentrations were apparently too low to obtain, or to find large aggregates, but occasionally two or more particles were noted which were clearly linked by antibody bridges (Fig. 3 and 4). Through the Special Virus Leukemia Program of the National Cancer Institute, additional virus suspensions were made available which were prepared at the John L. Smith Memorial for Cancer Research, Chas. Pfizer & Co., Inc. Maywood, N.J. One of these, derived from sonically treated and ficin-digested EB3 cells by potassium tartrate gradient centrifugation, proved cleaner than those obtained in this laboratory. Agglutination tests with this suspension and several of the sera listed in Table 1 confirmed the reactions described.

It is evident from these observations that the three sera from Burkitt tumor patients as well as the American γ globulin contained antibodies to the capsid antigen of the herpeslike virus particles present in the majority of cultured Burkitt cell lines. Antibodies to herpes simplex, varicella, and

TABLE 1. *Comparison of results obtained with selected sera in indirect immunofluorescence and viral agglutination tests*

Serum	Adsorption of serum	Indirect immunofluorescence			Antibody coating and agglutination of virus particles
		Herpes group viruses[a]	EB3 cells		
			Intensity[b]	Titer	
Burkitt lymphoma no. 5	Raji cells[c]	HSV +	++	1,280	+
Burkitt lymphoma no. 7	Raji cells	HSV +	++	160	+
Burkitt lymphoma no. 11	Raji cells	HSV +	++	640	+
Burkitt lymphoma no. 11	Raji cells and HSV	HSV −	++	640	+
Human γ globulin	Raji cells	HSV +	++	320	+
Leukemia LK-3	None	HSV −	−	<8	−
Leukemia LK-5	None	HSV +	−	<8	−
Leukemia LK-35	None	HSV −	−	<8	−
Cytomegalic inclusion disease CMV-518	None	CMV +[d]	−	<8	−
Varicella VA-2	None	VAV +	−	<8	−

[a] Herpes simplex (HSV); cytomegalo (CMV); varicella (VAV).
[b] At a serum dilution of 1:10
[c] Cells (2×10^8) were suspended in 1 ml of serum 1:10, incubated for 1 hr at 37 C and overnight at 4 C, and centrifuged at 50,000 × *g* for 30 min.
[d] Complement-fixing titer, 1:256

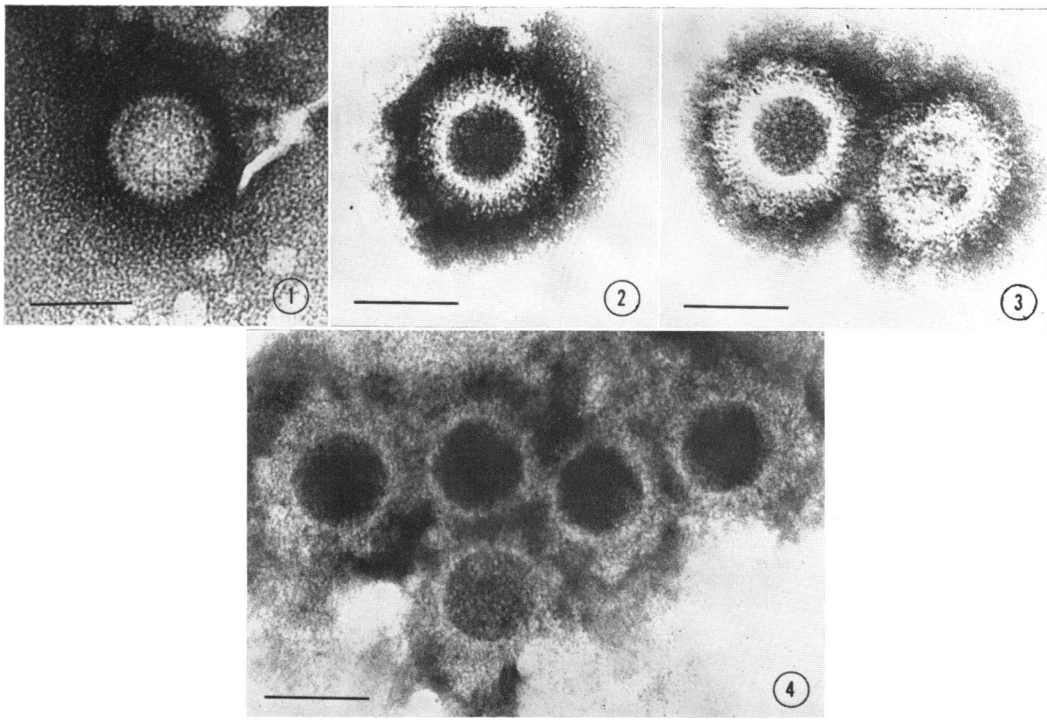

FIG. 1–4. *Electron micrographs of virus particles after exposure to various sera or γ globulin. Fig. 1: Serum LK-5, negative in the immunofluorescence test. Note absence of antibody coating. Fig. 2: Burkitt lymphoma serum (adsorbed with Raji cells and herpes simplex virus), positive in dilution 1:640 in the immunofluorescence test. Note fringe of antibody molecules surrounding the virus particle. Fig. 3: Same serum as Fig. 2. Note antibody bridges linking the virus particles. Fig. 4: Human γ globulin, positive in dilution 1:320 in the immunofluorescence test. Immune aggregate of five virus particles. The bar in each figure represents 1,000 A.*

cytomegaloviruses failed to combine with the virus particles. The results of the agglutination tests, limited as they are, appear to parallel those obtained by immunofluorescence. The extent to which the viral capsid antigen contributes to the staining has not been determined. The relative paucity of formed virus particles in infected cells suggests that noncapsid antigens may dominantly account for this reaction.

The serological data indicate that the herpeslike virus present in Burkitt cell lines is widely distributed in Africa as well as the United States. Indeed, similar virus particles have now been detected in cultured lymphoblasts derived from a lymphoma of an American patient (O'Conor and Rabson, J. Natl. Cancer Inst. **35**:899, 1965), and the reagents employed for staining of Burkitt cells also elicited fluorescence in some of the cells of this AL_2 line (G. Henle and W. Henle, *unpublished data*). The identity of the herpeslike virus particles, as well as their role, if any, in the etiology of African, or American lymphomas, remains to be determined.

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