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Surface marker characteristics and Epstein–Barr virus studies of two established North American Burkitt's lymphoma cell lines*

(African Burkitt's lymphoma/lymphocyte markers)

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ABSTRACT **Tumor cell lines have been established in continuous culture from two North American Burkitt's lymphomas. The SU-AmB-1 line, derived from a patient with low serum antibody titers to Epstein–Barr virus (EBV), was devoid of EBV genomes by the reaction for EBV-associated nuclear antigen (EBNA), could not be induced to express EBV antigens, and was highly refractory to EBV superinfection. Conversely, the SU-AmB-2 cell line, derived from a patient with "African type" serology, yielded a positive EBNA reaction and was readily inducible and superinfectable. Although both cell lines possessed B (bone-marrow-derived) cell characteristics, they had different surface marker patterns. It is postulated that two different classes of undifferentiated B cell lymphomas exist, one of which is positive for the presence of EBV genomes and occurs endemically in Africa and New Guinea and sporadically in other parts of the world, the other of which is EBV-negative and occurs sporadically throughout the world, including the endemic areas.**

Although Burkitt's lymphoma was first identified in North American patients by its striking resemblance to African cases (1, 2), a number of significant differences have emerged which justify a critical comparison of these malignancies at the cellular level. Whereas, both varieties have similar clinical presentations (3, 4), morphologies (5), and B (bone-marrow-derived) cell characteristics (6), cases of American Burkitt's lymphoma (AmBL), unlike those of African origin, are not endemic to certain regions (4), respond poorly to chemotherapy (7), often have lower serum antibody titers to Epstein–Barr virus (EBV) (8, 9), and have not contained cellular EBV genomes detectable by nucleic acid hybridization (10, 11). To characterize the malignant cell population in these patients, two North American Burkitt's lymphoma cell lines were established in continuous culture. Immunologic and virologic evidence is presented indicating that these represent two distinctively different B cell malignancies, one resembling the African (endemic) variant, the other the North American (sporadic) variant.

MATERIALS AND METHODS

Patients

K.P., SUMC 42-66-70. A 12-year-old boy was admitted in July, 1973 with fever, weight loss, and abdominal pain. An abdominal mass was palpable, which at laparotomy involved the cecum, appendix, and soft tissue of the mesentery and omentum. The neoplastic lymphoid cells were uniform in size, and had relatively large nuclei and a thin rim of amphophilic, pyroninophilic, periodic acid-Schiff reagent negative cytoplasm. There was no evidence of abdominal lymph node involvement. Smears prepared from a peritoneal effusion revealed malignant cells containing oil red O positive cytoplasmic vacuoles. Final diagnosis by Dr. Ronald F. Dorfman was malignant lymphoma, undifferentiated, Burkitt's type.

D.S., SUMC 47-23-08. A 16-year-old boy presented with a history of abdominal cramping, fever, night sweats, weight loss, fatigue, and left cervical adenopathy. Radiographic examination revealed small bowel masses with intussusception. Cervical exploration disclosed a white, fleshy necrotic mass infiltrating the muscles of the neck without definite node involvement. On microscopic examination, the lymphomatous process was predominantly in extranodal tissue and a lymph node was only focally involved. The tumor cells were uniform, round, and highly pyroninophilic, with tiny, often multiple, basophilic nucleoli. A starry sky pattern was prominent. Final diagnosis by Dr. Hun Kim was diffuse lymphoma, undifferentiated, strongly suggestive of the Burkitt's type.

Cell lines

Su-AmB-1. A peritoneal effusion sample was obtained by paracentesis from patient K.P. After the red blood cells were removed by the Ficoll-Hypaque method (14), the tumor cells were resuspended in clarified effusion fluid with 10% dimethylsulfoxide at a concentration of 5×10^6 cells per ml and frozen in liquid nitrogen at $-1°$/min. With the aid of feeder layers, tumor cells from cold storage were established in stationary suspension culture as described elsewhere§.

SU-AmB-2. An inguinal lymph node obtained by biopsy from patient D.S. was teased apart in cold medium. The disaggregated tumor cells were placed directly on agar plates with feeder layers and established in continuous culture§.

Other Lines. Three African Burkitt's lymphoma cell lines (Raji, HR-1, and EB3) and three lymphoblastoid lines (SU-LB-1, -4, and -5) were used for comparative studies. The lymphoblastoid lines were established in our laboratory from uninvolved Hodgkin's disease spleens. All cultures were grown at 37° in stationary suspension culture in RPMI-1640 medium with 20% fetal calf serum, vitamins, and antibiotics (15). The SU-AmB-1 and SU-AmB-2 lines also required a 10% human serum supplement. For the superinfection and transformation experiments, human serum from an EBV-negative donor was used.

Abbreviations: AmBL, American Burkitt's lymphoma; B cells, bone-marrow-derived cells; NPC, nasopharyngeal carcinoma; IM, infectious mononucleosis; EBV, Epstein–Barr virus; VCA, viral capsid antigen; D and R, diffuse and restricted components of the early antigen; EBNA, EBV-associated nuclear antigen; ACIF, anticomplement immunofluorescence test; IF, immunofluorescence; IdUrd, 5-iododeoxyuridine; E, spontaneous erythrocyte rosette test; EA, EAC, erythrocyte–antibody and erythrocyte–antibody–complement rosette tests; SRBC, sheep red blood cells.

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§ A. L. Epstein and H. S. Kaplan, manuscript in preparation.

Lymphocyte marker studies

Surface Immunoglobulin. The presence of surface immunoglobulins was determined on live culture cells and on fixed cell preparations by established methods (16, 17). The sera used to grow the tumor lines were freed of aggregated γ globulins (18) by centrifugation at 130,000 × *g* for 2 hr. The cells were harvested 16 hr later and tested with fluorescein-conjugated goat anti-human immunoglobulins specific for IgM, IgA (Antibodies Incorporated, Davis, Calif.), and IgG (Cappel Laboratories, Inc., Downingtown, Pa.).

Fc Receptors. The cell lines were tested for the presence of Fc receptor sites by the aggregated γ globulin technique (19). The sera used to grow the cells were freed of aggregates as described above.

IgMEAC and IgGEA Rosette Tests. The cell lines were assayed for the binding of IgMEAC and IgGEA rosettes by the procedures of Shevach *et al.* (20). IgMEA served as a control reagent for the nonspecific binding of sheep red blood cells (SRBC). The rabbit anti-SRBC 19S IgM and 7S IgG antisera were purchased from Cordis Labs, Miami, Fla., the SRBC from Microbiological Media, Concord, Calif., and the complement was obtained fresh from normal mouse serum. A rosette was scored if three or more SRBC were seen adhering to the surface of a cultured cell.

Spontaneous Erythrocyte Rosette Test. The cell lines were tested for their ability to form spontaneous (E) rosettes with SRBC by the method of Bentwich *et al.* (21).

Epstein–Barr virus studies

Antibody Determinations. The patients' sera were titrated for antibodies to four EBV-related antigens. Antibodies to the Epstein–Barr viral capsid antigens (VCA) and to the diffuse (D) and restricted (R) components of the EBV-induced early antigen complex were measured by indirect immunofluorescence (IF) tests (22, 23) and antibodies to the EBV-associated nuclear antigen (EBNA) were measured by anti-complement immunofluorescence (ACIF) tests (24).

Detection of EBV-Related Antigens. Smears of the cultured cells on coverslips were air dried, fixed for 3 min at −20° in acetone or acetone and methanol, and tested by the above-mentioned techniques for VCA, D and R, and EBNA.

Activation of EBV by 5-Iododeoxyuridine (IdUrd). The cell cultures were incubated with 20 or 50 μg/ml of IdUrd for 1 or 3 days. Parallel control cultures were incubated in the absence of IdUrd. Smears were prepared at 4 and 7 days and processed for ACIF and indirect IF with sera from several sources including patients K.P. and D.S., nine other patients with AmBL, six patients with nasopharyngeal carcinoma (NPC), and eight patients with infectious mononucleosis (IM). The sera fell into five groups: those containing (*1*) no antibodies to EBV; (*2*) anti-VCA only; (*3*) anti-VCA and anti-EBNA; (*4*) anti-VCA and anti-D; and (*5*) anti-VCA, anti-D, and anti-EBNA antibodies.

Infection of AmBL Cells with EBV. The technique of laboratory infection with EBV has been previously described (25). The virus was derived from culture media of HR-1 subline cultures of the P3J African Burkitt's lymphoma. The B95-8 virus, which is reportedly capable of inducing transformation but not productive infection (26), was used in some experiments. The infectivity of the virus preparations was ascertained by simultaneous exposure of Raji cells. The Raji line, which has been shown to contain EBV genomes, is a highly EBV-susceptible, nonproducer African Burkitt's lymphoma culture (27). The infectivity of EBV from SU-AmB-2 cultures was also tested on the Raji cells. SU-AmB-2 media, collected from 4- to 13-day cultures incubated at 32° or 37°, were used undiluted or at 10^{-1} dilution.

Table 1. Lymphocyte marker studies of North American Burkitt's lymphoma and other cell lines*

Cell lines	Lymphocyte marker IgM	IgG	IgA	Fc	EAC	EA	E
North American Burkitt's							
SU-AmB-1	95	0	0	12	1	0	0
SU-AmB-2	0	5	0	20	23	0	0
African Burkitt's							
Raji	38	0	15	0	92	11	0
EB3	40	0	0	0	0	4	0
HR-1	10	0	0	0	0	0	0
Lymphoblastoid							
SU-LB-2	30	6	0	0	55	0	0
SU-LB-4	25	8	15	2	59	2	0
SU-LB-5	25	22	8	0	56	0	0

* Data are mean percentage of positive cells.

Transformation of Fetal Lymphocytes. The capacity of SU-AmB-2 culture medium to transform cord blood lymphocytes was assessed by the incorporation of radioactive DNA precursors (28) and the establishment of EBV-positive fetal lymphoblastoid cells in continuous culture (29). Fetal lymphocytes, separated from fresh cord blood by the Ficoll–Hypaque method, were washed twice and adjusted to a concentration of 5×10^6 cells per ml. Three milliliters of cells were mixed with 1.8 ml of growth media from the SU-AmB-1, SU-AmB-2, and B95-8 cell lines. After 1 hr of incubation at 37°, 4.2 ml of fresh medium was added to the cultures. At 7, 14, and 21 days, 0.5 ml aliquots of the cultures were pulsed for 1 hr with 2 μCi of [^{3}H]thymidine to measure DNA synthetic activity. In addition, viable cells were counted by trypan blue dye exclusion at 21 days and the cultures were examined microscopically for the presence of cell aggregates.

RESULTS

Lymphocyte Marker Studies. The SU-AmB-1 line was strongly positive for surface IgM, had a low percentage of cells with Fc receptors, and did not form IgMEAC, IgGEA, or E rosettes (Table 1). In contrast, the SU-AmB-2 line was weakly positive for surface IgG, had a relatively high percentage of cells with Fc receptors, and formed IgMEAC but not IgGEA or E rosettes. The African Burkitt's lymphoma cell lines were all positive for surface immunoglobulins and had a variable number of cells with Fc and complement receptors. The lymphoblastoid cell lines, unlike the Burkitt's lymphoma cultures, had polyclonal surface immunoglobulins and a high percentage of cells forming IgMEAC rosettes.

Table 2. EBV titers of sera from two patients with North American Burkitt's lymphoma

Patient	anti-VCA	anti-D	anti-R	anti-EBNA
K.P.	1:20	<1:5	<1:5	1:5
D.S.	1:640	1:640	*	1:40

* Low titers of anti-R could not be detected in the presence of brilliant D staining by dominantly anti-D positive sera.

Table 3. EBV antigens in North American Burkitt's lymphoma and Raji cell lines*

Cell line	Indirect IF			ACIF	
	VCA	D	R	C' EBNA	C' Control
SU-AmB-1	0	0	0	0	0
SU-AmB-2	3–10	3–10	3–10	>90†	0
Raji	0	0	0	>90	0

* Data are % of positive cells.
† 3–10% of the cells stained brightly (VCA) and the remainder showed atypical, faint, punctate nuclear staining.

EBV Serology of AmBL Patients. Patient D.S. had high titers of antibodies to VCA, D, and EBNA, whereas patient K.P. had low anti-VCA and anti-EBNA antibody titers indicative of prior primary infection (Table 2).

EBV-Related Antigens in AmBL Cell Cultures. SU-AmB-1 cells, derived from patient K.P., were entirely negative for VCA, D, R, and EBNA, whereas SU-AmB-2 cells, cultured from patient D.S., were found to produce VCA, D, and R antigens (Table 3). Since no significant excess of D or R over VCA positive cells were observed, the EBV infectious cycle evidently was not aborted before VCA synthesis. Confirming this conclusion, viral nucleocapsids were readily detectable in these cells by electron microscopy.

The presence of EBNA in the SU-AmB-2 cells was not recognized initially because the fluorescent staining of more than 90% of the nuclei was weak and punctate, rather than brilliant and diffuse as in all other EBNA-positive lines and biopsies examined to date (24, 30, 31). However, the fact that this reaction, though atypical, was consistently found with eight VCA-positive AmBL sera and not with three VCA-negative AmBL sera established that it was due to EBNA. This conclusion was confirmed by the absence of nuclear staining with anti-VCA-positive but anti-EBNA-negative sera from patients with acute infectious mononucleosis and by staining with sera from nasopharyngeal carcinoma patients with high anti-EBNA titers.

Table 5. EBV antigen expression after superinfection of cell cultures

Cell line	Virus, dilution	Antigen*			
		VCA	D	R	EBNA
SU-AmB-1	None, —	0	0	0	0
	HR-1, 10^{-1}	0	⩽0.03+	0	⩽1+‡
	HR-1, 10^{-2}	0	R+	0	⩽0.1+‡
SU-AmB-2†	None, —	5+	5+	5+	>90±
	HR-1, 10^{-1}	80+	>90+	>90+	>90±
	HR-1, 10^{-2}	>90+	>90+	>90+	>90±
	B95-8, 10^{-1}	8+	12+	10+	>90±
	B95-8, 10^{-2}	7+	10+	8+	>90±
Raji†	None, —	0	0	0	>90+
	HR-1, 10^{-1}	20+	>90+	>90+	>90+
	HR-1, 10^{-2}	⩽1+	25+	25+	>90+
	B95-8, 10^{-1}	R±	2+	R±	>90+

* Data are mean percentage of positive cells at 48–72 hr; ±, + indicate the intensity of immunofluorescence; R = rare.
† Since it is not possible to discern EBNA in VCA-positive cells, the values listed for the SU-AmB-2 and Raji lines are estimates based on the number of VCA-negative cells.
‡ In these cultures, EBNA-positive cells had declined in number by 7 days and were absent by 14 days.

IdUrd Activation of EBV. Incubation of SU-AmB-1 cultures for various intervals with IdUrd (20 μg/ml) failed to induce antigen synthesis. In contrast, the percentage of brightly stained VCA-positive SU-AmB-2 cells increased up to 4-fold with several anti-VCA-positive, anti-D-negative sera after incubation with 20 or 50 μg/ml of IdUrd (Table 4). Since the anti-D-positive serum used in the first experiment stained a greater percentage of cells, it appears that the induction of EBV by IdUrd was largely abortive.

Superinfection of the AmBL Cell Lines with EBV. The SU-AmB-1 cells proved highly refractory to infection by EBV from the HR-1 line (Table 5). Three days after incubation with the 10^{-1} virus dilution, less than 1% of the cells were EBNA-positive, only rare cells (≤ 0.025%) were D-pos-

Table 4. Activation of EBV antigen expression in SU-AmB-2 cells by IdUrd

Exp. no.	Test sera and reciprocal titers						Indirect IF†		Anti-C' IF†			
									Control		IdUrd‡	
	Source	No. of sera	VCA	D	R	EBNA	Control	IdUrd‡	EBNA	Other	EBNA	Other
1	AmBL	1*	640	160	?	40	6+	40++	>90±	10++	>90±	80++
		7	10–160	10	10	2–40	3–6+	5–15+	>90±	5–7+	>90±	5–13+
		3	<2	<2	<2	<2	0	0	0	0	0	0
2	IM (acute)	4	320–640	40–320	?	<2	2–3+	10–12+	0	3–5++	0	10+
		4	80–160	<10	<10	<2	1–2+	0+	0§	3–5+	0	8–10+
	NPC	3	1280	160–640	?	160–320	3+	10–12+	>90+	3–5++	?+¶	10–15++
		3	80–320	<10	<10	160–320	1–2+	10+	>90+	3–5+	?+¶	8–10+
	Controls	1	80	<10	<10	160	1–2+	n.d.	>90±	3–5+	?+¶	8–10+
		1	<2	<2	<2	<2	0	n.d.	0	0	0	0

N.d. indicates not done.
* Autologous serum of patient D. S.
† Percentage of positive cells; ±, +, ++ indicate the intensity of immunofluorescence.
‡ 20 μg/ml of IdUrd for 24 hr followed by incubation in IdUrd-free medium for 6 days (Exp. 1) or 3 days (Exp. 2). Similar results were obtained using 50 μg/ml of IdUrd for 1–3 days.
§ Two of the four sera showed faint, possibly nonspecific nuclear staining.
¶ Due to toxicity of IdUrd in Exp. 2, EBNA had escaped from some of the cells.

Table 6. Failure of SU-AmB-2 culture media to transform cord blood lymphocytes

Source of medium	Dilution	Δ $\log_{10}$ cpm* at day 7	14	28	Cell count (10^4 cells/ml at day 28)
SU-AmB-2	None	−0.29	0.09	−0.10	3
B95-8	None	0.59	1.48	2.61	272
B95-8	10^{-1}	−0.06	0.44	1.63	67
Control	—	—	—	—	4

* $\log_{10}$ cpm of exposed cells − $\log_{10}$ cpm of control cells. Values of ≥0.2 represent transformation of the fetal lymphocytes.

itive, and none produced VCA. By 7 days, D-positive cells were no longer detectable and EBNA-positive cells were rare. Finally, by 14 days EBNA-positive cells were no longer seen. In contrast, the same virus dilution induced D and R antigen synthesis in nearly all the Raji cells and in 90% of the SU-AmB-2 cells at 2–3 days. VCA was present in 10–20% of the Raji cells and in 80–90% of the SU-AmB-2 cells. The SU-AmB-2 cells were also more susceptible than the Raji cells at higher virus dilutions. Surprisingly, the B95-8 virus, though reported to have only transforming acitvity, induced abortive infections in about 2% of the Raji cells and in about 5–10% of the SU-AmB-2 cells. The Raji cells could not be superinfected with media from control and IdUrd-treated SU-AmB-2 cultures.

Transformation Studies with Fetal Lymphocytes. In three separate experiments, SU-AmB-2 cell media failed to increase [^{3}H]thymidine incorporation in the fetal cultures or to induce the formation of cell aggregates (Table 6). Positive control media from B95-8 cells transformed the cord blood lymphocytes in 7–14 days. As expected, media from SU-AmB-1 cultures did not contain transforming activity.

DISCUSSION

The endemic cases of Burkitt's lymphoma in equatorial Africa and New Guinea have remarkably distinctive and homogenous clinicopathologic characteristics (32), among which are the presence of B cell surface markers and an association with Epstein–Barr virus. Until recently, all African Burkitt's lymphoma biopsies studied had been shown to contain EBV genomes by EBNA staining (31) and/or nucleic acid hybridization (33, 34). However, Lindahl *et al.* (30) recently observed four EBV-negative African lymphoma biopsies, at least one of which was a Burkitt's lymphoma. Moreover, Klein *et al.* (35) have reported the successful establishment of an EBV-negative African Burkitt's lymphoma cell line. Thus, a small percentage, estimated at 2–3% (36), of African Burkitt's lymphomas appears to be EBV-negative.

Criteria for the diagnosis of Burkitt's lymphoma in patients from nonendemic areas have been clearly defined (12, 13). The similarity of these nonendemic cases to those of African origin gained credibility when it was found that some patients had elevated serum antibody titers to EBV (8, 9) and that tumor cells from an AmBL patient had B cell characteristics (6). However, with the introduction of more stringent techniques for the detection of EBV-specific DNA (33) or nuclear antigens (24), studies of AmBL biopsies have consistently revealed the absence of demonstrable EBV genomes (10, 11). Accordingly, the pendulum has swung to the view that sporadic cases in nonendemic regions may differ categorically from the African type with respect to their relationship to EBV.

The two North American Burkitt's lymphoma cell lines described here, which to our knowledge are the first to have been established, provide a new and important link in the chain of evidence for the classification of these neoplasms. Fortuitously, these two cell lines proved to be distinctively different. The SU-AmB-1 line, which closely resembles previously reported cases of AmBL, has a high percentage of surface IgM-bearing cells and a low percentage of cells with Fc receptors. It gives a negative EBNA reaction and is highly resistant to superinfection by EBV and nonresponsive to IdUrd activation. In direct contrast, the SU-AmB-2 line expresses EBV-specific antigens and contains Epstein–Barr viral DNA. Like most African Burkitt's lymphoma lines studied, the SU-AmB-2 cells (*1*) do not release infectious or transforming virus, despite the high percentage of VCA-positive cells and the detection of viral nucleocapsids by electron microscopy; (*2*) are inducible by IdUrd, but the induced cycles of viral replication are largely abortive (37, 38); and (3) are highly superinfectible (25, 39, 40). In addition, the tumor cells, though having barely detectable surface immunoglobulins, had Fc and complement receptors indicative of their B cell character (20). The criteria used to establish the malignant nature of the two lines were the same as those discussed in a previous publication (15).

Patient K.P. had low titers of serum antibodies to EBV, of prior primary infection, whereas patient D.S. had high titers (anti-VCA 1:640, anti-D 1:160) consistent with "African-type" Burkitt's lymphoma serology (41). The data of Levine *et al.* (9) reveal a similar duality of serologic patterns in a series of 29 patients with American Burkitt's lymphoma; 14 had anti-VCA titers of ≥1:160, whereas, the remaining 15 had low or negative titers. These findings suggest the need for further studies of nonendemic Burkitt's lymphoma cases to establish the relation between EBV-related serologic patterns and the presence of EBV genomes in tumor biopsies or cell cultures. However, some Burkitt's lymphoma cell lines shown to possess EBV receptors (42) have been devoid of EBV genomes (35). Tumor cells which possess few or no EBV receptors would likely be EBV-negative even in patients with high titer infection. The U-715M line of Klein *et al.* (35), a recent Israeli case[¶], and our SU-AmB-1 cell line appear to be examples of Burkitt's lymphoma with few or no EBV receptors.

In the light of previously available information on Burkitt's lymphoma and the evidence presented here, we suggest the hypothesis that two different classes of undifferentiated B cell lymphomas exist, one of which is positive for the presence of EBV genomes and occurs endemically in Africa and New Guinea and sporadically in other parts of the world, the other of which is EBV-negative and occurs sporadically throughout the world, including the endemic areas. Consistent with this hypothesis is the recent observation of EBV DNA and "African type" serology in one European and two American patients[‖], as well as in the SU-AmB-2 cell line derived from patient D.S. Conversely, the 2–3% of EBV-negative African cases already mentioned, our SU-AmB-1 cell line, and a recent Israeli case from which an EBV-negative

[¶] N. Goldblum, personal communication.

[‖] H. zur Hausen, J. L. Ziegler, and G. Klein, and P. H. Levine, respectively, personal communications. The American studies are now described by M. Gravell, P. H. Levine, R. F. McIntyre, V. J. Land, and J. S. Pagano (1976) *J. Nat. Cancer Inst.*, in press.

cell line was established[¶] appear to be examples of the second category. Whether Burkitt's lymphoma is a single disease entity with two different etiologies, one of which is linked to EBV, or is a mixture of two fundamentally different neoplasms with identical clinicopathologic characteristics may be answered by a systematic analysis of future cases with respect to EBV serology, presence or absence of EBV genomes, cytogenetic abnormalities (43), and lymphocyte surface markers. Resolution of these alternatives may have significant clinical implications for the treatment and prognosis of Burkitt's lymphoma.

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1. Dorfman, R. F. (1965) *Cancer* **18**, 418–430.
2. O'Conor, G., Rappaport, H. & Smith, E. (1965) *Cancer* **18**, 411–417.
3. Cohen, M., Bennett, J., Berard, C., Ziegler, J., Vogel, C., Sheagren, J. & Carbone, P. (1969) *Cancer* **23**, 1259–1272.
4. Levine, P. H. & Cho, B. (1974) *Cancer Res.* **34**, 1219–1221.
5. Dorfman, R. F. (1967) *J. Nat. Cancer Inst.* **38**, 491–504.
6. Binder, R., Jencks, J., Chun, B. & Rath, C. (1975) *Cancer* **36**, 161–168.
7. Ziegler, J. L. & Carbone, P. P. (1966) *Blood* **28**, 982–983.
8. Hirshaut, T., Cohen, M. & Stevens, D. (1973) *Lancet* **ii**, 114–116.
9. Levine, P., O'Conor, G. & Berard, C. (1972) *Cancer* **30**, 610–615.
10. Nonoyama, M., Kawai, Y., Huang, C. H., Pagano, J. S., Hirshaut, Y. & Levine, P. (1974) *Cancer Res.* **34**, 1228–1231.
11. Pagano, J., Huang, C. H. & Levine, P. (1973) *N. Engl. J. Med.* **289**, 1395–1399.
12. Dorfman, R. F. (1968) *Cancer* **21**, 563–574.
13. Berard, C., O'Conor, G., Thomas, L. & Torloni, H. (1969) *Bull. WHO* **40**, 601–607.
14. Thorsby, E. & Bartlie, A. (1970) in *Histocompatibility Testing*, ed. Terasaki, P. (Munksgaard, Copenhagen, Denmark), p. 655.
15. Epstein, A. L. & Kaplan, H. S. (1974) *Cancer* **34**, 1851–1872.
16. Pernis, B., Forni, L. & Amante, L. (1970) *J. Exp. Med.* **132**, 1001–1018.
17. Rabellino, E., Colon, S., Grey, H. M. & Unanue, E. R. (1971) *J. Exp. Med.* **133**, 156–167.
18. Winchester, R., Fu, S., Hoffman, T. & Kunkel, H. (1975) *J. Immunol.* **114**, 1210–1212.
19. Dickler, H. & Kunkel, H. (1972) *J. Exp. Med.* **136**, 191–196.
20. Shevach, F. M., Herberman, R., Frank, M. M. & Green, I. (1972) *J. Clin. Invest.* **51**, 1933–1938.
21. Bentwich, Z., Douglas, S., Siegal, F. & Kunkel, H. (1973) *Clin. Immunol. Immunopathol.* **1**, 511–522.
22. Henle, G. & Henle, W. (1966) *J. Bacteriol.* **91**, 1248–1256.
23. Henle, G., Henle, W., & Klein, G. (1971) *Int. J. Cancer* **8**, 272–282.
24. Reedman, B. & Klein, G. (1973) *Int. J. Cancer* **11**, 499–520.
25. Rocchi, G., Hewetson, J., Henle, W. & Henle, G. (1973) *J. Nat. Cancer Inst.* **50**, 307–314.
26. Miller, G., Robinson, J., Heston, L. & Lipman, M. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4006–4010.
27. Epstein, M. A., Achong, B. G., Barr, Y. M., Zajac, B., Henle, G. & Henle, W. (1966) *J. Nat. Cancer Inst.* **37**, 547–559.
28. Robinson, J. & Miller, G. (1975) *J. Virol.* **15**, 1065–1072.
29. Miller, G., Lisco, H., Kohn, H. & Stitt, D. (1971) *Proc. Soc. Exp. Biol. Med.* **137**, 1459–1465.
30. Lindahl, T., Klein, G., Reedman, B., Johansson, B. & Surjit, S. (1974) *Int. J. Cancer* **13**, 764–772.
31. Reedman, B., Klein, G., Pope, J., Walters, M., Hilgers, J., Singh, S. & Johansson, B. (1974) *Int. J. Cancer* **13**, 755–768.
32. Burkitt, D. P. & Wright, D. H. (1970) *Burkitt's Lymphoma* (E and S Livingstone, London, England).
33. zur Hausen, H. & Schulte-Holthausen, H. (1970) *Nature* **227**, 245–248.
34. Nonoyama, M., Huang, C., Pagano, J., Klein, G. & Singh, S. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3265–3268.
35. Klein, G., Lindahl, T., Jondahl, M., Leibold, W., Menézes, J., Nilsson, K. & Sundström, C. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3283–3286.
36. Clements, G. B., Klein, G. & Povey, S. (1975) *Int. J. Cancer* **16**, 125–133.
37. Gerber, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 83–85.
38. Hampar, B., Derge, J., Martos, L. & Walker J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 78–82.
39. Klein, G. & Dombos, L. (1973) *Int. J. Cancer* **11**, 327–337.
40. Klein, G., Dombos, L. & Gothoskar, B. (1972) *Int. J. Cancer* **10**, 44–57.
41. Henle, G., Henle, W., Clifford, P., Diehl, V., Kafuko, G., Kirya, B., Klein, G., Morrow, R., Murrube, G., Pike, P., Tukei, P. & Ziegler, J. (1969) *J. Nat. Cancer Inst.* **43**, 1147–1157.
42. Jondal, M. & Klein, G. (1973) *J. Exp. Med.* **138**, 1365–1378.
43. Manolov, G. & Manolova, Y. (1972) *Nature* **237**, 33–34.