The Investigation of Epstein–Barr Viral Sequences in 41 Cases of Burkitt's Lymphoma from Egypt

Epidemiologic Correlations

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Background. **Epstein-Barr virus (EBV) is associated with many human neoplasms, including Burkitt's lymphoma (BL). Endemic BL in central Africa is more often EBV-associated than BL in the United States, where seroconversion for EBV occurs somewhat later than in Africa. Therefore, the EBV association rate in BL may correlate more with the socioeconomic status of the population studied, which influences the age of EBV seroconversion, than with such factors as malaria, which may relate to the overall higher incidence rate in endemic regions.**

Methods. **Forty-one patients with BL in Egypt, which differs both climatically and racially from central African countries (i.e., Kenya, Uganda) where BL is endemic, were analyzed. All biopsies were evaluated for EBV-encoded RNAs (EBER1) by RNA in situ hybridization, analyzed for p53 protein expression using the monoclonal antibody D07, and immunophenotyped using a panel of monoclonal antibodies that included L26 (CD20), Leu 22 (CD43), and A6 (CD45RO). Twelve cases were evaluable for EBV subtype by polymerase chain reaction with EBV-specific primers.**

Results. **The median age at diagnosis was 9 years (range, 2–22 years). The biopsy site was extranodal in 29 patients and nodal in 12 patients. All 41 cases were documented as B-cell neoplasms. A hybridization signal for EBER1 RNA was identified in greater than 95% of the neoplastic cells in 30 of 41 cases (73%), whereas no signal was observed in 11 cases (27%). Epstein-Barr virus subtype 1 was found in 10 patients, subtype 2 in two patients. Immunostaining for p53 was observed in greater than 5% of the neoplastic cells in 9 of 37 cases (24%). No significant correlation was observed between EBV positivity and sex, biopsy site, or p53 immunostaining.**

Conclusions. **The prevalence of EBV in BL from Egypt is slightly lower than in BL in endemic regions, but significantly higher than in sporadic BL. Epstein-Barr virus positivity probably reflects the socioeconomic status of the patient population, and age at seroconversion. The prevalence of EBV subtype 1 suggests that immunodeficiency does not play a role in Egyptian Burkitt's lymphoma, in contrast to endemic Burkitt's lymphoma, in which holoendemic malaria is thought to contribute to immunodeficiency, a higher incidence rate, and the observed prevalence of subtype 2. *Cancer* 1995;76:1245–52.**

Key words: Burkitt's lymphoma, Epstein–Barr virus (EBV), EBER1, in situ hybridization, p53, immunosuppression.

Burkitt's lymphoma (BL) is classified in the Working Formulation as a high grade, diffuse, small, noncleaved non-Hodgkin's lymphoma. Burkitt's lymphoma occurs with high frequency in equatorial Africa and Papua, New Guinea, where it is referred to as "endemic" BL. It occurs with much lower frequency (i.e., sporadically) elsewhere in the world. Endemic BL is almost always associated with Epstein–Barr virus (EBV)[1]; EBV DNA is present within the tumor cells of 95% or more of such tumors.[2] Epstein–Barr virus DNA is present in a variable fraction of tumors in other world regions and appears to be least often associated with tumors from the United States and Europe.[3]

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Infection with EBV occurs in very young children in endemic regions and other developing countries, whereas seroconversion occurs predominantly in the second and third decades of life in industrialized nations. It has been hypothesized that early EBV infection in equatorial Africa, coupled with holoendemic malaria (which causes temporary immunosuppression and is mitogenic to B cells), leads to an increase in the number of EBV-infected cell clones and may thus predispose people to the development of EBV-associated BL.[4,5] Burkitt's lymphoma is frequently associated with EBV in some other world regions. For example, some 50% to 60% of tumors from South America are EBV-positive,[3] and there is an even higher fraction of positive cases from Algeria.[6] Whether early EBV infection is sufficient to account for this or whether other environmental factors are relevant is not known. Malaria, however, is unlikely to be a significant factor in these regions because of its low incidence.

Egypt is a north African country that differs markedly from equatorial Africa, both ethnically and environmentally. Malaria, for example, occurs rarely in Egypt. Burkitt's lymphoma accounts for approximately 9.8% of all childhood cancers in Egypt, compared with 50% in equatorial Africa.[7] Clinically, the disease more closely resembles the sporadic variety, with a high frequency of abdominal involvement and infrequent jaw involvement (the hallmark of endemic BL). In view of these differences from endemic BL and the high frequency of EBV association reported from Algeria, we were interested in determining the frequency with which BL in Egypt is associated with EBV. The finding that a high proportion of cases are associated with EBV would support the notion that a factor common to widely disparate geographic regions (such as early EBV infection associated with low socioeconomic status) has an important influence on the likelihood of EBV association. Because we previously have demonstrated that endemic BL also differs from tumors in other world regions with respect to the higher frequency of association with subtype 2 (or B) EBV, we also examined EBV subtype in these cases to determine whether Egyptian BL conforms, in this respect, to the endemic or sporadic pattern.[5]

Finally, in view of our previous observation, also made in several other laboratories, that BL often contains mutated p53 genes, we examined p53 expression by immunohistochemistry in these tumors.[8]

Materials and Methods

Case Selection

Cases of BL, consecutively diagnosed between 1989 and 1993, were retrieved from the files of the Department of Pathology of the National Cancer Institute (Cairo, Egypt). The criteria for inclusion in this study were (1) a histologic diagnosis of malignant lymphoma, diffuse, small, noncleaved Burkitt's subtype; (2) pertinent clinical information, including age, sex, and biopsy site; and (3) availability of diagnostic paraffin tissue blocks for analysis by immunohistochemistry and RNA in situ hybridization. As controls, we included 10 cases of lymphoblastic lymphoma of T-cell phenotype and 8 cases of aggressive non-Hodgkin's lymphoma of B-cell phenotype, other than classic Burkitt's lymphoma.

Immunohistochemistry

Immunophenotypic studies were performed using fixed, paraffin embedded tissue sections and an avidin-biotin immunoperoxidase method previously described.[9] The antibody panel used for each biopsy included L26 (CD20, DAKO, Carpinteria, CA), A6 (CD45RO, Zymed, San Francisco, CA), and Leu22 (CD43, Becton Dickinson, San Jose, CA). Cases of lymphoblastic lymphoma were stained with anti-CD3 (DAKO) to confirm a T-cell phenotype. In addition, the neoplastic cells in each biopsy were evaluated for the p53 antigen using the monoclonal antibody DO7 (DAKO). Biopsies were reported as positive (+) for p53 if greater than 5% of the neoplastic cells showed strong nuclear staining for p53.[10] Biopsies were reported negative (−) if no immunostaining was observed in any of the malignant cells. Biopsies containing p53-positive malignant cells that represented 5% or less of the tumor population were reported as negative and further classified as showing either 2%–5% positive-staining cells (equivocal cases) or 1–5 positive cells per high-power field (occasional p53-positive cells identified).

In Situ Hybridization

The RNA in situ hybridization technique has recently been described in detail.[11,12] Briefly, 5-micron sections of paraffin embedded tissue were prepared on silanated slides. Tissue sections were deparaffinized, rehydrated, permeabilized with a nonionic detergent, and digested with proteinase K (10 μg/ml), then acetylated with triethanolamine and acetic anhydrite to reduce nonspecific electrostatic hybridization. The riboprobe was applied in a formamide buffer, and the slides were hybridized overnight. After stringent posthybridization washes, an antidigoxigenin alkaline phosphatase antibody conjugate was applied to each slide. The slides were then washed and placed into a color developing solution consisting of nitrobluetetrazolium and X-phosphate overnight. The reaction was stopped by briefly washing the slides in an appropriate buffer. The slides

were counterstained with eosin and coverslips were applied.

The integrity of the RNA in each tissue section was evaluated with a digoxigenin-labeled riboprobe (105 base pairs) directed at an abundant cellular RNA polymerase III transcript, U6 (gift from Dr. Richard Ambinder). Sections showing hybridization signal with the U6 probe were determined adequate for analysis with the EBER1 probe. The EBV EBER1 riboprobe was prepared as previously described.[11]

Formalin fixed, paraffin embedded tissue blocks were prepared from cultured RAJI and MOLT4 cell lines, which served as the positive and negative controls, respectively. As with case materials, 5-micron sections were prepared on silanated slides, and a positive and negative control accompanied each in situ hybridization run. Hybridization runs using hybridization buffer containing sense probe, antisense probe, and absence of probe were separately performed to confirm proper transcription of the digoxigenin-labeled riboprobe.

Polymerase Chain Reaction Analysis for EBNA Typing

To determine the EBV subtype in these Burkitt's lymphoma specimens, we used the method described previously.[5] The polymerase chain reaction was performed in 18 evaluable cases using EBNA-3 primers. The primers were common to both type 1 and type 2 EBV strains; however, the priming sites flanked regions of type-specific variation, such that the resulting fragments were of different size. DNA, previously extracted from paraffin embedded specimens, was amplified in a 100 μl reaction mixture containing Taq polymerase (Boehringer Mannheim, Indianapolis, IN). The reaction mixture consisted of a Tris-hydrochloride buffer at pH 8.4, 50 mM potassium chloride, 1.5 mM Mg^{2+}, and 250 μm of each of the deoxynucleoside triphosphates (Pharmacia/LKB; Alameda, CA). Reaction tubes were overlaid with mineral oil and placed in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT). An initial denaturing reaction was performed at 95°C for 5 minutes followed by a 90-second annealing step at 55°C, followed by a 2-minute extension step at 70°C. In the final cycle, the extension step was performed for 5 minutes. Amplified DNA was subjected to electrophoresis on a 4% (3% NuSieve and 1% SeaKem) agarose gel containing ethidium bromide. DNA from lymphoblastic cell lines were used as controls (EBV type 1 positive B95, EBV type 2 positive JLPC119, and EBV negative Molt4).

Results

Clinical Findings

The clinicopathologic findings are summarized in Table 1. All 41 patients were native Egyptians. Biopsy specimens were submitted to the Pathology Department, National Cancer Institute, Cairo, Egypt. There were 24 males and 17 females. The median age at the time of biopsy was 9 years (range, 2–22 years). Sites biopsied were lymph nodal in 12 cases (29%) and extranodal in 29 cases (71%); the latter included breast (1), maxilla (1), soft palate (1), ovary (3), intestine (9), and soft tissue (14). Four of the soft tissue masses occurred in the cheek. Control cases of T-cell lymphoblastic lymphoma and other aggressive B-cell lymphomas are reported in Table 2.

Immunohistochemical Findings

The immunohistochemical findings are summarized in Table 1. The cells in all 41 biopsies were documented as B cells by positive immunostaining for L26 (CD20). The tumor cells in two biopsies coexpressed CD43 (Leu22). Immunostaining for the T-cell–associated marker CD45RO (A6) was not observed in any of the neoplastic cells in any of the 41 biopsies. All 10 cases of lymphoblastic lymphoma were CD3-positive; Leu22 was positive in 7 of 8 cases in which staining was adequate. A B-cell origin was confirmed by L-26 positivity in the 8 control aggressive non-Hodgkin's lymphoma of either large-cell type (3 cases); small noncleaved, non-Burkitt's (SNC-NB) (4 cases), or malignant lymphoma (ML), not otherwise specified (NOS) (1 case).

Immunostaining for p53 was observed in greater than 5% of the neoplastic cells in 9 of 37 cases (24%). Four cases were technically unsatisfactory. The remaining 28 cases (76%) were reported as negative for p53 and were further classified as showing either 2%–5% positive-staining tumor cells (5 cases), 1–5 positive-staining cells per high-power field (2 cases), or complete absence of staining for p53 (21 cases). No correlation was observed between p53 immunostaining and age, biopsy site, or sex. Four of nine (44%) lymphoblastic lymphomas and five of seven aggressive B-cell lymphomas (non-Burkitt's) were p53-positive.

In Situ Hybridization

The EBV in situ hybridization findings are summarized in Table 1. Intact cellular RNA was present in each case as shown by a strong hybridization signal with the U6

Table 1. Clinical, Immunohistochemical, and In Situ Hybridization Data for Egyptian Burkitt's Lymphoma: 41 Patients

Patient no.	Age	Sex	Biopsy site	Diagnosis	Antibody				EBV	Subtype
					L26	*Leu22*	*A6*	*p53*		
7	3	M	ST, retroperitoneal	SNC, B	+	–	–	–	+	1
10	3	F	ST, retroperitoneal	SNC, B	+	–	–	+	+	1
11	3	F	Maxilla	SNC, B	+	–	–	–	+	1
13	4	M	LN, axillary	SNC, B	+	–	–	–	+	
16	4	M	ST, retroperitoneal	SNC, B	+	–	–	–	+	
28	4	F	ST, cheek	SNC, B	+	–	–	unsat	+	1
37	4	F	Ileum	SNC, B	+	–	–	–	+	1
3	5	M	LN, abdominal	SNC, B	+	—	—	—	+	
4	5	F	ST, retroperitoneal	SNC, B	+	–	–	–(1–5 HPF)	+	2
9	5	M	ST, retroperitoneal	SNC, B	+	–	–	–	+	
30	5	M	Ileum	SNC, B	+	–	–	–	+	
1	6	F	Ovary	SNC, B	+	–	–	–	+	
6	6	M	St, cheek	SNC, B	+	–	–	–(2–5%)	+	1
24	7	F	LN, abdominal	SNC, B	+	–	–	–(2–5%)	+	
35	7	M	ST, cheek	SNC, B	+	–	–	–	+	
36	7	F	Ileum	SNC, B	+	–	–	–(1–5 HPF)	+	1
41	7	M	ST, abdominal	SNC, B	+	–	–	unsat	+	
40	8	M	LN, abdominal	SNC, B	+	–	–	+	+	
8	9	M	LN, NOS	SNC, B	+	–	–	–	+	
5	11	F	Ovary	SNC, B	+	–	–	–(2–5%)	+	
38	11	M	ST, pelvis	SNC, B	+	–	–	+	+	
21	12	F	Ovary	SNC, B	+	–	–	unsat	+	
33	12	M	Small intestine, NOS	SNC, B	+	–	–	–	+	1
34	12	M	LN, abdominal	SNC, B	+	+	–	–	+	2
18	13	F	LN, abdominal	SNC, B	+	–	–	–	+	
26	13	M	Colon	SNC, B	+	–	–	–	+	
29	13	F	Ileum	SNC, B	+	–	–	+	+	
17	14	F	LN, NOS	SNC, B	+	–	–	+	+	1
25	14	M	ST, cheek	SNC, B	+	–	–	–	+	
22	22	M	LN, abdominal	SNC, B	+	–	–	–	+	1
39	2	M	ST, retroperitoneal	SNC, B	+	–	–	–(2–5%)	–	
15	4	M	Ileum	SNC, B	+	–	–	+	–	–
31	9	F	Ileum	SNC, B	+	–	–	–	–	–
23	10	M	LN, cervical	SNC, B	+	–	–	unsat	–	–
2	11	M	LN, NOS	SNC, B	+	–	–	–	–	
19	11	F	Ileum	SNC, B	+	–	–	+	–	–
27	12	M	LN, cervical	SNC, B	+	–	–	–	–	–
32	12	F	ST, gluteal	SNC, B	+	+	–	+	–	
20	14	M	ST, retroperitoneal	SNC, B	+	–	–	+	–	
12	19	M	Soft palate	SNC, B	+	–	–	–	–	–
14	21	F	Breast	SNC, B	+	–	–	–(2–5%)	–	

EBV: Epstein–Barr virus; ST: soft tissue; SNC, B: small noncleaved, Burkitt's subtype; +: positive; –: negative; LN: lymph node; NOS; not otherwise subclassified; unsat; unsatisfactory; HPF: high-power field.

riboprobe. A hybridization signal using the EBER1 riboprobe was observed in greater than 95% of the neoplastic cells in 30 out of 41 cases (73%), whereas no signal was observed in 11 cases (27%) (Fig. 1). The mean age of the patients with EBV + BL was 8.3 years, whereas the mean age of the patients with EBV was 11.4 years. Although this difference is interesting, it was not significant statistically ($P = 0.15$, Wilcoxon rank sum test). No significant correlation was observed between EBV-positive cases and age, sex, biopsy site, or p53 immunostaining. All 10 patients with lymphoblastic lymphoma were EBV-negative. Epstein–Barr virus was positive in one SNC-NB but negative in all other aggressive B-cell lymphomas.

Table 2. Clinical, Immunohistochemical, and In Situ Hybridization Data

Type of lymphoma	Patient no.	Age	Sex	Biopsy site	Diagnosis	Antibody				p53	EBV
						L26	*Leu22*	*A6*	*CD3*		
Egyptian T-cell lymphoblastic lymphoma											
	1	48	M	LN, NOS	LL	−	−	−	+	+	−
	2	6	M	LN, inguinal	LL	−	unsat	−	+	+	−
	3	7	F	Small intestine, NOS	LL	−	+	−	+	−	−
	4	49	M	LN, cervical	LL	−	+	−	+	+	−
	5	4	M	LN, cervical	LL	−	+	−	+	−	−
	6	7	M	Pleura	LL	−	+	+	+	unsat	−
	7	7	M	LN, cervical	LL	−	+	−	+	+	−
	8	14	M	LN, cervical	LL	−	+	−	+	−5–10 HPF)	−
	9	40	M	LN, cervical	LL	−	+	−	+	−(<1 HPF)	−
	10	48	M	LN, NOS	LL	−	unsat	unsat	+	−	−
Egyptian aggressive B-cell lymphomas (non-Burkitt's)											
	1	13	F	Nasopharynx	ML, LC	+	unsat	−		unsat	−
	2	12	M	LN, abdominal	ML, LC	+	−	−		+	−
	3	15	M	Rectum	ML, LC, LC, IBL	+	−	−		+	−
	4	8	F	Nasopharynx	ML, NOS	+	−	−		−	−
	5	42	M	LN, axillary	SNC, NB	+	−	−		+	−
	6	12	M	Small intestine, NOS	SNC, NB	+	−	−		+	+
	7	39	M	LN, abdominal	SNC, NB	+	+	−		−(2–5%)	−
	8	23	M	Oral mucosa	SNC, NB	+	−	−		+	−

EBV: Epstein-Barr virus; LN: lymph node; NOS: not otherwise subclassified; LL: lymphoblastic lymphoma; −: negative; +: positive; unsat: unsatisfactory; ML: malignant lymphoma; HPF: high-power field; LC: large cell; IBL: immunoblastic; SNC, NB: small noncleaved non-Burkitt's lymphoma.

EBNA Subtyping

Epstein–Barr virus subtype was successfully identified in 12 biopsies, all of which were positive for EBV by in situ hybridization. Ten were identified as type 1 and 2 as type 2. Six biopsies that were negative by in situ hybridization were also negative by polymerase chain reaction, confirming the absence of EBV sequences.

Discussion

The incidence rate of BL differs markedly in different geographic regions. In equatorial Africa, the rate varies between 5 and 10 per 100,000 children younger than 16 years, whereas in the United States it is closer to 2 per million, a difference of 25-fold to 50-fold.[13] In Egypt, although there is no population-based registry, BL constitutes 4.25% of all cases of non-Hodgkin's lymphomas.[7]

However, in patients younger than 22 years (the age group of the present study), the frequency is distinctly higher (16.9%). Interestingly, BL differs both clinically (distribution of tumor sites) and with respect to its pathobiology (EBV association and the location of the chromosome breakpoint arising from the myc/lg translocations) in different world regions[4,14] (Hamdy et al., unpublished data). Interestingly, the clinical pattern in our patients, as in other North African patients, is more consistent with the sporadic variety of BL. In the present study, 63% of cases presented with an abdominal mass and only 15% with a facial or jaw tumor. Similar findings were observed in other parts of the Middle East.[15,16] A study reported by Daniel[17] revealed that the abdomen is also the most commonly affected site in Ethiopian children. Whether the low frequency of jaw tumors is a consequence of a changing pattern of presentation of BL in Africa, as suggested by Taqi and Yakubu,[18] is not known, although it would appear that lifestyle, environment, and perhaps heredity are the major determinants of the clinical pattern observed, because jaw tumors are more common in endemic regions. In sporadic Burkitt's lymphoma, the majority of patients present with intra-abdominal tumors, frequently arising from lymphoid tissue in the ileocaecal region, or from mesenteric lymph nodes.[19]

The median age at presentation (9 years) was also comparable to that reported in other studies from the Middle East.[15] In endemic regions, the median age at presentation is 7 years, whereas in the United States it is slightly higher, 10 years.[20,21]

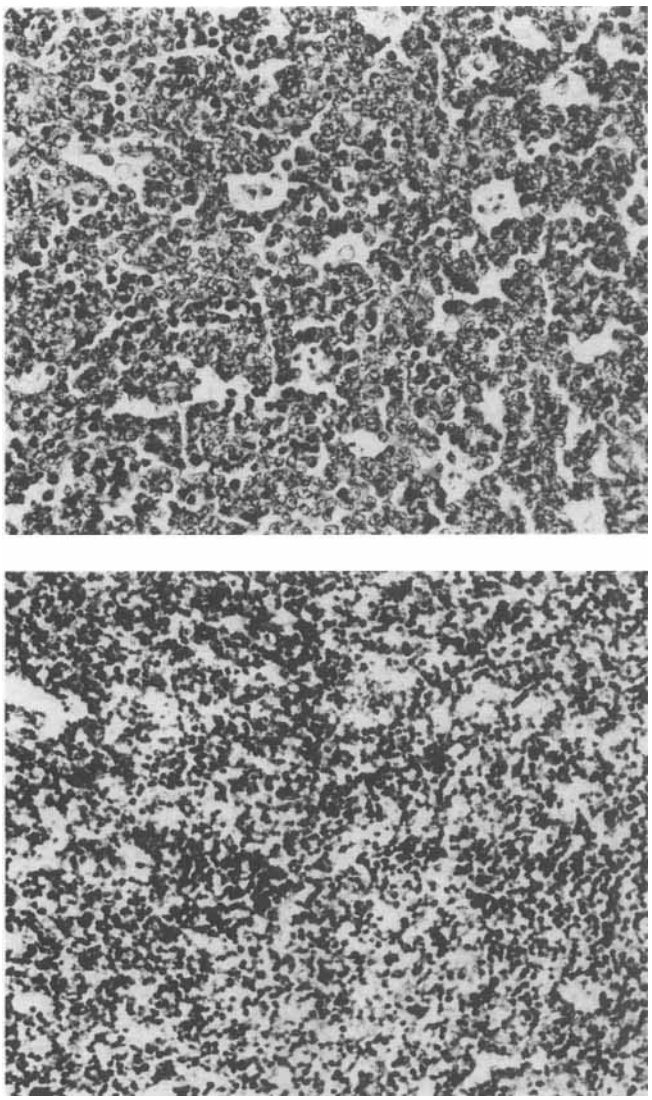

Figure 1. Table 1, Case 29. Top, biopsy of ileum shows a diffuse small noncleaved cell Burkitt's lymphoma with characteristic starry sky pattern. Bottom, the tumor cells are monomorphic and exhibit a high mitotic rate with marked karyorrhexis. Epstein–Barr virus EBER1 RNA is expressed in >95% of the tumor cells (top, H & E, original magnification ×200; bottom, EBER1 RNA in situ hybridization, original magnification ×199).

In the present study, 73% of cases were EBV-associated, which is a much higher frequency than in the United States but a significantly lower frequency than that observed in endemic cases. Selection bias might play a role in contributing to this high frequency, as the Egyptian National Cancer Institute is a government hospital, and affluent patients tend to go to private hospitals. Our data, in conjunction with previous data from Algeria and South America, suggest that EBV association per se is not the determinant of the clinical spectrum.[3,6] Jaw tumors have been infrequent in all of these series, despite a high rate of EBV positivity. Of interest is a study from Turkey in which 14 of 15 patients with BL examined were EBV-positive.[22] A significant fraction of patients from rural areas of Turkey present with African-type jaw tumors (25%) or with orbital tumors (20%). It would appear, as previously surmised, that each geographic region so far studied, consists of a different mixture of Burkitt's lymphoma subtypes—whether defined clinically, by EBV association, or by chromosomal breakpoint location.

We observed only patients with type 2 EBV among the 12 patients examined. This pattern is similar to that observed in sporadic BL and differs from the pattern reported in both endemic BL and human immunodeficiency virus-associated BL, suggesting that immunosuppression in Egyptian patients may not have a role in lymphomagenesis.[23–25] Alternatively, the predominance of EBV strain type 1 observed in these cases may reflect a relative infrequency or absence of strain type 2 in the Egyptian population.

In African patients, malaria is postulated to induce intermittent immunosuppression, which may increase the "body load" of EBV-containing cells by inhibiting EBV-specific responses. In this scenario, the frequency of EBV-associated tumors is increased. In the absence of malaria, the frequency of EBV-positive tumors may simply reflect the proportion of individuals with EBV-containing target cells (i.e., cells in which genetic changes leading to BL can occur) in the relevant age group in the population. An earlier report indicated that African children in whom Burkitt's lymphoma will develop show an elevated titre of viral capsid antibodies 1 to 2 years preceding the emergence of the tumor.[26] Furthermore, Shibata et al.[27] reported that the presence of detectable amounts of EBV DNA in reactive lymph nodes from human immunodeficiency virus-infected patients with generalized lymphadenopathy was associated with an increased incidence of EBV-associated lymphoma at another site or time. It is entirely possible that EBV infection before the age of 3 may increase the risk of developing BL in all populations, and that other factors that influence the proportion of EBV-infected cells in an individual also alter the likelihood of the development of EBV-associated lymphomas. Malaria may be the most important of these factors, but perhaps there are others in developing countries, such as Egypt and Turkey, in which there is not a high incidence of malaria.

The role of EBV in the induction of BL is still unresolved. Nor is it known whether the deregulation of c-myc, which is a consequence of a myc/lg chromosomal translocation and which clearly has a central role in pathogenesis, precedes or follows EBV-infection of a cell.[28] On balance, it seems more likely that the genetic

changes, occurring in a single EBV-infected cell, result in monoclonal malignant proliferation.[4] In sporadic cases of BL, EBV is often absent from the tumor, indicating that genetic changes alone are capable of inducing the neoplasm. However, it is probable that EBV acts as a cofactor, increasing B-cell proliferation and increasing the likelihood that the necessary genetic lesion will occur. In addition, in EBV-positive BL, EBNA-1 may increase c-myc expression.[4] Thus, EBV-positive clones may be selected for during lymphomagenesis.

In the present series, we found no correlation between the expression of p53 and EBV association. Moreover, the fraction of tumors in which p53 expression was detectable was similar to the fraction, reported in previous series, in which p53 mutations were observed by single strand conformation polymorphism analysis and nucleotide sequencing.[8] This suggests that p53 expression correlates, in Burkitt's lymphoma, with the presence of p53 mutations. Immunohistochemistry, however, has the added advantage that the fraction of cells expressing p53 can be assessed. Our observation that many tumors express high levels of p53 in only a fraction of tumor cells implies that p53 mutation is associated with progression rather than with primary tumor pathogenesis. Although it is not possible to exclude rigorously the possibility that all cells in any given tumor contain a mutation but not all overexpress p53, this is very unlikely. This conclusion is supported by recent findings that p53 mutation is associated with both in vitro evidence of resistance to radiation and chemotherapy and clinically, with a poor prognosis.[29,30]

We conclude that EBV is frequently associated with Egyptian BL, as is the case in other developing countries, and hypothesize that this is a consequence of infection with EBV before the age of 3 in these countries. The low frequency of EBV type 2 in this series suggests that immunosuppression does not have a role in the genesis of BL in Egypt, whereas our observation of a heterogeneous pattern of expression of p53 in tumor cells is consistent with the idea that p53 mutations are associated with tumor progression in BL.

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