Elevated Immunofluorescence Antibody Titers to Several Herpesviruses in Burkitt's Lymphoma Patients: Are High Titers Unique? [1,2]

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SUMMARY—Antibody titers for viral capsid antigens of all four human herpesviruses were measured by immunofluorescence in the sera of 16 Burkitt's lymphoma (BL) patients, 16 age- , sex- , and locality-matched controls, and 136 family members from the West Nile District of Uganda. Among family members, titers greater than 1:4 were found in 98% for herpes simplex virus (HSV), 86% for varicella-zoster virus (VZV), 100% for cytomegalovirus (CMV), and 94% for Epstein-Barr virus (EBV). Titers in patients averaged ≈2 logs (fourfold) higher than those in matched controls for EBV, VZV, and CMV (P=0.001); titers for HSV were only slightly higher in cancer patients. The mothers of patients had somewhat higher EBV titers ($0.05 \leq P \leq 0.01$) than the mothers of controls, but no other differences between patient and control families were found. By immunofluorescence, a method which apparently has not been used for all four human herpesviruses in BL patients, the patients had elevated antibody titers not only to EBV but also to CMV and VZV. The elevated titers to three of the four human herpesviruses were not due to serologic cross reactions.—J Natl Cancer Inst 54: 49-51, 1975.

HIGH TITERS OF ANTIBODY to Epstein-Barr virus (EBV) were found by the fluorescent antibody (FA) test in a high percentage of Burkitt's lymphoma (BL) patients (*1*, *2*), and EBV is involved in most etiologic hypotheses about BL (*2–5*). Few serologic studies of other herpesviruses were reported in BL patients (*6*, *7*), and apparently none measured antibodies to all four viruses by the indirect immunofluorescent method (*1*, *8*).

In a recent field study in West Nile, Uganda (*9*), the clinical histories of many BL patients suggested that herpes simplex infection had occurred shortly before onset of the tumor. FA titers for herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), and EBV were therefore measured in sera from the patients with BL and from controls and their families. Not only EBV but also CMV and VZV titers were higher in patients than in controls.

MATERIALS AND METHODS

Sera were collected in West Nile from 16 BL patients, from 16 controls of the same age and sex, and from 136 other individuals in patient and control families. Controls were selected by random numbers from tax lists in the same subcounty as the patients (*9*). Blood specimens were allowed to clot before overnight storage at 4° C. The sera were stored at −20° C and then placed in liquid nitrogen for shipment and further storage.

Antibody to EBV was tested at the IARC with standard methods for the viral capsid antigen (VCA) test (*1*, *8*) and fourfold serum dilutions beginning at 1:10. Antibody against the other three viruses was measured at The Netherlands Cancer Institute by a similar indirect immunofluorescence method (*10*) with a 1:40 dilution of fluorescein-conjugated swine antihuman immunoglobulin obtained from Nordic, Tilburg, The Netherlands (in preparation). The antigens were as follows: HSV type-2 obtained from a vaginal specimen and grown in RK13 cells; VZV (isolate 69-115) from a zoster patient, grown in diploid human embryonic lung fibroblasts; and a CMV isolate, "Reints," grown in the same strain of cells. The VZV and CMV strains were provided by Dr. K. W. Slaterus, Wilhemina Gasthuis, Amsterdam, The Netherlands, who identified them by their cytopathology and reactions with known antisera in complement-fixation tests. Infected cells were mixed with an equal number of uninfected cells before being applied to the microscope slides, dried overnight, and then fixed in acetone for 10 minutes before storage at −20° C. Sera were diluted in fourfold steps beginning with 1:4. HSV type-1 and -2 gave similar results in this test, and a single antigen (type-2) was therefore used for convenience. Patient and control sera identified only by number were tested together; the clinical histories were unknown to those performing the test.

RESULTS

All patients and controls had titers of 1:16 or more to HSV and CMV and 1:10 or more to EBV (1 of the 16 patients was not tested for EBV). Two patients and five controls had no titer (<4) against VZV; the others had titers of 1:4 or above. Table 1 indicates the geometric mean titers (GMT) of all groups tested. Titers were ≈4 times (2 logs) higher in patients than in controls for VZV, CMV, and EBV (table 2). The differences were statistically significant at the $P \cong 0.001$ level for all three by the Student's t-test. HSV titers were slightly higher in patients than in controls. The mothers of patients had higher titers

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TABLE 1.—*GMT of matched BL patients and controls with their families for four human herpesviruses*

Test group	HSV	VZV	CMV	EBV, VCA
BL patients	224. 8(16)[a]	32(16)	378. 1(16)	249. 1(18)
Controls	167. 6(18)	9. 7(18)	133(18)	55. 5(18)
Patient families	141(93)	17. 2(93)	191. 4(93)	65. 3(94)
Control families	147(90)	14. 1(90)	206. 3(90)	54. 8(88)

[a] Number of sera tested *in parentheses*.

TABLE 2.—*Differences in FA titer between matched BL patients and control individuals for four human herpesviruses*

Subjects	HSV	VZV	CMV	EBV
BL patients+controls	0.38[a] (16)[b]	2.00(16) ($P \cong 0.001$)	1.75(16) ($P \cong 0.001$)	1.87(15) ($P \cong 0.001$)
Siblings	−.45(38)	0.13(38)	−0.61(38)	−0.12(39)
Mothers	.75(12)	.83(12)	.50(12)	1.35(10)
Fathers	.33(12)	1.08(12)	−.58(12)	−0.09(11)

[a] Average $= (\log \frac{\text{case titer}}{\text{control titer}})$. This is equivalent to the average number of "tubes" difference in titer for twofold dilutions.

[b] Numbers of pairs tested *in parentheses*.

for EBV than the mothers of controls. In case and control families, statistically significant titer differences were found only between cases and their controls, not between their mothers, fathers, and siblings, and the differences were almost equal in magnitude for VZV, CMV, and EBV.

DISCUSSION

Numerous studies have been done on EBV titers of groups of patients and controls with the FA method, and elevated EBV titers have been associated with BL (*1*, *2*), nasopharyngeal carcinoma (*11*), infectious mononucleosis (*12–14*), Hodgkin's disease (*15*, *16*), lupus erythematosus (*17*), and Izumi fever (*18*), though only in the first three does the association seem solidly established. A few investigations have reported antibody levels for other herpesviruses in the same sera but have used complement fixation rather than FA for this work (*7*, *16*). One report mentions elevated complement-fixation titers for VZV in Hodgkin's disease (*16*). The present study is the first report of FA titers for all four human herpesviruses in a group of BL patients and controls. The elevation of CMV and VZV titers and those for EBV suggests that testing of other serum collections with the FA technique might produce valuable information and shed some light on the meaning of a "high" titer to a herpesvirus—the central unanswered question which underlies EBV serology.

Our results might be due to chance, to variability of the FA test, or to factors peculiar to the ecology of West Nile. FA tests in the EBV field have shown that variability up to two or even three dilutions can be obtained from one testing to another, depending, among other factors, on the antigen batch (type of cell line and proportion of immunofluorescent cells) (*19*). When CMV, VZV, and HSV antibodies were tested, similar variability was observed. However, the titers were obtained in tests in which coded patient and control sera were interdigitated and the sera were from a small but carefully matched series of cases and controls. The elevation of FA titers for VZV, CMV, and EBV can be looked for in larger groups of BL patients and controls and by other laboratories.

Cross-reactions among the human herpesviruses in the FA test could produce high titers to several viruses in the same serum. Other workers have not found such cross-reactions (*8*) except for a partial serologic relationship between HSV and VZV (*20*), though a macroglobulin reacting with CMV was detected in the serum of some patients with other herpesvirus infections (*21*). Our results for the 68 children, 15 years and under, in control families tested the cross-reaction hypothesis. The titer for each virus was plotted against the titer of one of the other three viruses in the same serum. The results for each possible pair of viruses showed a complete scatter; there was no evidence of relationship between a high titer for one virus and high or low titers for any of the others. For each possible pair of viruses, sera could be found with high titers for one virus (HSV-256, VZV-128, EBV-160, CMV-512) which had low titers for the other (HSV and VZV-4, EBV-10, CMV-32). Finally, 20 sera from patients with nasopharyngeal carcinoma and 20 controls, which were tested by our laboratories together with the present BL series with the same antigens, showed highly elevated titers for EBV but not for the other human herpesviruses. These findings have been extended and will be reported separately (in preparation). Serologic cross-reaction does not seem a reasonable explanation of the findings.

Dean et al. (*9*) previously suggested, from a clinical-epidemiologic survey, that lesions resembling herpes simplex infection occur with unusual frequency just before the development of BL tumor. Since these

herpetic-type lesions have healed by the time the patients came to the hospital, it was impossible to attempt isolation of HSV from these lesions; it was hoped that serology would yield clues about the cause of the oral ulcers and unilateral conjunctivitis described by the patients (*9*). However, since all the patients had HSV titers of 1:64 or higher and all the controls had titers of 1:16 or more, HSV infection can neither be ruled in nor out. Patients with a history of herpes-like lesions did not have higher HSV titers than did controls or the other patients. Douglas and Couch (*22*) showed, however, that patients with recurrent herpes infections do not necessarily have high neutralizing antibody titers or rises in titer with recurrences, and there is no reason to believe that FA titers would be different in this regard. Hence the failure to find "elevated" HSV titers in our BL patients does not prove or disprove the clinical hypothesis of an association between BL and HSV. The hypothesis that the tumors are caused by an interaction between EBV and another herpesvirus would be difficult to test in a population in which nearly everyone is infected by several herpesviruses at an early age.

The association between BL and EBV is not only supported by seroepidemiologic data, but also by the regular presence in African BL tumor cells of EBV fingerprints, i.e., EBV genomes (*23*, *24*) and EBV-specific nuclear antigen (*25*). The presently reported FA titers for other herpesviruses should be completed by other tests directed against early antigen, membrane antigen, and soluble antigen of CMV, VZV, HSV-1, and HSV-2. BL biopsy specimens should be examined for virus genomes other than EBV. The on going prospective seroepidemiologic study of BL in the West Nile district of Uganda (*26*) would provide the proper material from BL cases and controls to perform such seroepidemiolgic and molecular virologic studies.

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