A Longitudinal Comparison of Antibodies to Epstein-Barr Virus and Clinical Parameters in Chronic Lymphocytic Leukemia and Chronic Myelocytic Leukemia

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Elevated titers to the Epstein-Barr virus have previously been reported in a number of lymphoproliferative diseases, including Burkitt's lymphoma, infectious mononucleosis, and Hodgkin's disease. This study also demonstrates a significantly higher titer against EBV in a group of patients with chronic lymphoproliferative disease (CLL) than in a group of patients with chronic myeloproliferative disease (CML) or normal individuals. No significant antibody changes were detected in the 20 CML patients or 23 of the 24 CLL patients who were followed for a period of time up to 5 yr. It appears that the elevated EBV titers seen in patients with CLL reflect an event or process occurring prior to the onset of disease or in the very early stages rather than a nonspecific rise paralleling the increase in total-body lymphocytes.

RECENT STUDIES have demonstrated elevated antibody titers to a herpes-type virus (Epstein-Barr virus or EBV) in several lymphoproliferative diseases.[1-5] Although similar herpes viruses have been etiologically related to lymphomas in chickens,[6,7] monkeys,[8] and rabbits,[9] the evidence that EBV is responsible for any human lymphomas remains circumstantial. The factors determining EBV antibody levels in humans are largely unknown, and since EBV replicates more readily in lymphoblastoid cell lines than other tissues, antibody levels might be a reflection of the mass of lymphoid tissue in the patients.

One disease that allows an opportunity to compare accumulating lymphoid mass with EBV titers is chronic lymphocytic leukemia (CLL), where the peripheral white blood count (WBC) reflects the progression of the disease process[10] and where treatment is frequently withheld for years until the patient becomes symptomatic. The purpose of this study was to follow patients

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Submitted March 22, 1971; revised May 18, 1971; accepted May 19, 1971.

Supported in part by Contract PH 43-65-639 with the Special Virus Cancer Program.

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Table 1.—EBV Titers by Diagnosis*

Diagnosis	Number of Patients	Mean Age	EBV Titer			GMT
			< 10	10–640	> 640	
CLL	34	63.5	1	23	10	1:444 †
CML	20	49.4	1	19	0	1:84
N1 ‡	24	64.0	2	22	0	1:87
N1 §	61	27.2	4	53	4	1:93

* Initial samples only.
† Statistically higher than all control groups ($p < 0.001$).
‡ Simultaneously tested age-matched controls.
§ Previously tested normal individuals.

with CLL and determine whether progression of disease was associated with changes in levels of antibody to EBV. A group of patients with chronic myelocytic leukemia (CML) were studied simultaneously to compare the results obtained in a chronic lymphoproliferative disorder and a chronic myeloproliferative disorder.

Materials and Methods

Ninety-one samples of serum were obtained from 34 patients with CLL who were followed in the hematology clinic of the University of Colorado Medical Center and the Denver Veterans Administration Hospital between 1964 and 1969. All patients accepted into the study had a progressive lymphocytosis of at least a year's duration. Those presenting initially with massive lymphadenopathy or splenomegaly were excluded from the study in order to eliminate lymphosarcoma patients with a late leukemic phase. Since immunoglobulin abnormalities were expected in the CLL group, immunoglobulin levels were determined on each sample titered for EBV antibody.

Serum from 20 CML patients were provided by Dr. C. J. Zarafonetis, Chief of the Hematology Division, University of Michigan Medical Center. The CML samples were selected from the serum bank from patients who had been followed at the University of Michigan between 1960 and 1969 and included five patients who entered a blastic phase of their disease during the course of study.

Twenty-four samples, matched for age and sex with 24 CLL patients, were provided from clinically normal individuals by Mr. Charles Peters from the serum bank of the National Communicable Disease Center.

All sera used in this study were coded and tested blindly for EBV antibody by indirect immunofluorescence.[11] The cell line used as the source of viral antigen was the HR1K clone of P3J. The conjugate used was goat-antihuman IgG prepared by Hyland Laboratories. Immunoglobulin levels were measured in the CLL patient by a modification of the micro-Oudin tube method of single diffusion in agar.[12]

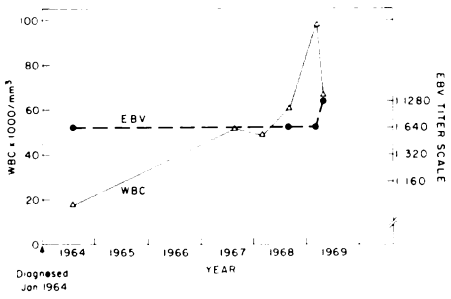

Fig. 1—Serial EBV titers and white blood counts are shown for one untreated patient with CLL (A.C.). In the 5 yr she was followed subsequent to diagnosis 1964–1969, EBV titers remained stable although the white blood count was rising progressively.

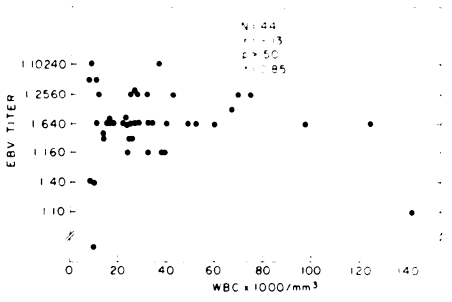

Fig. 2—The relationship between EBV titer and white blood count is shown for each blood sample collected from 18 CLL patients followed serially prior to treatment. There is no correlation between EBV titer and WBC ($r = -0.13$), indicating that titer is not directly related to lymphoid mass.

Results

A comparison of EBV titers in the leukemic patients and normal individuals showed that the geometric mean titer (GMT) of the CLL patients (1:444) was significantly higher than the GMT of the CML patients (1:84) and the matched normal controls (1.87) ($p<0.001$). There was no difference between either of the control groups in this study and the GMT of normal individuals (1:93) who had been studied earlier in this laboratory (Table 1).[5,13]

Serial samples were available from 24 of the CLL patients and all 20 CML

Table 2.—EBV Titer Ranges for 24 CLL Patients With Serial Serum Samples

Identification	Age	Sex	Date of Diagnosis	Date of First Serum Sample	Date of Last Serum Sample	Interval in Months	Range of EBV Titers*
G.B.†	70	F	4/67	4/67	8/67	4	2560(3)‡
A.C.	68	F	1/64	7/64	3/69	55	640–1280 (6)
H.C.†	69	M	1961	11/64	3/68	40	20–40 (6)
E.C.	84	M	2/65	3/65	10/67	31	2560–10, 240 (3)
E.G.†	72	F	4/62	1/64	5/68	52	80–2560 (4)§
M.H.†	79	F	1950	1/64	11/65	22	640–5120 (3)
C.H.	61	M	6/66	9/66	2/68	17	320–640 (2)
E.Ha.†	64	M	12/57	3/66	3/69	36	160 (3)
E.He.†	61	M	12/57	10/64	8/65	10	160 (3)
J.J.	79	M	1962	2/68	2/68	12	320–640 (3)
N.K.†	56	F	1949	3/65	2/66	11	40–160 (2)
J.L.	58	M	7/64	8/64	1/69	53	160 (3)
F.L.	59	F	1959	10/64	10/68	48	640–2560 (3)
M.M.†	43	M	3/56	6/65	1/66	7	320 (2)
E.M.	56	M	11/63	1/64	6/64	5	320 (2)
L.S.	48	M	1962	12/65	4/68	28	640 (5)
W.S.†	72	M	1/65	10/66	3/68	17	640–2560 (3)
R.S.	71	M	3/56	5/65	1/69	44	2560–10, 240 (5)
V.S.	52	M	6/64	8/67	1/69	17	640 (4)
A.O.†	61	M	12/66	8/66	1/69	29	640–2560 (6)
C.T.	70	M	6/64	3/66	3/68	24	40–640 (2)
J.B.†	61	F	7/67	1/68	12/68	11	10,240 (3)
K.M.†	52	F	5/67	12/67	4/68	5	640–1280 (2)
H.C.†	69	F	1953	7/66	8/68	25	1280–5120 (3)

* Differences of a fourfold dilution are within the limits of test reproducibility.
† On chemotherapy during study.
‡ Number of serum samples tested are in parentheses.
§ See Fig. 3.

Table 3.—EBV Titers by WBC in CLL

WBC	Number of Samples	EBV Titer			GMT°
		< 10	10–640	> 640	
Initial samples only					
0–40,000	21	1	16	4	1:334
41,000–100,000	7	0	3	4	1:936
> 100,000	6	0	4	2	1:501
All samples					
0–40,000	58	1	41	16	1:467
41,000–100,000	16	0	9	7	1:891
> 100,000	17	0	11	6	1:657

° No differences are statistically significant at $p < 0.05$.

patients in this study. The titers appeared to be consistent in almost all cases over the entire period of follow-up, which continued up to 4½ years (Table 2, Fig. 1). There was no correlation between WBC and EBV titer in the CLL or CML patients (Tables 3 and 4, Fig. 2). Chemotherapy did not appear to affect the EBV titers in any of the patients (Table 2).

Although a decline in immunoglobulin levels occurred as the disease progressed in several of our patients, in only one patient with severe IgG depression late in the course of disease did there appear to be a definite decline in EBV titer (Fig. 3).

Discussion

The association of EBV with human lymphomas is an intriguing observation requiring further study. This virus, first isolated by Epstein et al. in 1964,[14] was detected in a tissue culture preparation derived from the lymph node of a patient with Burkitt's lymphoma. Subsequent virological and immunological studies have shown that the virus is ubiquitous, however, and can be isolated from the tissues derived from healthy normal individuals as well as from patients with a variety of diseases.[3]

Antibodies to the virus can be detected in more than 90% of healthy adults,[15,16] and the significance of high antibody levels in Burkitt's lymphoma,[1]

Table 4.—EBV Titers by WBC in CML

WBC	Number of Samples	EBV Titer			GMT°
		< 10	10–640	> 640	
Initial samples only					
0–40,000	11	1	10	0	1:64
41,000–100,000	4	0	4	0	1:94
> 100,000	5	0	5	0	1:138
All samples					
0–40,000	68	4	64	0	1:88
41,000–100,000	12	0	12	0	1:106
> 100,000	9	0	9	0	1:100

° No differences are statistically significant at $p < 0.05$.

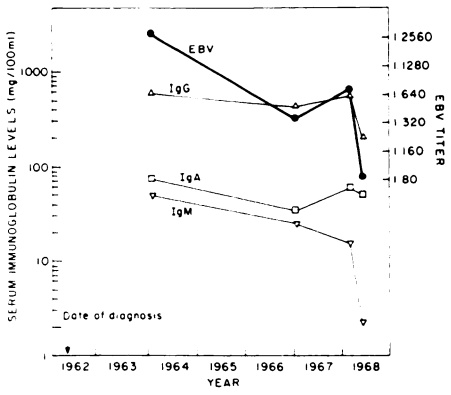

Fig. 3.—A comparison of EBV titers and immunoglobulin levels are shown for patient EG. (1964–1968) with advanced chronic lymphocytic leukemia. Shortly prior to her death in June 1968, immunoglobulin levels and EBV titer declined significantly.

postnasal carcinoma,[3] Hodgkin's disease,[5] and sarcoidosis[17] is uncertain. The possibility that titers to EBV are related nonspecifically to the proliferation of lymphoid cells led to the current study.

Since the peripheral blood generally reflects the body burden of leukemic cells in the CLL patient, this study used the WBC as an index of the stage of the disease. The data indicate that EBV antibody levels are higher in patients with CLL, a chronic lymphoproliferative disorder, than CML, a chronic myeloproliferative disorder. There was no correlation, however, between total white blood count or absolute lymphocyte count and EBV levels in our CLL patients (Fig. 1). Furthermore, antibody levels remained constant despite chemotherapy and were generally unaffected by the immunoglobulin changes found in the CLL patients. It appears, therefore that the elevated EBV titers seen in patients with CLL reflect an event or process occurring prior to the onset of disease or in the very early stages rather than a nonspecific rise paralleling the increase in total-body lymphocytes.

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