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EBV in Situ Hybridization Study for Non-Hodgkin's Lymphomas

Young-Hyeh Ko, M.D., and Jung-Dal Lee, M.D.

Department of Pathology,
Hanyang University College of Medicine, Seoul, Korea

Epstein-Barr virus(EBV) has been implicated in the pathogenesis of B-lymphoproliferative disorders, T-cell lymphomas and Hodgkin's disease. In this report, we performed an in situ hybridization study on EBV genome in 10 cases of nasal non-Hodgkin's lymphoma(NHL), 20 cases of Waldeyer's ring(WR) NHL, and 20 cases of nodal NHLs to document EBV association with lymphomas in Koreans. For immunophenotyping, monoclonal antibodies for CD 20, MB 2, CD 45Ro & CD 43 were used. For in situ hybridization study, EBV DNA probe for Bam HI 'V' fragment and EBV RNA probe for EBER and BHLF were used.

Twenty two cases(44%) of malignant lymphomas were positive for EBV genome. Generally, T-cell lymphomas showed a higher positive rate(61%) than B-cell lymphomas(24%). Among T-cell lymphomas, nasal lymphomas showed a higher positive rate(80%) than WR(50%) or nodal lymphomas(50%).

Of 22 EBV genome positive cases, 10 cases were positive for EBER, 10 cases for BHLF, and 2 cases for both EBER and BHLF. The histologic types by Working Formulation(WF) were not correlated with EBV genome positive rate, whereas lymphomas showing the histologic spectrum of polymorphic reticulosis(PR) showed a higher positive rate(65%) than lymphomas without PR-like features(40%). These results indicate that nasal T-cell lymphomas with the histologic spectrum of PR are strongly associated with EBV and that the anatomic site may be an important factor in this association.

Key Words : *EBV, Non-Hodgkin's lymphoma, Polymorphic reticulosis, In situ hybridization.*

INTRODUCTION

The pathogenesis of malignant lymphomas is heterogeneous, some involving oncogenic virus, dominantly acting oncogenes or tumor suppressor gene. Epstein-Barr virus is a representative oncogenic virus related to malignant lymphoma and has a particular tropism for epithelium of the pharynx, and salivary gland duct cells. The virus infects through the oropharynx, invading B lymphocytes that have CD 21 receptors(Young et al., 1986). Although this virus has been implicated in the pathogenesis of B-lymphoproliferative disorders in immuno-deficiency, recent studies suggest EBV is associated with a wide spectrum of lymphoma

Address for correspondence : *Young-Hyeh Ko, M.D., Department of Pathology. Hanyang University Medical College, 17, Haengdang-dong, Sungdong-gu, Seoul, 133-791, Korea. Tel : (02) 293-2111(Ext : 2640), Fax : (02) 296-7502.*

including T-cell lymphomas and Hodgkin's disease (Jones et al., 1988 ; Anagnostopoulos et al., 1989).

The aims of this study are two fold : one is to document the association of EBV with malignant lymphoma in Korea and the other is to document the relation of EBV to anatomic site, cell-lineage or histologic subtype.

MATERIALS AND METHODS

Paraffin blocks from 50 cases of malignant lymphomas were used, which included 10 cases arising in the nasal cavity, 20 cases in the waldeyer's ring(WR), and 20 cases in the lymph nodes. All the cases were reviewed and classified by Working Formulation(WF). The nasal and WR T-cell lymphomas were reclassified into polymorphic reticulosis(PR), PR-like lymphoma, and pleomorphic T-cell lymphoma according to Ho et al.(1990)(Fig. 1, 2, & 3). For immunophenotyping, all cases were stained for CD 20(Dakopatts, Denmark), MB 2(Biotest, USA), CD 45RO(Dakopatts, Denmark), CD 43(Biotest, USA) and CD 68(Dakopatts, Denmark) by a standard streptavidin-alkaline phosphatase method.

In situ hybridization studies to detect EBV genome used biotinylated EBV DNA, Bam HI "V" fragment(Enzo, USA), FITC-labelled EBER oligonucleotide(Dakopatts, Denmark), and FITC-labelled BHLF oligonucleotide as probes.

The methodology for EBV DNA in situ hybridization is as follows ; After deparaffinization, sections were digested for 15 minutes with proteinase K(Sigma, USA)and then washed and air dried. The EBV DNA probe diluted in hybridization mixture(Amresco, USA) was applied to each slide. The covered slides were placed onto the 95 heating block for 5

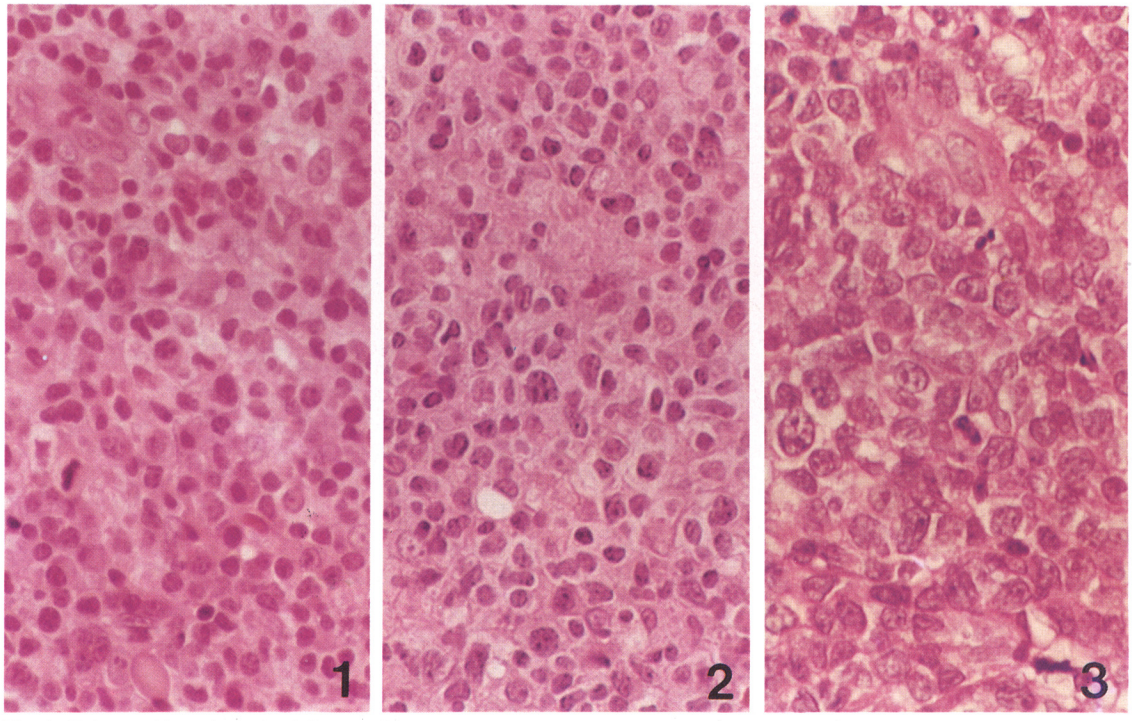


Fig. 1. Polymorphic reticulosis. A few medium sized atypical lymphocytes and numerous small lymphocytes are admixed with many plasma cells.

Fig. 2. Polymorphic reticulosis-like malignant lymphoma. In comparison to polymorphic reticulosis, the number of neoplastic cells with obvious cytologic atypism is markedly increased. A few plasma cells are present.

Fig. 3. Pleomorphic T-cell lymphoma. Relatively monotonous neoplastic cells show nuclear irregularity similar to those of neoplastic cells in polymorphic reticulosis.

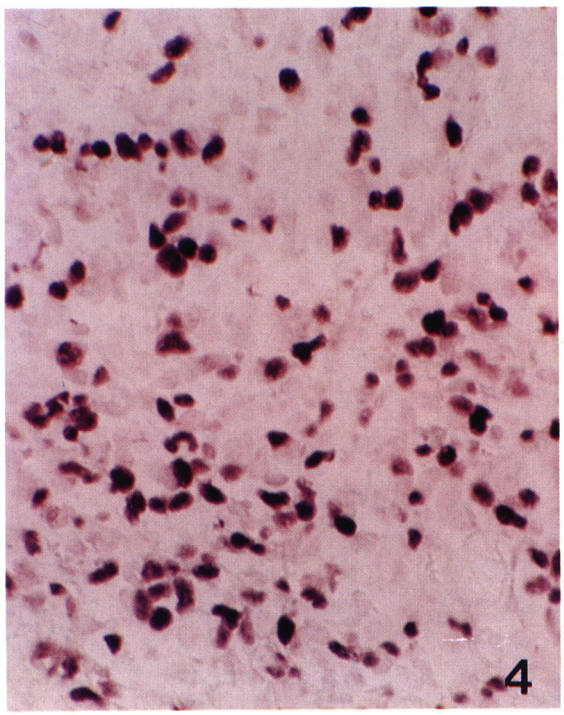
minutes and then incubated at 37℃ for 90 minutes. After removal of the cover slip the slides were incubated in post hybridization buffer and then washed. Detection of the hybridization signal was performed with an in situ hybridization detection kit(Dakopatts, Denmark).

The EBV RNA in situ hybridization studies using FITC-labelled EBER and BHLF oligonucleotide were performed as directed in the specification sheet provided by Dakopatts.

RESULTS

Distribution of EBV genome according to the location and immunophenotype(Table 1)

EBER and BHLF signals were mostly moderate to strong on neoplastic lymphocytes(Fig. 4 & Fig. 5). Because the signal of EBV DNA was weaker than the EBER and BHLF signal and because it was often difficult to interpret the results, most of the data was



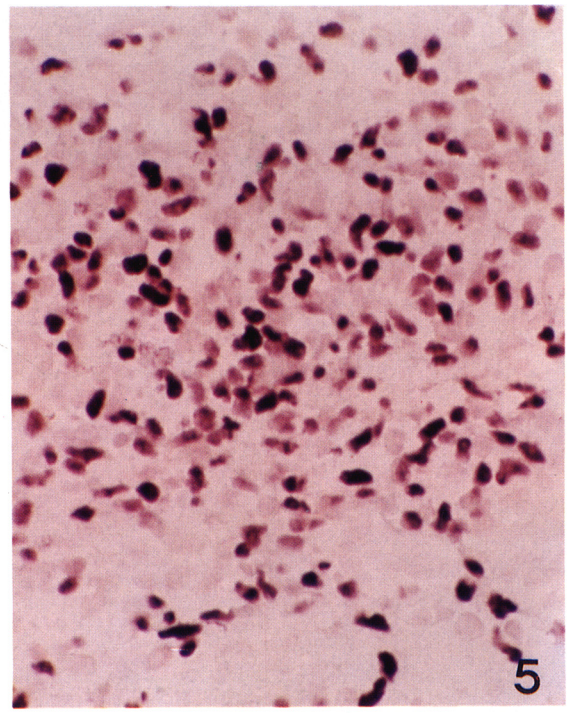


Fig. 4. Strong nuclear staining for EBER probe in the nasal T-cell lymphoma.
Fig. 5. Strong nuclear staining for BHLF probe in the nasal T-cell lymphoma.

Table 1. Distribution of EBV Genome according to Location and Immunophenotype

Pattern	Nasal cavity	WR		LN		Total
	T	T	B	T	B	
DNA+EBER+BHLF−	6	0	1	2	1	10
DNA+EBER−BHLF+	1	3	0	3	2	9
DNA+EBER+BHLF+	1	0	0	0	1	2
DNA−EBER−BHLF+	0	1	0	0	0	1
DNA−EBER−BHLF−	1	2	7	3	6	19
DNA+EBER−BHLF−	1	2	4	2	0	9
Total	10	8	12	10	10	50

WR : Waldeyer's ring, LN : Lymph node

Table 2. EBV RNA Positivity in T-Cell Lymphomas arising in the Nasal Cavity & WR according to Histologic Spectrum of PR

Histologic type	Nasal cavity		WR		Total
	+	−	+	−	
PR	1	1	0	0	2
PR-like lymphoma	4	0	3	2	9
Pleomorphic lymphoma	3	1	0	2	6
Conventional lymphoma	0	0	1	0	1
Total	8	2	4	4	18

WR : Waldeyer's ring, PR : Polymorphic reticulosis

Table 3. EBV RNA Positivity related to Histologic Type by Modified WF

Histologic type	Nasal cavity		WR		LN		Total
	+	−	+	−	+	−	
SL	0	0	0	0	0	1	1
FL	0	0	0	1	0	0	1
Intermediate	0	0	0	0	0	1	1
Mixed	0	0	0	3	3	3	9
PR lymphoma	5	1	3	2	0	0	11
AILD-like	0	0	0	0	0	2	2
Large	0	0	2	6	5	1	14
PR lymphoma	3	1	0	2	0	0	6
IBL	0	0	0	1	1	1	3
Burkitt	0	0	0	0	0	2	2
Total	8	2	5	15	9	11	50

WF : Working Formulation of NCI, SL : Small lymphocytic, FL : Follicular lymphoma
PR : Polymorphic reticulosis, IBL : Immunoblastic lymphoma

analysed based on the EBER and BHLF findings.

Among 50 cases of NHL, 22 cases(44%) were positive for either EBER or BHLF. EBV RNA positivity was markedly different between T and B cell NHL, being positive in 17 of 28 T-NHL(61%) and in 5 of 22 B-NHL(24%). The T cell nasal-NHL showed a higher positive rate(80%) than Waldeyer's ring(50%) or nodal T-NHL(50%). Among 22 EBV RNA positive cases, 10 cases were positive for EBER which is actively transcribed in latent infection, 10 cases for BHLF being transcribed in lytic infection, and 2 cases were positive for both EBER and BHLF probe.

EBV RNA positivity according to histologic spectrum of PR(Table 2)

The nasal and WR T-cell lymphomas were classified into PR, PR-like lymphoma, and pleomorphic T-cell lymphoma. There is no consistent correlation in EBV genome positive rate with these histologic types.

EBV RNA positivity according to histologic types by WF(Table 3)

By modified Working Formulation, the cases were subdivided into one small lymphocytic lymphoma, one follicular, mixed, small cleaved and large cell lymphoma, one intermediate lymphocytic lymphoma, 22 diffuse, mixed, small cleaved and large cell lymphomas including 11 lymphomas with histologic features of PR, and 2 AILD-like T-cell lymphomas, 20 large cell lymphomas including 6 lymphomas with histologic features of PR, 3 immunoblastic lymphoma and 2 Burkitt's lymphoma. EBV RNA positive rates were noted in 11 of 22 diffuse, mixed small cleaved and large cell lymphomas(50%) and 10 of

20 diffuse large cell lymphomas(50%). Among mixed small and large, and large cell lymphomas, lymphomas showing the histologic spectrum of PR were positive for EBV RNA in 65% in contrast to the lower rate(40%) of lymphomas without PR-like features. Other subtypes were too small in number to compare with the result.

DISCUSSION

EBV has been associated with a number of malignant lymphomas including endemic Burkitt's lymphoma, posttransplant B-cell lymphomas, and lymphomas arising in AIDS patients. Although those lymphomas were mostly of B-cell origin, the EBV genome has been recently detected in Hodgkin's disease, T-cell lymphomas and Ki-1 lymphomas (Jones et al., 1988 ; Anagnostopoulos et al., 1989 ; Ho et al., 1990).

In this study, the EBV genome was detected in 44% of malignant lymphomas in which T-cell lymphomas rather than B-cell lymphomas, especially arising in the nasal cavity showed a much higher positive rate for EBV genomes. Along with recent studies performed in the United States, Hong Kong and in Japan documenting a high prevalence of EBV in nasal lymphoma(Ho et al., 1990 ; Harabuchi et al., 1990 ; Weiss et al., 1992), this result indicates that association of EBV with nasal lymphomas is not restricted to one geographic location.

High frequency of the EBV genome in nasal lymphomas raises many questions ; what is the factor leading to the high association of nasal lymphoma with EBV? ; is it cell lineage, anatomic site, or histologic type of polymorphic reticulosis? ; Is EBV oncogenetically involved?

Malignant lymphomas arising in the nasal cavity are more common in the Orient and are mostly T-cell lymphomas with a spectrum of histologic features from polymorphic reticulosis to PR-like lymphomas, to pleomorphic T-cell lymphomas being thought of as the most advanced stage evolved from an earlier phase of PR(Ho et al., 1990 ; Ko et al., 1990).

Although histologic subtype by WF has no correlation with EBV, lymphomas within the histologic spectrum of PR showed a higher positive rate for EBV than other histologic types. Likewise, previous EBV studies on polymorphic reticulosis showed a strong association with the EBV genome(Harabuchi et al., 1990). These findings suggest that polymorphic reticulosis has a particular association with EBV. However, in our cases which belong to the spectrum of PR, EBV showed a stronger association with cases arising in the nasal cavity than in WR, which indicated that anatomic site is a more important factor associated with EBV.

The association of EBV with nasal T-cell lymphoma is an unexpected finding and cannot be explained with current knowlege. EBV infects the oropharyngeal epithelial cells and underlying B lymphocytes which are sites for chronic EBV replication(Young et al., 1986). If EBV imparts a neoplastic growth advantage to infected cells in vivo, lymphoid tumors containing EBV DNA can be expected to arise in this location. Furthermore, usually B-cells harbor EBV receptors on their surface, but T-cells have been thought to lack the CR2 molecule. Although recent studies have documented that the CR2 molecule exists on the T-cells with the immunophenotype of immature cortical thymocytes and raises the possibility that these lymphocytes may be subject to EBV-targeting in vivo(Tssoukas and Lambris, 1993), there is no reasonable explanation of the preference of EBV for T-cells rather than B-cells.

These contradictory findings can be explained if EBV is not a prerequisite for lymphomagenesis. Because EBV infection is ubiquitous and various kinds of malignant neoplasms harbor EBV genome in their neoplastic cells(Nonoyama et al., 1973 ; Wu and Kuo, 1993) there has been a doubt as to the role of EBV in lymphomagenesis. But obvious EBV episome clonality demonstrated by Southern blot hybridization suggests that infection must have been established at an early stage of lymphomagenesis prior to clonal expansion of the latently infected cells which subsequently transmits identical viral cell progeny and supports the pathogenetic role of EBV(Katz et al., 1989).

In this study, the histologic grade of PR lymphomas has no correlation with EBV positive rate. Medeiros et al. (1992) reported that EBV was frequently associated with angiocentric immunoproliferative lesions, particularly within high-grade lesions which correspond to PR-like lymphomas and pleomorphic T-cell lymphomas in the present study. While PR was placed in a category of angiocentric immunoproliferative lesions, angioinvasion or angiocentricity is not a peculiar feature of PR and is frequently seen in peripheral T-cell lymphoma occuring in other extranodal sites as well as the

lymph nodes. Furthermore, all PR does not always show angioinvasiveness(Ho et al., 1990 ; Ko et al., 1992). Therefore PR and angiocentric lymphomas seem not to be an identical entity although they share some pathologic and clinical features.

In this study, the EBV genome in neoplastic cells exists in the latent or replicating forms or in both. Although latently infected EBV plays a role in oncogenesis, up to 40% of patients with EBV--associated malignant lymphoma show productive infection at least when studied at one point during the course(Katz et al., 1989). Viral replication may be an indicator that the immunologic mechanisms involved in keeping EBV latency are impaired. The clinical implication of this viral replication in malignant lymphoma should be investigated.

In conclusion, Among NHL, nasal T-cell lymphoma has a peculiar association with EBV in that the anatomic site may play a most important role. This phenomenon cannot be explained with our current knowledge of EBV. The pathogenetic mechanism of EBV in lymphomagenesis should be further elucidated.

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