Detection of Epstein-Barr Virus Encoded RNA in Cutaneous T-cell Lymphoproliferative Disorders

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Background: Recent reports suggest that Epstein-Barr virus (EBV) may play an important role in such a wide spectrum of human neoplasia. Recently, peripheral T cell lymphomas and particularly, angiocentric lymphomas (ACL), increasingly are reported to be associated with EBV. Nasal-type and nasal T/NK cell lymphoma (TNKL) have recently been reported to comprise most of ACLs. The prognosis of these tumors has been extremely poor.

Objective: The purpose of this study was to investigate EBV association in primary or secondary cutaneous T-cell lymphoproliferative disorders and to identify any prognostic association.

Methods: Thirty six patients with primary or secondary cutaneous T-cell lymphoproliferative (CTCL) disorders were examined to evaluate the presence of Epstein-Barr virus using in situ hybridization for EBV-encoded RNA (EBER).

Results : EBER was detected in tumor cells in one third of the total cases (13/36); 4/4 secondary skin lymphoma from nasal TNKL, 8/8 primary cutaneous nasal type TNKLs and 1/5 mycosis fungoides (MF). EBER was not detected in the following disease: 6 cases of anaplastic large cell lymphomas (ALCL) including 2 cases of probable NK-like T cell lineage, 3 lymphomatoid papulosis, 2 CD56(-) T cell ACLs and 7 subcutaneous panniculitic T-cell lymphomas (SPTL) by Revised European-American Lymphoma (REAL) classification and recent concept of further classification into NK-cell lineage. One case of T-cell pseudolymphoma as a negative control was also negative in EBER.

Conclusion : High incidence of EBV was observed in primary or secondary CTCLs in Koreans, with predilection for nasal and nasal type TNKL. In MFs, an erythrodermic MF with fatal outcome was associated with EBV and the EBV detection might reflect worse prognosis in MFs as seen in an aggressive course of nasal and nasal type TNKLs.

(Ann Dermatol 12(3) 173~178, 2000).

Key Words : Cutaneous T-cell lymphoproliferative disorders, Epstein-Barr virus

Recent reports suggest that Epstein-Barr virus (EBV) may play an important role in a wide spectrum of human neoplasia[1-4]. Recently, peripheral T cell lymphoma and especially, angiocentric lymphomas (ACL) increasingly have been reported to be associated with EBV[3-6]. Nasal-type and nasal T/NK cell lymphoma (TNKL) have recently been reported to comprise most of ACLs[3,7,8]. The prognosis of these tumors has been extremely poor[7-10]. The purpose of this study was to investigate EBV association in primary or secondary cutaneous T-cell lymphoproliferative disorders and to identify any

Received October 20, 1999.
Accepted for publication December 30, 1999.
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prognostic association.

MATERIALS AND METHODS

Clinicopathologic study

During the period between 1989 and 1998 in the Asan Medical Center, a total of 36 cases of primary or secondary cutaneous T-cell lymphoproliferative disorders with sufficient paraffin tissues were selected for this study. Immunohistochemical analyses were performed with the labelled streptoavidin-biotin method using paraffin tissue specimens of the skin. The antibodies used included polyclonal CD3 (Dako), L26 (CD20) (Dako), UCHL-1(CD45RO) (Dako), and CD56 (Beckton-Dickinson). Review of H&E and immunohistochemical slides was performed by 1 dermatologist and 2 pathologists. The diagnosis and classifications were based on Revised European-American Lymphoma (REAL) classification and recent concept of further classification into NK-cell lineage[7]; 4 secondary skin lymphomas from nasal TNKLs, 8 primary cutaneous nasal type TNKLs and 5 mycosis fungoides (MF), 6 anaplastic large cell lymphomas (ALCL) including 2 cases with coexpression of CD56, 3 lymphomatoid papulosis, 2 angiocentric T-cell lymphomas (ACL-T), 7 subcutaneous panniculitic T-cell lymphomas (SPTL) and 1 T-cell pseudolymphoma. Clinical informations was obtained from the medical records and clinical follow-up.

RNA in situ hybridization

In situ hybridization (ISH) was performed using fluorescein-conjugated oligonucleotide probes for Epstein-Barr virus early regions (EBER) (cocktails of EBER 1 and 2, Dakopatts, Denmark). Briefly, deparaffinized, formalin-fixed 5μm-thick skin biopsy sections were digested with proteinase K (10μg/ml) for 30 min and hybridized for 2 hr with the probes. Detection was performed using alkaline phosphatase-conjugated rabbit anti-FITC and enzyme substrate (BCIP/NBT). The positive reaction was determined by a dark blue color in the nucleus. The test was interpreted as positive when more than rare tumor cells were stained (rare cells: 1-2 cells/medium power). Semiquantitative analysis of the staining results were analyzed according to the following; (+) up to 10% of the tumor cells; (2+) > 10% to 30% of the tumor cells; (3+) > 30% of the tumor cells.

RESULTS

Clinical Findings

The diagnosis of each patient in primary or secondary cutaneous T-cell lymphoproliferative disorders are summarized in the Table 1. Six patients out of 8 primary cutaneous nasal type TNKLs, 3 out of 4 cases with lymphomas secondary to nasal TNKLs, 4 out of 7 SPTLs and 1 case with erythrodermic MF had died with progressive disease. Among the remaining TNKLs, 3 primary cutaneous TNKLs, 1 lymphoma secondary to nasal TNKLs showed progressive disease with no response to combination chemotherapy at the last follow-up. In contrast to the very poor prognosis in primary or secondary TNKLs, CD56(-)/CD3(+) cutaneous T-cell angiocentric lymphoma patients were alive and free of disease at the last follow-up period.

Results of EBV studies

The results of EBV studies are also shown in Table 1. In situ hybridization using antisense EBER probe showed a positive reaction in the tumor cell nuclei in 36% of cases (13/36).

1. EBV positivity according to histologic subtypes

EBER was detected in tumor cells in one third of the total cases (13/36); 4/4 secondary TNKLs, 8/8 primary TNKLs and 1/5 of MFs. All TNKLs showed positive staining although the mean grade of staining is higher in secondary TNKLs than primary TNKLs. EBER was not detected in the following diseases: 6 cases of ALCLs, 3 Lymphomatoid papulosis, 2 angiocentric T-cell lymphomas and 7 SPTLs.

2. EBV positivity according to CD56 positivity

All TNKLs with CD56 positivity showed EBER positivity. However, the 2 cases of ALCL with substantial coexpression of CD56 and CD30 were not associated with EBV expression. The other 22 cases didn't express CD56, NK-cell marker in the tumor cells. 12 out of the 13 cases with EBV expression were CD56(+) TNKLs, except an erythrodermic MF.

Table 1. Cases, diagnosis, outcome and EBER results

No.	Sex / Age	Diagnosis	EBER[13]	Outcome
1	F / 50	ALCL[1]	-	AND[14]
2	F / 57	ALCL	-	AND
3	F / 53	ALCL	-	AND
4	M / 62	ALCL	-	AWD[15]
5	M / 59	ALCL-NK[2]	-	AWD
6	M / 36	ALCL-NK	-	AND
7	M / 35	Lyp[3]	-	AWD
8	F / 47	LyP	-	AND
9	M / 21	LyP	-	AND
10	M / 55	MF-PAT[4]	-	AND
11	M / 48	MF-PLA[5]	-	AND
12	F / 30	MF-PLA	-	AND
13	M / 60	MF-NOD[6]	-	AWD
14	M / 38	MF-ERY[7]	3+	DWD[16]
15	M / 61	ACL-T[8]	-	AND
16	F / 64	ACL-T	-	AND
17	M / 36	PL-T9)	-	AND
18	M / 42	SPTL[10]	-	FL17)
19	M / 39	SPTL	-	DWD
20	M / 28	SPTL	-	FL
21	M / 30	SPTL	-	DWD
22	M / 31	SPTL	-	FL
23	M / 57	SPTL	-	DWD
24	M / 40	SPTL	-	DWD
25	M / 77	P-NK/T[11]	3+	DWD
26	M / 55	P-NK/T	1+	FL
27	M / 61	P-NK/T	3+	DWD
28	M / 17	P-NK/T	3+	FL*
29	F / 42	P-NK/T	2+	DWD
30	F / 26	P-NK/T	1+	DWD
31	M / 35	P-NK/T	2+	FL*
32	M / 33	P-NK/T	3+	DWD
33	M / 52	S-NK/T[12]	3+	AWD**
34	M / 70	S-NK/T	3+	DWD
35	M / 55	S-NK/T	3+	DWD
36	M / 37	S-NK/T	3+	DWD

Aberrations : 1) ALCL: anaplastic large cell lymphomas, 2)ALCL-NK: anaplastic large cell lymphomas with NK marker expression, 3) LyP: lymphomatoid papulosis, 4) MF-PAT: mycosis fungoides-patch stage, 5) MF-PLA: mycosis fungoides-plaque stage, 6) MF-NOD: mycosis fungoides-nodular stage, 7) MF-ERY: mycosis fungoides-erythrodermic stage, 8) ACL-T: T cell angiocentric lymphomas without NK marker, 9) PL-T: pseudolymphoma-T cell, 10) SPTL: subcutaneous panniculitic T-cell lymphomas, 11) P-NK/T: primary cutaneous TNKL, 12) S-NK/T: TNKL secondary to nasal lymphoma, 13) EBER: EBV-encoded RNA 14) AND: alive with no disease, 15) AWD: alive with disease, 16) AWD: dead with disease 17) F/L: follow-up loss,

* : The cases with asterisks were suffering from progressive disease without response to combination chemotherapy.

** : This case responded to combination chemotherapy but had a recurrence after 4 months.

3. EBV positivity according to clinical outcome

At the last follow-up, 15 patients had passed away due to the progression of the disease. The TNKLs with fatal outcome was associated with EBER expression. EBER was detected in only 1 erythrodermic MF out of 4 MFs and a fatal outcome was shown in the case with EBER positivity. Although 4 out of 7 SPTLs died with progressive disease, no SPTLs were associated with EBER expression. All the survivors with EBER positivity except one case (double asterisk, Table 1) at the last follow-up were suffering from progressive disease without response to combination chemotherapy.

DISCUSSION

EBV is a human lymphotropic herpesvirus that infects the vast majority of the world's adult population with establishment of a lifelong infection in all infected individuals[1]. EBV has been demonstrated to be etiologically associated with various peripheral T-cell lymphomas[1,3]. However, the etiologic role of viruses in CTCLs is still controversial[1,2] and there are many differences of the expression rate of EBV in the previous reports depending on the subtype of CTCLs, racial background and other factors[4-15].

In benign cutaneous pseudolymphomas of the human skin, EBV was suggested as an etiologic factor but EBER was not demonstrated.1 One case of T-cell pseudolymphoma was EBER-negative in our study.

In the 7 cases of SPTL above, EBER was not shown. In the previously reported cases of SPTL, EBER results were contradictory[2]. However, in the diagnosis of past years, much confusion was made about cytophagic histiocytic panniculitis (CHP), SPTL and angiocentric TNKL of subcutis[2,3]. This confusion might be the cause of EBV detection in some previously reported SPTL[2].

Among our series of 8 primary cutaneous nasal type TNKLs, at least 6 patients showed multiple subcutaneous nodules and subcutaneous lymphomatous patterns with/without angiocentricity histopathologically. Final diagnosis (either angiocentric TNKL involving deep subcutis or SPTL) was very difficult to determine before CD56 detection. SPTL which is characterized by subcutaneous nodules, systemic signs and symptoms, and an aggressive clinical course with death due to a hemophagocytic syndromes was also identified and must be differentiated with subcutaneous angiocentric nasal type TNKL[5-7]. Increasing use in recent years of monoclonal antibodies of CD56 in the diagnosis of malignant lymphomas has led to the recognition of another subtype of cutaneous lymphoma with a NK cell phenotype.[7] Instead of the previous term, CD56(+) angiocentric T-cell lymphoma, a new designation of T/NK-cell lymphoma was adopted, emphasizing the expression of antigens associated with both of T-and NK-cell type. Because the nasal area is the most common site of presentation, "nasal T/NK-cell lymphoma" was favored as the primary term of choice for midline facial lesions. Tumors with identical morphology, phenotype, and genotype presenting in another anatomic site would be called as nasal type T/NK-cell lymphomas and these tumors are reported to be highly associated with EBV[7-9]. Of note, all the nasal and nasal type TNKL of our series showed EBV-positivity although stronger expression was observed in secondary cutaneous TNKL from nasal cavity TNKL than primary cutaneous TNKL. The diagnosis of ACL involving deep subcutis was easily confused with SPTL since ACL showed, in part, various degree of angiocentric pattern with rather diffuse cellular infiltration in the subcutis.[3,7] Furthermore, the pattern of both ACL and SPTL have been reported to be associated with fatal outcomes and a hemophagocytic syndrome[3,5-7]; thus there may be some clinicohistopathologic overlap between these two types of lymphomas. Based on our results of great difference of EBER positivity between SPTLs and TNKLs, EBV detection may be an important clue to differential diagnosis.

In the previous groups of ACL primarily involving skin, nasal-type TNKL has recently been reported to comprise most of them.[4,7] ACL has been classified as a group of uncommon peripheral T-cell lymphoma, which frequently involves the extranodal site involving skin. In the heterogenous group of ACL, there might be at least 3 different immunophenotypes ; those are, mostly nasal-type CD56(+) TNKL, true T without NK markers and finally NK-like T-cell (CD56(+) with clonal rearrangement of the T cell receptor gene)[7]. In our study of both primary and secondary TNKLs, all the cases were EBV-associated including one case of NK-like T-cell with clonal rearrangement of the T cell receptor gene. However, 2 cases of T cell

ACLs without NK markers (ACL-T in the following table) were completely negative on EBER staining. Only a few studies have investigated the presence of EBV in ACLs separating CD56(+) and CD56(-) immuonophenotype. One recent EBV study11 included only 1 case of CD56(+) ACL (namely TNKL) in 14 primary cutaneous ACLs and reported primary cutaneous ACLs was not much less associated with EBV in contrast to secondary cutaneous ACLs. Their results further support our observation that 12 CD56(+) ACLs (4 secondary and 8 primary) were EBV-associated in contrast to CD56(-) T cell ACLs. Although this has to be further studied with more cases to confirm, the negative association of EBV in our 2 cases of CD56 (-) T cell ACLs may reflect the favorable outcome compared to the fatal outcome in the primary and secondary TNKLs.

Although a case report of EBV positivity in refractory, systemic anaplastic large cell lymphoma (ALCL) with reactive hemophagocytic syndrome exist,11 primary cutaneous ALCL is not likely to be associated with EBV expression[12]. In our study, 6 primary cutaneous ALCLs and 3 cases of lymphomatoid papulosis were completely negative on EBV staining.

In the mycosis fungoides (MF), the EBV is associated with the lesions in 0 to 32 % according to the published series.[13] In the previous reports, one MF with mutilating facial tumors and an erythrodermic MF course with enlarged peripheral lymph nodes, the genome of EBV was demonstrated by polymerase chain reaction in the diseased skin[13]. In one recent study in China, EBV was detected in 12 cases (42.9%) including the secondary T-cell lymphomas of skin, large cell lymphoma, mycosis fungoides, adult T-cell leukemia/lymphoma, and angiocentric lymphoma by EBER in situ hybidization..14 In their study, no overall correlation could be found between the presence of EBV and the prognosis or the severity of the skin lesion[14]. However, the prognostic role of the EBV has been demonstrated in peripheral aggressive T-cell lymphomas[1,7,13]. EBV associated T-cell lymphomas have a poor survival rate and the EBV infection may be associated with the expression of the multidrug resistant gene-1 and the risk of a terminal hemophagocytosis[13]. In our study, only one MF with aggressive course was associated with EBV and the EBV detection might reflect worse prognosis in MFs as seen in an aggressive course of nasal and nasal type TNKLs. In our patient of erythordermic fatal MF with enlarged peripheral lymph nodes, the presence of the EBV in the tumor cells of the skin lesions is also an argument in favour of the pathogenic role of the virus.

The clinicopathologic characteristics of malignant lymphomas vary according to geography. In contrast to the western reports[15,16] showing very low or no detection of EBER in primary cutaneous lymphomas, the higher association of EBER in Korea[17] may merely reflect the racial, regional and epidemiological characteristics of CTCL. The main difference exists in the fact that CTCLs of Asians are characterized by high incidence of nasal and nasal type TNKL compared to those of Western countries[7,17]. Overall, in Korea, cutaneous T-cell lymphomas which represent a heterogeneous group of peripheral T-cell lymphomas arising in the skin, EBV was demonstrated in 13 cases (36%) by EBER in-situ hybridization; particularly high rates were exhibited by primary and secondary TNKLs. EBV seems to play a relatively significant role in the development of cutaneous lymphomas in Korea. Whether high prevalence of EBV latent infection exists in Korea has to be further studied.

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