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Epstein-Barr Virus in Nasal Angiocentric Lymphoma with Malignant Histiocytosis-like Hemophagocytic Syndrome*

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악성조직구증과 유사한 혈구탐식증후군을 동반한 코의 혈관중심위 림프종과 Epstein-Barr 바이러스의 관련성 연구

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서 론 : 혈구탐식증후군은 고열, 범혈구감소증, 간비장종대, 림프절비대 및 혈액응고장애 등을 동반하는 전신적 질환으로 대부분 면역억제 상태에서 바이러스 및 각종 병원체의 감염에 의해서 유발되고 예후가 불량하다. 조직학적으로 림프세망기관의 조직구의 증가와 혈관탐식현상이 빈번히 관찰되고 이와 같은 현상을 보이는 악성조직구증식증과의 감별이 어렵다. 코의 혈관중심위 림프종은 거의 일정한 Epstein-Barr 바이러스(EBV)양성을 보인다고 보고되고 있고 병의 경과중 혈관탐식증후군이 빈번하게 발생되는데 이는 EBV감염에 의해서 유발된다고 보아지고 있다.

재료 및 방법 : 1985년 1월부터 1995년 12까지 강남성모병원과 성바오로병원에서 코의 혈관중심위 림프종으로 진단 받았던 환자 42명 중 혈구탐식증후군을 동반한 10명을 대상으로 임상양상을 관찰하고, 조직표본에 면역조직화학염색법과 교잡반응을 사용하여 악성세포의 표현형을 살펴보고 EBV와의 관련성을 관찰하였다.

결 과 : 10명의 환자 중 5명은 혈관중심위 림프종 진당당시, 3명은 재발시기, 2명은 관해 시기에 혈구탐식증후군을 동반하였다. 모든 환자에서 실시된 치료방법에 상관없이 치명적인 경과를 보였으며 중

*본 논문은 1996년도 가톨릭 암센터 연구비 지원에 의한 것임.

앙생존기간은 18일(2-44일)이었다. 대상 모두에서 T형세포 표현형과 교잡반응상 EBV양성을 보였으며, EBV는 주로 악성림프종세포에 분포양상을 보였다.

결 론 : 혈구탐식증후군은 코의 혈관중심위 림프종의 흔한 합병증으로 불량한 예후를 보인다. 임상적 양상 및 조직학적 검사상 악성 조직구증가증과 유사한 소견을 보여 감별이 어렵고, 치명적 결과를 초래하므로 치료에 어려움을 주고 있다. 코의 혈관중심위 림프종과 밀접한 관계를 보이는 EBV에 의해서 유발된다고 보아지고 있으며 치료의 개선을 위하여 앞으로 병인적연구가 필요하다고 생각된다.

KEY WORDS : Angiocentric lymphoma · Hemophagocytic syndrome · Malignant histiocytosis · Epstein-Barr virus.

Introduction

Hemophagocytic syndrome(HS) is a systemic disease characterized by fever, pancytopenia, hepatos-plenomegaly, lymphadenopathy, coagulopathy and histologically proliferation of histiocytes in the lymphoreticular system[1)2)]. It is usually associated with viral infection such as Epstein-Barr virus(EBV), cytomegalovirus and adenovirus in the immunocompromised patients[3)4)]. Recently it has been reported that EBV-associated HS is frequently observed in lymphoma of T-cell lineage and shows fatal outcome[5)6)]. It is difficult to distinguish HS from malignant histiocytosis(MH) and providing the suggestion of previously diagnosed MH may include HS in T-cell lymphoma. Nasal angiocentric lymphoma (AL) has been classified as a peripheral T-cell lymphoma, but recently classified as nasal or nasal type T/NK cell lymphoma and highly infected with EBV[7)8)]. MH-like HS dose not infrequently occur in nasal AL and usually has a rapidly fatal course[7)]. In this study we retrospectively analyzed AL with HS who had initially suspected to have MH to assess the clinical significances and the pathogenetic association with EBV and that would help to change our concept of MH and distinguish HS from MH.

Materials and Methods

1. Patients

From 1987 to 1996 a total of 12 patients admitted to Catholic Cancer Center were established to have HS. The diagnosis of HS was based on a combination of the following clinicopathologic features : ① fever and splenomegaly, ② cytopenia of at least two hematopoietic series, and ③ over 2% of hemophagocytic histiocytes in the bone marrow[1)9)]. The finally established causes of HS were nasal AL(10 cases) and unidentified etiology(2 cases). Nine of 10 patients of AL were initially suspected as MH. Lymphoma staging was done according to the Ann-Arbor system. The clinical and laboratory records of AL with HS were evaluated retrospectively. The survival time of patients with HS was defined as the duration from the onset of HS to death.

2. Diagnosis of angiocentric lymphoma

The histopathologic diagnosis of AL was done by examination of paraffin-embedded tumor section with hematoxylin-eosin stain. For immunophenotypical study, sections of the paraffin-embedded blocks were cut at 6 μm thickness and stained with specific monoclonal antibodies by an avidin-biotin complex(ABC) peroxidase methods described previously[10)]. Mouse anti-human T-cell(CD45RO)(DAKO® Carpinteria, CA) and Mouse anti-human B-cell(IgG2a)(DAKO® Carpinteria, CA)were used for the immunophenotyping of T and B lineage respectively.

3. Association of angiocentric lymphoma with EBV

Serologic study of EBV-antibodies(Ab) : Serologic tests of IgG and IgM Abs against EBV-viral capsid antigen(VCA), early antigen(EA), and Abs against EBV nuclear antigens(EBNA) were performed in an available patient using the indirect immunofluorescent methods as previously described[11)].

Table 1. Patients' clinical features

Patient	Age/Sex	Initial CS	Timing of HS	Duration Dx-HS(M)	Treatment of HS	Outcome	Survival after HS(D)
1	51/M	I	at Dx	0	supportive	died	20
2	63/M	II	at Dx	0	CHOP	died	25
3	38/M	IV(skin)	at Dx	0	supportive	died	2
4	57/M	II	at Dx	0	supportive	died	27
5	31/M	IV(BM)	at Dx	0	supportive	died	16
6	43/M	II	relapse	19	CHOP	died	44
7	23/F	I	relapse	36	CHOP	died	14
8	30/F	I	relapse	9	CHOP	died	14
9	42/F	II	remission	7	supportive	died	7
10	45/F	II	remission	10	supportive	died	21

CS : clinical stage, BM : bone marrow, HS : hemophagocytic syndrome, Dx : diagnosis

In situ hybridization(ISH) : The EBV RNA in situ hybridization studies were performed using a biotinylated 26bp oligonucleotides (Research Genetics) complementary to the most abundant early RNA sequence in EBV infections. 6 μm sections cut from paraffin block, placed on organosilane pretreated glass slides were deparaffinized, dehydrated, predigested with pepsin solution, and hybridized for 30 min at a concentration of 0.5 μg/ml of probe. After washing and blocking of endogenous peroxidase, detection was accomplished using streptavidin alkaline-phosphatase conjugate followed by development of signal with stable fast red TR/stable naphthol phosphate (Research Genetics) and counterstaining with hematoxylin. A red color within the nucleus over background levels was considered a positive reaction. A known EBV-positive neoplasm was served as a positive control and an EBV-negative lymphoid tissue as a negative control in each run.

Results

1. Patients' clinical features

The basic clinical features of all patients were summarized in Table 1. There were 6 male and 4 female patients with median age of 43 years (range 23 – 63 years). The initial clinical stage of lymphoma were stage I(3 cases), II(5 cases), and IV(2 cases) and the extranodal involvement sites were skin(1 case) and bone marrow(1 case). Five patients had HS as an initial manifestation, three had at the time of relapse of lymphoma, and two had during the clinical remission of lymphoma. Four patients were treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy and other six had only supportive care. The median survival of all patients, supportively cared patients, and patients with CHOP were 18 days(range 2 – 44 days), 18 days(range 2 – 27 days), and 19.5 days(range 14 – 44 days), respectively.

2. Histopathologic features

Polymorphic cellular composition was observed in six cases and other 4 cases had relatively monomorphic composition. Angiocentricity was observed in only 4 cases, but necrosis was in all cases. Immunophenotypical studies showed that all cases expressed T-cell phenotype(Fig. 1). Representative histologic features of HS are shown in Fig. 3 & 4.

3. Association of angiocentric lymphoma with EBV

The data of serological test in one available patient showed the active EBV infection with positive anti-EBNA Ab, anti-VCA IgG Ab titer over 1 : 640, and positive anti-EA IgG Ab(1 : 320).

The in situ EBER hybridization showed the EBV transcripts in the nuclei of most of the atypical cells in all cases(Fig. 2).

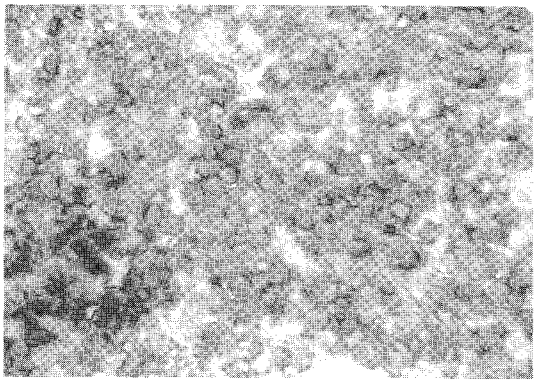

Fig. 1. UCHL1(CD45RO) expression in atypical lymphoid cells(Haematoxylin counterstaining, ×400).

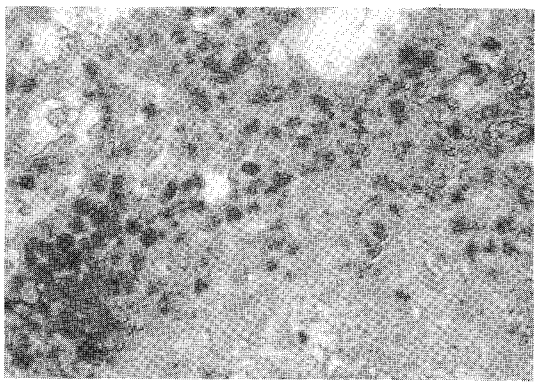

Fig. 2. Nuclear staining of the atypical lymphoid cells after EBER in situ hybridization(Haematoxylin counterstaining, ×400).

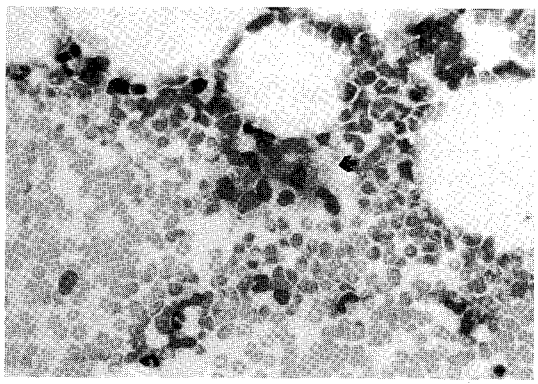

Fig. 3. BM aspiration cytology shows increased histiocytes (arrow)(Wright stain, ×400).

Discussion

We recently experienced a patient with nasal angiocentric lymphoma(AL) in whom as a terminal event, the hemophagocytic syndrome mimicking malignant histiocytosis(MH) developed. MH is a systemic malignancy derived from cells of the mononuclear phagocytic system and the essential diagnostic features include disseminated and progressive proliferation of morphologically atypical histiocytes and presence of phagocytosis by these neoplastic cells. Hemophagocytic syndrome(HS) has the similar clinical manifestations, but the cytologic atypia in hemophagocytic cells is not present[12]. Recently reassessment of patients previously diagnosed as having MH revealed that most cases are lymphoma of T-cell lineage or others, such as a virus associated hemophagocytic syndrome(VAHS)[13]. Retrospective analysis in this study revealed that 11 of a total 16 patients with MH previously had diagnosed were MH-like HS and 9 of them, initially had suspected MH, were caused by nasal AL.

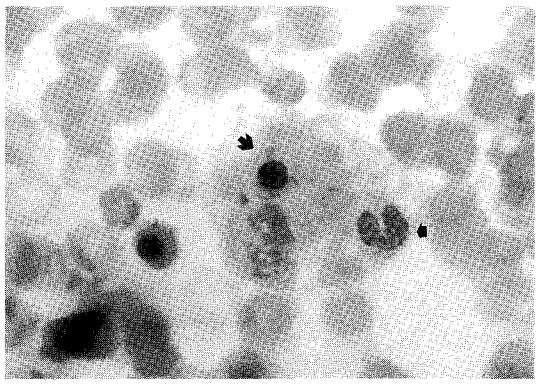

Fig. 4. Histiocyte shows hemophagocytosis of normoblast & segmented neutrophil(Wright stain, ×400).

HS has been frequently reported in various neoplastic disorders of T-cell including peripheral T cell lymphoma(PTCL), T-acute lymphoblastic leukemia and the angiocentric character of the neoplastic lymphoid infiltrates have been frequently observed in PTCL with HS[12-14]. The most common cause of HS was nasal AL(10/12 cases) in this presentation. HS was developed mostly in the active disease-status, but even in the clinical remission of two cases, too. We could not confirm the postmortem pathologic staging of all cases due to lack of the autopsy-specimen. But, Jaffe et al.[12] had reported that malignant lymphoma with erythrophagocytosis simulating MH had prevalence of lymphoma-involvements in lymph nodes,

spleen, liver, lung, skin, and kidney and hepatosplenomegaly was a common feature in all cases of HS even in the patients with clinical remission. Therefore pathologic staging including laparoscopic biopsy would be needed for the exact staging of lymphoma with MH-like HS.

Nasal AL has been classified as a peripheral T-cell lymphoma, but, recently it is proving that it may include true natural killer(NK) cell lineage[7][8]. And although angiocentricity is common to these tumors, it is not universally seen. Therefore it is proposed that the term 'nasal or nasal type T/NK cell lymphoma' should replace AL as a choice[7]. In this study, we only used pan T-cell marker(CD45RO) to distinguish from B-cell and all showed T-cell phenotype and did not performed NK-cell phenotying due to lack of fresh specimens. More phenotypical studies including immuphenotyping and T-cell receptor gene rearrangement would be needed to distinguish the true T-cell lymphoma from NK-cell lymphoma. In this histologic study, necrosis and cellular polymorphism were more common feature than angiocenricity and monomorphism, respectively.

Although HS was originally described in immunocompromised patients with viral infection, patients with vast bacterial, fungal, and parasitic infections have also developed HS, too[3]. Risdal et al.[3] have described a HS in patients with immunodeficiency and viral infection that also simulates MH. Among various infectious agents, herpesvirus, especially EBV, have been frequently implicated in the pathogenesis of VAHS[3][15]. EBV is ubiquitous Herpes virus with tropism for B-lymphocytes and oropharyngeal epithelium and has a strong association with endemic Burkitt's lymphoma, B-cell lymphoproliferative lesions in immunocompromised patients, and nasopharyngeal carcinoma[11][16]. But recently, it appears that EBV has also been linked to about 40% of peripheral T-cell lymphoma and Hodgkin's disease[17-19]. And EBV is more regularly detected in more than 80% of nasal AL and in these tumors virtually all tumor cells harbour the virus. In this study, the presence of EBV was detected in all cases particularly in the most of atypical lymphoid cells by in situ hybridization and the serologic test in one case indicated an active infection. Although a causal relationship between EBV and AL is still undefined, the characteristic clinicopathologic features strongly suggest that EBV may contribute to the lymphomagenesis and the biologic features of AL and in situ hybridization and serologic study would be helpful for diagnosis and prediction of AL with HS [7][11]. The mechanism of HS in AL is not fully understood yet, but it is thought to be caused by the cytokines, especially interferon-γ, tumor necrosis factor, and interleukin-1 released from EBV infected lymphocytes[20][21]. Further evaluation of the EBV-viral oncogenes and the microenvironment in HS would be needed to define the pathogenesis and the role of EBV in these tumors.

HS contributes the high mortality of AL and usually has a rapidly fatal outcome[6][14]. We observed a significantly different survival between nasal AL with HS and without HS and HS can be proposed as a poor prognostic factor in nasal AL. It is difficult to predict HS in the course of nasal AL, but symptomatic recurrence and the histologic progression of the primary nasal lesion may be cadidates. In the patient's number 8 showed symptomatic recurrence and the histologic progression of nasal lesions from angioimmunoproliferative lesion(AIL) grade 1 to grade 3 and finally she died due to HS in nasal AL(AIL grade 3). So the repeated biopsy of the nasal lesion in the symptomaticaly recurrent patients would be essential.

There is no effective treatment for HS and in literatural review, the combination of high-dose intravenous immunoglobulin and etoposide or high dose steroid were effective in some cases of HS[2][6][22][23]. In one case described a young patient successfully treated with CHOP chemotherapy in the early phase of clinical course[6]. But, in this study, all patients showed rapidly progressing into fulminant course despite CHOP chemotherapy or palliative steroid pulse therapy. It seems that more aggressive treatment is needed in this condition and the third generation combination chemotherapy regimen including etoposide & high-dose methylprednisone, such as ESHAP(etoposide,

methylprednisone, cytarabine, and cisplatin) may be appropriate as an initial treatment, but further investigation of pathogenesis and biology of AL with HS should be pursued ahead to improve the prognosis.

Summary

Malignant histiocytosis(MH)-like hemophagocytic syndrome(HS) is a fatal complication of nasal angiocentric lymphoma(AL) and difficult to distinguish from MH. Ten of total 42 patients with nasal AL had HS and 9 of them were initially suspected to have MH. Five patients had HS as initial manifestation, 3 at the time of relapse, and 2 during the clinical remission of lymphoma. Four patients were treated by combination chemotherapy(CHOP) and others had only supportive care. Immunohistochemical study and in situ hybridization were performed on the specimen obtained from 10 patients.

The median survival of all patients from HS was 18 days(range 2 - 44 days) and all had fatal outcome regardless of the treatment-modality. All cases were positive for UCHL1(CD45RO) and Epstein-Barr virus (EBV) by EBER in situ hybridization.

MH-like HS is a fatal complication of nasal AL and has a high association with EBV. Reactivation of EBV may contribute to HS and further investigation of predictive factors and effective treatment of HS should be pursued in future.

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