兩個致命性中線肉芽腫病例：鼻部T/NK細胞淋巴瘤合併Epstein-Barr病毒感染

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Nasal T/NK Cell Lymphoma Associated with Virus Infection in Two Cases of Lethal Midline Granuloma

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Nasal or nasal-type T/NK cell lymphoma often follows an aggressive course and has a poor prognosis. Radiation therapy may provide good local control of the disease in an early stage, while the disease is always resistant to chemotherapy. Failure to recognize this disease often leads to inappropriate management. We report two cases of nasal T/NK cell lymphoma with the unique feature of a crusted necrotic ulcer surrounded by erythematous infiltrative rim at midfacial region, clinically presented as so-called lethal midline granuloma. Histopathologically, the atypical lymphoid cells infiltrated diffusely and displayed angiocentricity. CD56 and latent membrane protein-1 (LMP-1) were identified by immunohistochemistry. The presence of Epstein-Barr virus DNA in the lesional skin was confirmed by polymerase chain reaction. (Dermatol Sinica 18 : 88-94, 2000)

Key words: Nasal T/NK cell lymphoma, Lethal midline granuloma, Epstein-Barr virus

鼻部或鼻型T/NK細胞淋巴瘤通常病情惡化快速且預後不良，放射治療對早期的局部病灶可有效控制，但化學治療的反應則不佳，未即時有正確的診斷常導致不當的治療。我們提出兩例鼻部T/NK細胞淋巴瘤，臨床表現爲所謂的致命性中線肉芽腫，病人在臉部的中央有一壞死結痂的潰瘍，周圍有泛紅的浸潤性病灶。病理切片下有異常淋巴細胞廣泛地浸潤，特別是在血管的周圍。免疫組織化學分析中，CD56及LMP-1皆呈陽性，EB病毒的DNA可進一步藉由聚合酶鏈性反應而確定其存在於病灶的皮膚組織中。(中華皮誌18 : 88-94, 2000)

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INTRODUCTION

Lethal midline granuloma (LMG) is a clinical term used to describe a rare condition characterized by rapidly progressive midfacial tissue destruction.[1-3] Most recent investigations indicated that the underlying lesions of most cases are lymphoma, which often express the natural killer (NK) cell marker CD56 and show a germline configuration of the T cell receptor (TCR) gene. Nasal or nasal-type T/NK cell lymphoma has been recently proposed to be highly associated with Epstein-Barr virus (EBV) infection. We herein report two cases of nasal T/NK cell lymphoma presenting as LMG, and assessed the association of EBV infection by serologic test, immunohistochemical staining, and polymerase chain reaction (PCR) technique.

CASE REPORT

Case 1

A 49-year-old man had experienced a painful, fetid, necrotic ulcer with an erythematous infiltrative rim on the left nasal ala for 2 months (Fig. 1A). Constitutional symptoms were absent. Rhinoscopy exhibited yellow-gray friable granulation tissue arising from the left inferior turbinate, and nasal floor necrosis. Nasal cavity and palate were intact at that time. Nasal computerized tomography (CT) revealed a left nasal mass encroaching the vestibule. Patient was referred to dermatologic department for skin biopsy since repeated mucosal specimens were nondiagnostic.

There was no lymphadenopathy and other skin lesion. Laboratory examination was unremarkable except for the positive serologic tests of antibodies to EBV (Table I). A skin biopsy specimen of the erythematous rim showed an extensive infiltration of atypical lymphoid cells in dermis and subcutaneous layer with angiocentricity, and marked tissue necrosis (Fig. 1B). According to Ann Arbor staging system, the stage I_EA nasal T/NK cell lymphoma was diagnosed but he refused to receive any treatment. Six months later, he was readmitted due to perforation of nasal septum and severe disfiguration of nose and upper lip, and was staged II_EA. Concurrent chemotherapy (CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisolone) and radiotherapy (total 56Gy) was intended.

After three courses of CHOP and local irradiation of a cumulative dosage of 2880 cGy were administered, he refused to receive further treatment because of side effects of the radiochemotherapy. The lesion showed rapid shrinkage without evidence of dissemination.

Case 2

A 70-year-old man was diagnosed to have sinusitis and treated for 6 months because of increasing nasal obstruction, purulent rhinorrhea and left maxillofrontal pain. Erythematous painful swelling of left paranasal area appeared 2 months later, along with occasional fever and epistaxis. He was seen 2 weeks later because of progressive ulceration and crusting of the paranasal skin lesion.

Physically, neither lymphadenopathy nor other skin lesion was found elsewhere. The nasal CT showed a soft tissue mass with low density on left nasal cavity and cloudiness of ethmoid and maxillary sinuses. The hemogram, biochemistry examination, and chest x-ray were grossly normal. Repeated biopsies taken from the nasal mass revealed only necrosis. During admission, necrosis and perforation of the palate developed and skin ulceration kept on progression even under intensive antibiotic treatment (Piperacillin 4g Q6h, and Amikacin 250mg Q8h). Skin biopsy was requested.

A skin biopsy specimen from the periphery of necrotic lesion disclosed atypical lymphoid cells of various sizes infiltrating diffusely in dermis and subcutaneous fat with tissue necrosis. Immunohistochemical staining of the T and NK cell marker was positive (Table I)(Fig. 1C).

Nasal T/NK cell lymphoma stage II_EB was diagnosed. Two courses of cyclophosphamide and prednisolone were administered, but got no response. The patient died of sepsis and active nasal bleeding 2 months later.

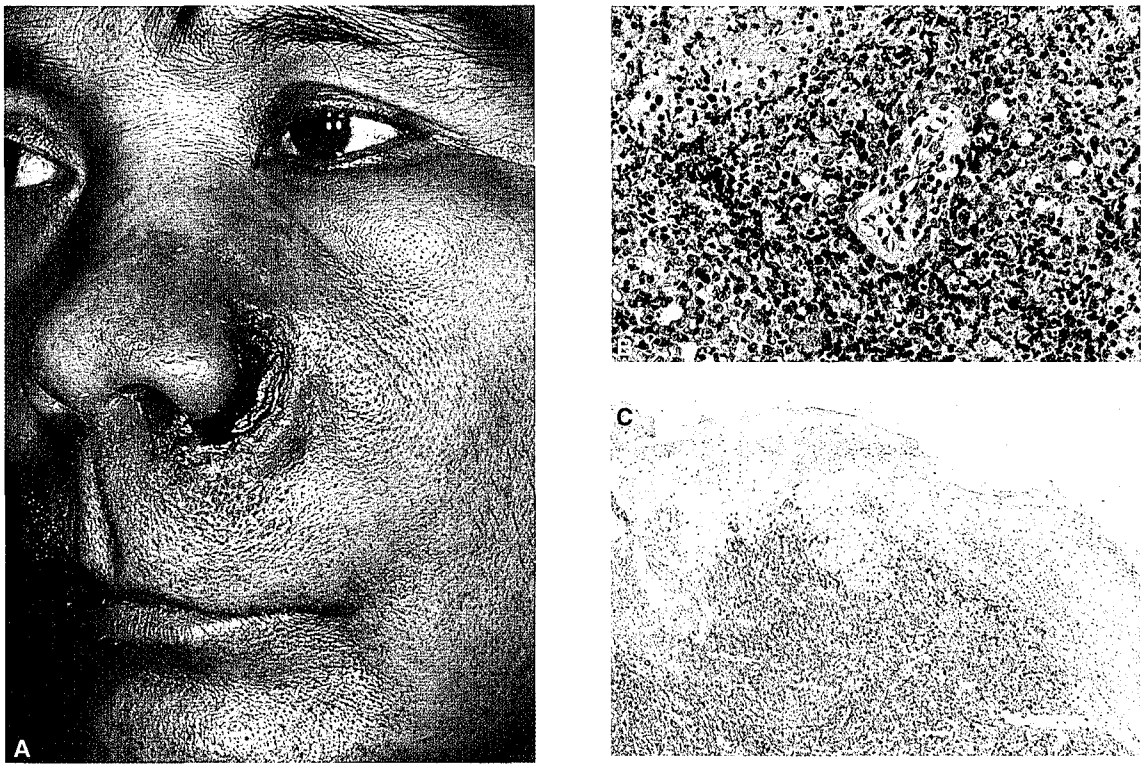

Fig. 1
(A) A crusted necrotic ulcer surrounded by an erythemetous infiltrative rim on left nasal ala with mild swelling of left cheek. (case 1) (B) Infiltration of large lymphoid cells with pleomorphic nuclei exhibitng angiocentricity. (case 1) (C) Diffuse infiltration of CD56-positive lymphoid cells throughout the dermis and subcutis. (case 2)

MATERIALS AND METHODS

Clinical samples, histologic examination, and immunohistochemical study

In 1998, two cases of nasal T/NK cell lymphoma with LMG syndrome were diagnosed (both are male, 49 years and 70 years) in Kaohsiung Chung-Gaung Memorial Hospital. The presentation, staging, treatment, and clinical course were reviewed. In each patient, a biopsy specimen of the lesional skin was obtained, formalin fixed, and stained with hematoxylin and eosin. Immunohistochemical stains for cytoplasmic CD3, CD4, CD8, CD20, CD43, CD45Ro, CD56, LMP-1, and EBV nuclear antigen-2 (EBNA-2) were also performed. Serologic studies of EBV associated antigens were checked in one patient.

DNA extraction, PCR, cloning and sequencing

The biopsy specimens of both patients were deparaffined and homogenized in lysis buffer containing 10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.5% SDS, 200 μ g/ml proteinase K and was incubated at 55°C for 2 hours. Sodium chloride was added to the solution to a final concentration of 0.2M. DNA was then extracted twice with equal volume of phenol-chloroform (1:1) and followed by chloroform extraction. The aqueous phase was removed from the top layer and RNase was added to a final concentration of 25 μ g/ml. The mixture was incubated at 37°C for 1 h. DNA was further purified by phenol-chloroform extraction and was precipitated with ethanol. The DNA was finally suspended in 100 ml of 10 mM Tris-HCl., 1mM EDTA. Primers corresponding to the BKRF-1 (BamH1 K fragment right open reading frame 1) gene and BNLF-1 (BamH1 N fragment left open reading frame 1) gene of B95-8 strain

were used to detect EBNA-1 and LMP-1 respectively. PCR was carried out in a Perkin-Elmer Cetus Model 4800 Thermal Cycler. The reaction mixture contained 500 mM KCl, 100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$, 1% (w/v) gelatin, 0.1 μm of primer, 0.125 mM of dNTP and 5 units of Taq DNA polymerase. PCR was carried out for 30 cycles at 94℃ for 30 sec, 50℃ for 1 min, and 72℃ for 1 min. Restriction enzymes were purchased from Promega and were used according to the condition recommended by the supplier. The products were analyzed by electrophoresis in a 2% agarose gel and were visualized over UV light.

RESULT

The clinical presentations and laboratory results were summarized in Table I. Immunophenotyping of the lymphoma cells showed NK cell pattern. The presence of EBV infection was confirmed in the immunohistochemical and PCR study (Fig. 2).

DISCUSSION

Nasal or nasal-type T/NK cell lymphoma is a distinct lymphoproliferative disease commonly affecting the nasal cavity and paranasal sinuses.[3,4] Clinically it may present as "lethal midline granuloma" syndrome with progressive ulceration and necrosis of the central face.[5-7] The underlying causes of LMG syndrome include infection, collagen vascular diseases, Wegener's granulomatosis, idiopathic midline destructive disease, and malignant lymphoma.[7,8] In 1966, Eichel described this confusing syndrome pathologically as nonspecific tissue necrosis with angioinvasive infiltrates of atypical lymphocytes, and named it "polymorphic reticulosis".[6] Similar changes of angiocentric infiltration and angioinvasion occur also in

Table I. Clinical characteristics, laboratory findings and immunophenotypes of both cases.

	Case 1	Case 2
Sex/Age (year)	M/49 M/70	
Primary lesion	Left nasal cavity	Left nasal cavity
Other region involvement	Left nasal ala skin and upper lip necrosis; nasal septum perforation	Left paranasal skin necrosis; palate perforation
Stage at diagnosis	II_EA	II_EB
Treatment	CHOP+radiotherapy	Cyclophosphamide+prednisolone
Response	Good response	No response
Prognosis (month)	11 (alive)	4
Serologic Studies of EBV-Ab	EBVCA-IgG > 1:1250 (+) EBVCA-IgA > 1:120 (+) EBVCA-IgM (-) EBNA-Ab (+) EBEA-IgG > 1:80 (+)	N/A
Immunophenotype		
CD3ε n(CD3, cytoplasmic)	(+)	(+)
CD4	(-)	(-)
CD8	(-)	(-)
CD43	(-)	(+)
CD45Ro	(+)	(+)
CD20	(-)	(-)
CD56	(+)	(+)
Immunohistochemistry		
LMP-1	(+)	(+)
EBNA-2	(-)	(-)
Polymerase chain reaction		
LMP-1	(+)	(+)
EBNA-1	(+)	(+)

N/A: nonavailable

lymphomatoid granulomatosis. DeRemee even regarded the two conditions as one disease in different organs.[9] The concept of "angiocentric immunoproliferative lesions, AIL" was proposed by Jaffe, who classified these angiocentric and angioinvasive lesions into 3 grades (Table II).[10,11] AIL commonly affects the upper respiratory tract, and 90% of the cases occurred in the nose and paranasal sinuses.[12] LMG consists of the grade II and grade III AIL changes, and pathologic changes of different grades may occur in different depth of the same lesion.[12] Since the primary nasal neoplastic lesion is frequently encountered with extensive necrosis, as in our cases, repeated biopsies frequently reveal only necrosis. Skin biopsy from the infiltrative rim would be more helpful for the evaluation of atypical lymphocytes.

Although nasal T/NK cell lymphoma is rare in western countries, occasional cases could be found among orientals. Males are affected predominantly and patients had a relatively young median age of 50 years compared with other non-Hodgkin's lymphoma.[4,12,13] Twenty four percent (18 over 76 cases reported in Taiwan) of the patients of nasal T/NK cell lymophoma clinically present as LMG.[12] B symptoms (fever, body weight loss, and night sweat) occur in 21.6% of the patients.[12] Local symptoms of the nose and paranasal sinuses are the features and cervical lymph node enlargement is seldom encountered.[12] Diagnosis is often delayed while the patient is treated for presumed rhinitis or sinusitis. The prognosis is generally poor though it often presents initially with a localized disease. Five-year survival rate of nasal T/NK cell lymphoma after combined radiotherapy and chemotherapy is about 40%.[12]

Table II. Pathologic grading of AIL

Grade I	Polymorphous infiltrate of lymphocytes, plasma cells and histiocytes with or without eosinophilia. No cytologic atypia present.
Grade II	polymorphous infiltrate of small lymphoid cells with some cytologic atypia in a polymorphous inflammatory background
Grade III	Monomorphic small and/or large lymphoid cells infiltrate with marked cytologic atypia, necrosis and inconspicuous polymorphous inflammatory background

Histologically, the atypical lymphoid cells may show a broad spectrum of cell sizes with a common feature of angiocentricity. Coagulative necrosis is nearly always present. Immunophenotyping of these cells commonly show postive CD56, CD2, cytoplasmic CD3, but generally surface CD3 staining is negative and clonal TCR gene rearrangement is not found.[3,13-15]

CD56 (neural cell adhesion molecule) is a surface glycoprotein found in neural/ neuroendocrine tissue, NK cells, and NK-like T cells. It is also expressed on normal dermal microvascular endothelial cells, which might well explain the angiocentricity of CD56-positive tumor cells and their invasion and destruction of dermal vessels.[15] Detection of CD56, one of the most simple and effective method for diagnosing nasal T/NK cell lymphoma,[13,14,16] is suggested to be routinely performed especially when angiocentricity is present histopathologically.

It is interesting that in nasal T/NK cell lymphoma EBV infection was virtually identified,[3] and was suggested to play a role in the pathogenesis. Kanavaros et al. suggested that it is likely that EBV infection has occurred before clonal expression of T cells and might contribute to cellular proliferation and neoplastic transformation.[17] The precise mechanism of EBV infection of neoplastic cells remains speculative since EBV receptor (CD21) is generally not detected. Some authors reported CD21 has been noted during early T-cell ontogeny.[18,19] Su *et al.* presented EBV may induce the expression of many cytokines, such as tumor necrotic factor α nand nuclear factor kappa-B (NF-κB),[3] which could lead to the tumor necrosis in addition to the contributory of angioinvasion. In our cases, EBV-related products of lesional cutaneous tissue were detected directly by immunohistochemistry and PCR technique (Fig. 2).

It is well known that nasopharyngeal

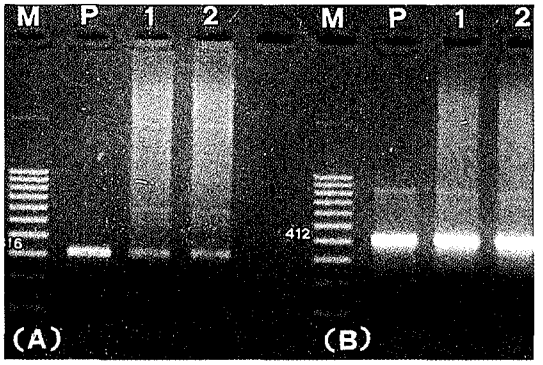

Fig. 2
The PCR products were seperated on a 3% agarose gel with ethidium bromide staining. (**M**, 100bp DNA ladder marker; **P**, B95.8 EBV-containing Marmoset cell line as positive control; **1**, patient 1 genomic DNA; **2**, patient 2 genomic DNA) (A) Primer sequence from BNLF-1 gene: visible bands at 316 bp indicating the presence of LMP-1 in both patients. (B) Primer sequence from BKRF-1 gene: the expected bands at 412 bp indicating the presence of EBNA-1 in both patients.

carcinoma, another EBV-associated malignancy, is also prevalent in south-east Asia with predilection of the same anatomic area. Curiously, they seldom occur in the same patient. It might hint that there are different pathogenetic interactions of the genetic, viral and other possible environment factors between the two diseases.

Nasal T/NK cell lymphoma runs a rapid and fatal course. There has been no general consensus in the standard treatment regimens according to the clinical stage presently. Yet radiation therapy had been generally considered as the main treatment modality.[20-22] It may provide improvement of the local control and disease-free survival especially in those with early stage (Stage I_E). Some authors suggested the primary lesion site should be treated with high-dose radiation therapy of at least 50 Gy since the angiodestructive nature of tumor may increase the numbers of radioresistant hypoxic cells.[22,23] Many reports have discouraged the use of chemotherapy alone or in combination with radiotherapy because of little benefit and a high risk of bleeding and septic complication.[21]

Patients with early stage of disease, those without B symptoms and age younger than 60 years have better response to treatment.[4] High proportion of Ki-67 proliferating cell index[14] and presence of EBV genome[17] were postulated to be correlated with poor prognosis. Anyway, a grave prognosis exists in most cases, especially after dissemination. In case 1, we chose concurrent chemoradiotherapy regarding the good prognostic factors he had as Liang *et al.* suggested.[4] The clinical response was satisfactory but the patient could not tolerate the side effects of concurrent chemoradiotherapy.

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