Table 1. Summary of clinical findings and outcome in 26 patients with duodenal follicular lymphoma

Case	Age/sex	Primary symptoms	Site in duodenum	Stage	Treatment	Relapse (month)[1]	Outcome (month)[2]
1	68/F	Medical check-up	Second portion, multiple	I	Eradication+R		AWOD (75)
2	50/F	Epigastralgia	Near Vater's papilla, multiple	I	Irradiation	LN, submandibular (4)	AWOD (58)
3	49/F	Medical check-up	Near Vater's papilla, single	I	Irradiation		AWOD (62)
4	60/M	Medical check-up	Second portion, single	I	CHOP		AWOD (69)
5	66/M	Medical check-up	Second portion, multiple	I	Observation		AWD (11)
6	52/F	Medical check-up	Near Vater's papilla, single	I	Eradication		AWD (51)
7	60/F	Medical check-up	Near Vater's papilla, single	I	Observation		AWD (39)
8	58/F	Epigastralgia	Near Vater's papilla, single	I	R monotherapy		AWOD (32)
9	72/M	Medical check-up	Second portion, multiple	I	R monotherapy		AWOD (32)
10	53/M	Medical check-up	Second portion, multiple	I	R monotherapy		AWOD (32)
11	46/F	Medical check-up	Second–third portion, multiple	II	R-CHOP		AWD (31)
12	63/M	Medical check-up	Second portion, single	II	R monotherapy		AWD (22)
13	42/M	Left flank pain	Second portion, multiple	II	CHOP		AWOD (57)
14	45/M	Medical check-up	Second portion, multiple	II	R monotherapy		AWD (30)
15	58/M	Right cervical LN enlargement	Near Vater's papilla, single	III	R-CHOP		AWOD (21)
16	52/F	Systemic LN enlargement	Near Vater's papilla, single	IV	C-MOPP		AWOD (96)
17	58/M	Medical check-up	Near Vater's papilla, single	IV	R-C-MOPP		AWOD (77)
18	61/F	Malaise	Second portion, single	IV	R-CHOP	Leukaemic change (12)	DOD (23)
19	49/F	Medical check-up	Bulb–third portion, multiple	IV	R-CHOP		AWOD (49)
20	52/F	Appetite loss and diarrhea	Second portion, multiple	IV	R-CHOP		AWOD (23)
21	62/M	Medical check-up	Bulb–second portion, multiple	IV	R-CHOP		AWD (16)
22	36/F	Abdominal distension	Unknown	IV	R-CHOP		AWOD (42)
23	45/M	Subcutaneous nodule on right upper arm	Near Vater's papilla, single	IV	Irradiation		AWOD (20)
24	46/M	Epigastric discomfort	Second portion, multiple	IV	Unknown		Unknown
25	50/F	Left inguinal LN enlargement	Bulb–second portion, multiple	IV	R-CHOP		AWOD (24)
26	37/M	Weight loss	Near Vater's papilla, multiple	IV	R-CHOP		AWOD (17)

AWOD, alive without disease; AWD, alive with disease; DOD, died of disease; LN, lymph node; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; R, rituximab; C-MOPP, cyclophosphamide, vincristine, procarbazine and prednisone.
[1]Recurrent sites and months after treatment.
[2]Outcome at last follow-up and months after initial diagnosis.

their diagnoses were switched to FL during follow-up. Immunohistochemically, all of the DFLs were positive for CD20, CD10 and Bcl-2, and negative for CD3, CD5 and cyclin D1 (Figure 1). *H. pylori* infection was frequent in both primary and systemic DFLs: eight of 12 (67%) and seven of nine (78%), respectively.

FISH analysis revealed IGH/BCL2 fusion in nine of the 12 cases (75%) of primary DFL and 10 of the 12 cases (83%) of systemic DFL.

Initial therapies for patients with primary DFL included cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) (two cases), rituximab (R) plus CHOP (R-CHOP) (1), irradiation (2), R monotherapy (6) and observation (3). For patients with systemic DFL, initial therapies included R-CHOP (eight cases), cyclophosphamide, vincristine, procarbazine and prednisone (C-MOPP) (1), R-C-MOPP (1) and irradiation (1) (Table 1). Two of the primary DFLs were initially treated by *H. pylori* eradication because their initial diagnosis was MALT lymphoma, and the clinical response to eradication was no change (NC) in both cases. After a median follow-up duration of 40 months (range 11–96 months), 17 of 25 patients were alive without disease, seven were alive with disease and one had died of lymphoma. The latter patient died due to leukemic change of FL 12 months after the diagnosis of systemic DFL. One of the patients alive with disease experienced transformation to diffuse large B-cell lymphoma in a submandibular lymph node 4 months after the diagnosis of primary DFL. The information on the outcome for one patient was not available.

DISCUSSION

Primary FL of the GI tract is rare. Misdraji et al. (16) reported the first case of primary DFL in 1997. Since then,

Table 2. Summary of morphologic, immunohistochemical and FISH analyses in 26 cases of duodenal follicular lymphoma

Case	Grade	Follicular/diffuse[1]	CD20	CD10	Bcl-2	CD3	CD5	Cyclin D1	FISH: IGH/BCL2	*H. pylori*
1	1	Follicular	+	+	+	−	−	−	+	+
2	1	Follicular	+	+	+	−	−	−	+	+
3	1	Follicular	+	+	+	−	−	−	ND	ND
4	1	Follicular	+	+	+	−	ND	ND	ND	ND
5	1	Follicular	+	+	+	−	−	−	−	−
6	2	Follicular	+	+	+	−	ND	ND	−	+
7	1	Follicular	+	+	+	−	ND	ND	+	−
8	1	Follicular	+	+	+	−	−	ND	+	+
9	1	Follicular and diffuse	+	+	+	−	−	ND	−	+
10	1	Follicular	+	+	+	−	−	−	+	−
11	1	Follicular	+	+	+	−	ND	ND	+	+
12	1	Follicular	+	+	+	−	−	ND	+	+
13	1	Follicular	+	+	+	−	ND	ND	+	+
14	1	Follicular	+	+	+	−	−	−	+	−
15	1	Follicular	+	+	+	−	ND	−	−	+
16	1	Follicular	+	+	+	−	ND	ND	+	ND
17	1	Follicular	+	+	+	−	−	−	+	+
18	1	Follicular	+	+	+	−	−	ND	+	+
19	1	Follicular	+	+	+	−	−	−	+	+
20	1	Follicular	+	+	+	−	−	−	+	−
21	2	Follicular	+	+	+	−	−	−	+	−
22	2	Follicular	+	+	+	−	ND	ND	−	+
23	1	Follicular	+	+	ND	−	ND	−	+	ND
24	1	Follicular	+	+	+	−	ND	−	+	ND
25	2	Follicular	+	+	+	−	−	−	+	+
26	1	Follicular	+	+	+	−	ND	ND	+	+

FISH, fluorescence *in situ* hybridization; *H. pylori*, *Helicobacter pylori*; ND, not done.
[1]Categorization as follicular: >75% of lymphoma has follicular pattern; follicular and diffuse: 25–75% follicular pattern.

several other studies have suggested that DFL may be a characteristic clinicopathologic entity due to its localized nature and good prognosis (9,10). Patients usually present with symptoms related to bowel thickening. In some cases, the symptoms are mild and non-specific; patients often have relatively long-standing symptoms before seeking medical attention. In this series, 79% (11 of 14) of primary DFLs were detected at routine medical check-ups. However, most of the patients with systemic DFL complained of symptoms such as lymph node enlargement and weight loss, and DFL was detected through the staging procedure.

All cases involved the second portion of the duodenum, and in 40% of cases, the lesions were found in the vicinity of the Vater's papilla. Yoshino et al. (9) speculated that occurrence of primary DFL at this site might be related to bile duct diseases, as suggested by the female predilection of the disease. However, in the present study, both primary and systemic DFL involved the second portion of duodenum, the speculation was not acceptable for systemic DFL.

DFL is reported to have histologic and immunohistochemical features that are similar to those of nodal FL, and not those of cutaneous FL (10). In the present study, all 26 DFLs showed low-grade histology, a predominant follicular growth pattern and immunohistochemical expression of CD20, CD10 and Bcl-2. *H. pylori* infection was frequent in both primary and systemic DFLs. Toyoda et al. (17) reported regression of DFL after eradication of *H. pylori*. Antibiotic therapy may be effective for the treatment for some patients with DFL, but in two of our primary DFL, the clinical response to eradication was NC.

The majority of FLs arise in lymph nodes and possess a characteristic chromosomal translocation, t (14;18)(q32;q21), juxtaposing the BCL2 gene with an immunoglobulin gene. The translocation is present in up to 85% of cases in Europe and the USA (12), and in ~60% of cases in Japan (13). Among extranodal FLs, cutaneous FL (18) and salivary gland FL (19) have a low incidence of, or lack, t (14;18)(q32;q21). In GI tract FL, previous reports with small

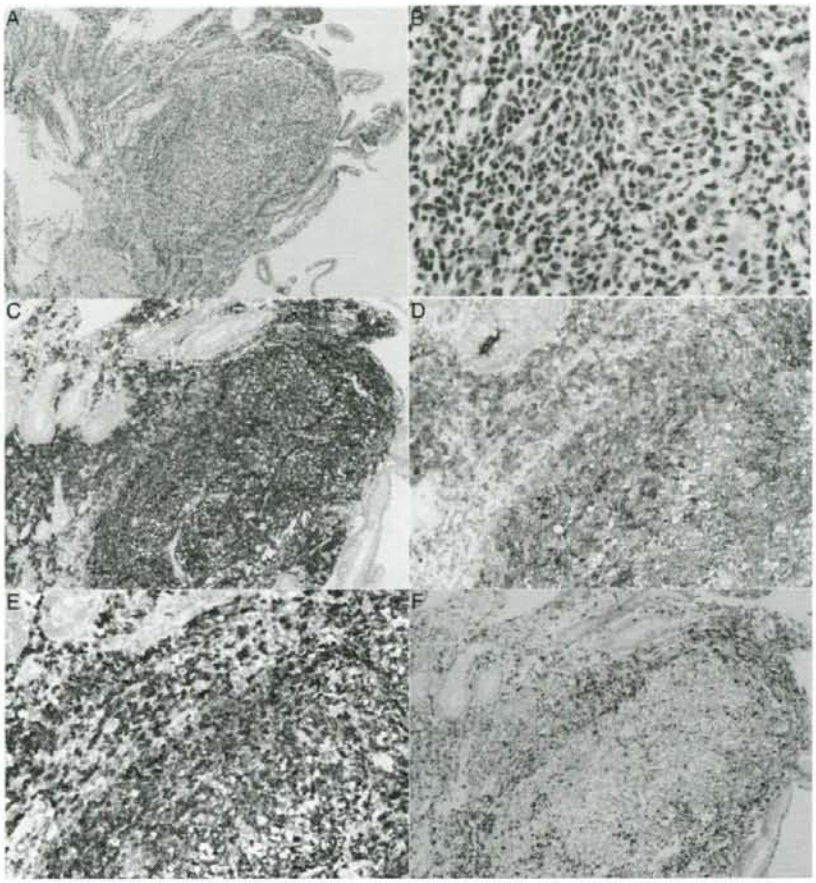

Figure 1. A case of primary duodenal follicular lymphoma, grade 1. The lesion shows well circumscribed follicles (A, HE, ×40), composed of a uniform population of small cleaved cells (B, HE, ×400). Immunohistochemically, CD20 (C, ×100), CD10 (D, ×200) and Bcl-2 (E, ×200) are positive in the follicular area, but CD3 is negative (F, ×100).

numbers of cases suggested occurrence of t (14;18)(q32;q21) (9,10). Our FISH analysis indicated a high incidence of IGH/BCL2 fusion, which was detected in nine (75%) of the 12 primary DFLs, and 10 (83%) of the 12 systemic DFLs. DFL is suggested to resemble nodal FL more closely than FL at other extranodal sites. Moreover, in Japan, the frequency of t (14;18) in DFL tends to be higher than that of nodal FL. It is speculated that IGH/BCL2 fusion-positive cells are likely to involve the duodenum. On the other hand, Sato et al. (11) recently reported that most cases of DFL are localized, and appear to have characteristics intermediate between MALT lymphoma and nodal FL according to IGH/BCL2 and VH usage analyses. They detected IGH/BCL2 fusion using the major break point by polymerase chain reaction (PCR) in 27% of DFLs, which was a lower frequency than our result obtained using FISH. The difference might be due to the low sensitivity of PCR using their primer sets, which detect only about half of the translocations.

Patients with DFL underwent chemotherapy, R-containing chemotherapy, irradiation or observation. Although the follow-up period was short, all patients with DFL except one of systemic DFL were alive at the last follow-up, suggesting that DFL is an indolent disease with a favourable outcome, irrespective of whether it is primary or systemic. As R monotherapy was reported to be an effective treatment for a stage I DFL (20), we have a schedule to evaluate the issue in patients with primary DFL in our institute in the future.

In conclusion, we have revealed the characteristics of DFL; primary DFL is found incidentally at medical check-ups, occurs in the second portion of the duodenum in the vicinity of the Vater's papilla, and appears to have a favourable outcome. It is histologically low grade, and phenotypically and genotypically similar to nodal FL. The characteristics of systemic DFL are similar to those of primary DFL histologically and genotypically. It is found at the time of systemic survey for pre-treatment staging.

Primary DFLs resemble systemic and nodal FLs, except that the former has a high incidence of early stage disease and low-grade histology. Primary and systemic DFLs may constitute a continuous spectrum. The duodenum may be a frequently involved extranodal site of FL with IGH/BCL2.

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Conflict of interest statement

None declared.

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CASE REPORT

Nocardia exalbida brain abscess in a patient with follicular lymphoma

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Abstract Nocardial brain abscess is a rare but severe complication in patients with malignancy. *Nocardia exalbida* was isolated in Japan and characterized within the genus *Nocardia*. We present the first report of *N. exalbida* brain abscess in a 63-year-old male patient with follicular lymphoma. He developed abnormal neurological findings during follicular lymphoma treatment, brain CT revealed ring-enhancing, multiloculated lesions, and *N. exalbida* was detected by aspiration of the lesion. He was successfully treated with trimethoprime–sulfamethoxazole (TMP–SMX) and meropenem without craniotomy or repeat aspirations. It should be noted that such an infection can occur in patients treated with conventional chemotherapy against malignant lymphoma.

Keywords Follicular lymphoma · *Nocardia* · Brain abscess

1 Introduction

Nocardia exalbida is a gram-positive rod and belongs to a genus of aerobic actinomycetes. The genus was initially isolated from cattle in 1888, and the first infected human case was reported in 1890. Thereafter, human nocardiosis has been more frequently reported due to the increase of immunocompromised hosts [1].

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Molecular methods for diagnosis have also enabled us to identify new species of the genus *Nocardia*. At least 13 species which cause human infection have been identified [2]. In Japan, nine species caused nocardial infections in patients from 1999 to 2001 [3]. We herein present a case of follicular lymphoma, developing a brain abscess due to *N. exalbida*, which was a newly identified species isolated from a Japanese case in 2006 [4].

2 Case report

The patient was a 63-year-old male. In September 2000, he was diagnosed as having stage IV follicular lymphoma, grade 2, with systemic lymph node swelling and bone marrow infiltration. The biopsy specimen of a lymph node revealed an increase in follicles composed of large, atypical lymphocytes. On immunohistochemical analyses, the lymphoma cells were CD3 (−), CD5 (−), CD10 (+), CD19 (+), CD20 (+), and bcl-2 (+). Cytogenetic analysis revealed 47, XY, t(14;18)(q32;q21), +18. Soluble IL-2 receptor was not analyzed throughout his clinical course. He was treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP regimen) followed by cyclophosphamide, vincristine, and prednisolone (COP regimen). Complete remission was achieved, and, in January 2001, he was referred to our institution for further follow-up. At his initial visit, laboratory data showed: immunoglobulin (Ig) G of 1,073 mg/dl, IgM of 76 mg/dl, IgA of 156 mg/dl, and a CD4 count of 6 per μl. Thereafter, he developed relapse three times, and each time entered remission with chemotherapy of cyclophosphamide, vincristine, prednisolone, procarbazine (C-MOPP regimen), and/or rituximab.

In 2005, he received three cycles of cladribine monotherapy, for the fifth relapse. Continuous intravenous

infusion of cladribine at 0.09 mg/kg on days 1–7 led to remission, eliminating tumor cells in the peripheral blood and lymph node shrinkage. Upon the start of cladribine therapy, prophylaxis for *Pneumocystis jiroveci* was initiated with oral trimethoprime–sulfamethoxazole (TMP–SMX) twice a week of 320 mg/day as TMP and 1,600 mg/day as SMX in two divided doses, tumor cells in the peripheral blood were eliminated, and lymph nodes were reduced in size.

In December 2005, the C-MOPP regimen was again started due to tumor regrowth. He received six cycles of C-MOPP until August 2006. During the sixth course of C-MOPP in August 2006, he was admitted to our hospital because of febrile neutropenia. Regarding laboratory data, the CD4 count was 14 per μl, IgG 515 mg/dl, IgM 32 mg/dl, and IgA 88 mg/dl. A chest X-ray revealed a nodule in the middle of the left lung field. A CT scan of the chest showed a nodule of 3 cm in diameter in the left inferior segment (S3) of the upper lobe (Fig. 1). All bacteriologic cultures of blood and sputum including ones for acid-fast bacilli were negative. He also underwent bronchoscopy, and culturing including that for acid-fast bacilli and the cytology of bronchoalveolar lavage were negative. He received empirical antibiotic therapy with imipenem for febrile neutropenia, and the lung nodule slightly reduced. Although the nodule did not resolve completely, he became afebrile and his performance status improved. He was subsequently discharged and continued C-MOPP chemotherapy.

He received another three cycles of C-MOPP, but the size of the lung nodule did not change on chest X-ray. His condition was stable, but during the last cycle in November 2006, he developed high-grade fever, headache, nausea, and vomiting and was admitted to our hospital for further examinations. On physical examination, superficial lymph nodes were palpable, he was slightly drowsy, and had a left visual-field defect. Lumbar puncture failed to detect lymphoma cells and bacteria in the cerebral spinal fluid, with a cell count of 14 per μl per three visual fields. A brain CT scan, revealed a ring-enhancing, multiloculated lesion of 3.5 cm in diameter with marginal brain edema in the right occipital lobe (Fig. 2).

Laboratory findings were as follows: hemoglobin 9.3 g/dl; white blood cell count, 5,300/μl; platelets, 15×10^4 per μl; serum sodium, 123 mEq/l; serum chloride, 89 mEq/l; C-reactive protein, 2.0 mg/dl. He developed complete left hemianopsia detected on a visual-field perimetry test, and a CT scan of the chest showed that the nodule remained in the left lung. As differential diagnoses of the brain lesion, the possibilities of lymphoma invasion, infection including acid-fast bacilli and others, fungus, and protozoa, and other malignancies, etc., were raised. In order to make a diagnosis, we performed needle aspiration of the brain lesion. Pus was drawn and its culture yielded gram-positive, aerobic, filamentous bacteria. The bacterium was an aerobic, gram-positive actinomycete, which produced a faint brown to gray-brown vegetative growth with abundant aerial hyphae off-white to whitish-gray with fringes of an orange tinge. Aerial hyphae had a tendency to break up into fragments of irregular length, with the formation of typical chains of spores on the aerial and vegetative mycelium characteristic of *Nocadia* species, consistent with the identification of *Nocardia* (Figs. 3, 4). Chemotaxonomical characteristics confirmed the diagnosis: wall chemotype IV

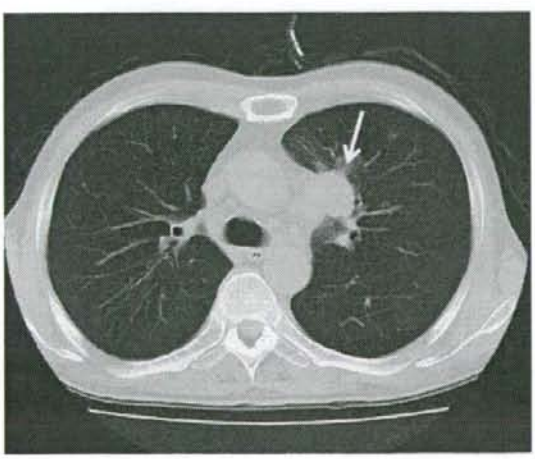

Fig. 1 CT scan of the lung before treatment. A nodule of 3 cm in diameter in the left inferior segment (S3) of the upper lobe is shown

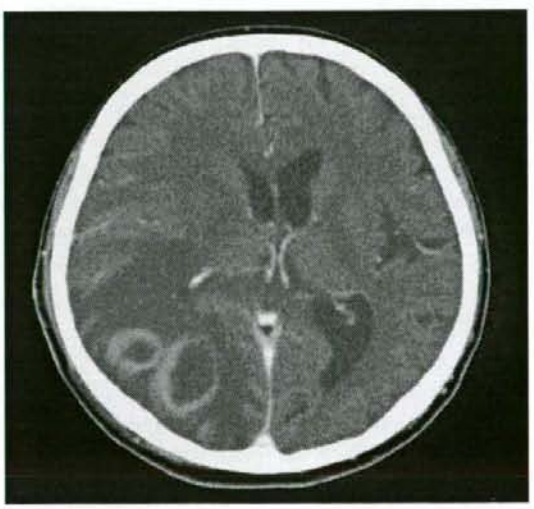

Fig. 2 CT scan of the head before treatment. Ring-enhancing, multiloculated lesions are shown

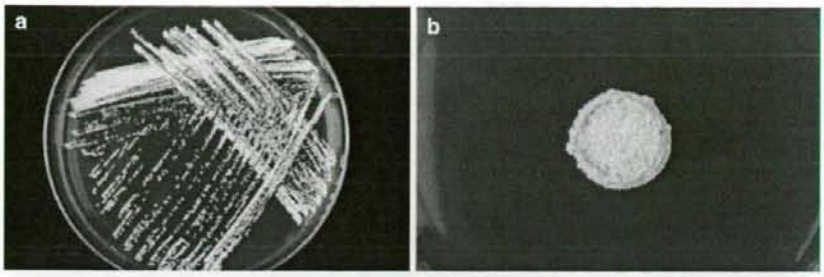

Fig. 3 Colonies of the strain IFM 10794. **a** Microcolonies on BHI agar cultured at 30°C for 7 days; **b** macrocolony on BHI agar cultured at 30°C for 14 days

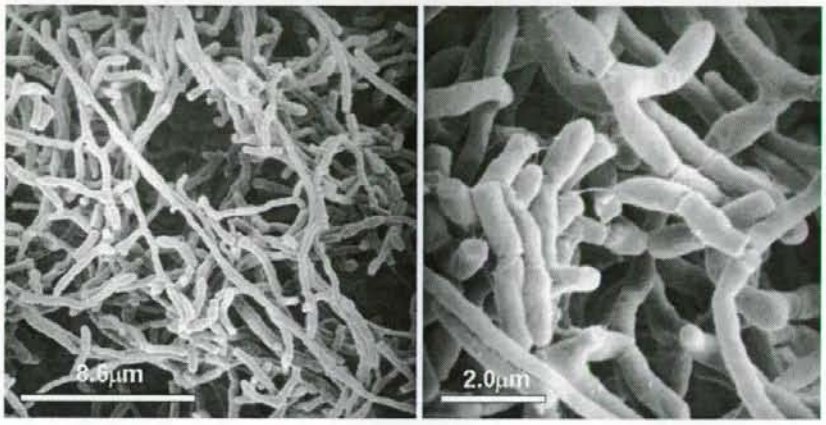

Fig. 4 Scanning electron micrograph of the strain IFM 10794 grown on BHI agar at 30°C for 7 days

contained meso-diaminopemelic acid and MK-$8_{(H4\omega\text{-}cyc)}$, and the mycolic acid type was *Nocardia*. Later, it was identified as *N. exalbida* by a molecular diagnostic method using 16S ribosomal ribonucleic acid (rRNA) gene sequencing, which revealed 99.9% homology with *N. exalbida* (data not shown).

Intravenous TMP–SMX and meropenem at 4 g/day in four divided doses were initiated. The dose of TMP–SMX was 13 mg/kg/day TMP (Total per day: 720 mg) and 65 mg/kg/day SMX (Total per day: 3,600 mg) in three divided doses. The sensitivity test later demonstrated that bacteria were sensitive to both TMP–SMX and carbapenem (imipenem) in Table 1. One month after the start of antibiotic administration, a CT scan of the head and chest showed resolution of the brain abscess and shrinkage of the lung nodule. His neurological symptoms also improved. Totally 2 months later, a CT scan of the head showed the decreased size of the brain abscess and absence of the lung nodule. TMP–SMX was switched to oral administration at a dose of 640 mg/day of the TMP component in two divided doses, and meropenem was discontinued. He was discharged in a stable condition without neurological deficit after four months of therapy. Six months after starting the antibiotic therapy, while this manuscript was in preparation, he was continuing oral TMP–SMX with no recurrence of the brain abscess (Figs. 5, 6).

3 Discussion

Nocardiosis has increased in incidence due to the rise in the number of immunocompromised hosts with steroid therapy, chemotherapy, hematopoietic stem cell and solid organ transplantation, HIV infection, and through improvements in diagnostic techniques [1, 5]. Occasional cases of nocardial brain abscess have been previously reported [6–11]. Bearman et al. [12] reported that CNS nocardial infection showed an incidence as high as 44% among all systemic nocardiosis cases. However, it is still a rare infection as a complication of cancer chemotherapy. According to a previous report, the incidence of nocardiosis in cancer patients was 0.06%, and, among those, CNS nocardiosis occurred in 2% of all cases [13]. Nocardial brain abscess accounts for about 2% of cerebral abscesses [14].

To our knowledge, this is the first reported case of *N. exalbida* brain abscess. Little is known about the clinical behavior of *N. exalbida* infection. This pathogen was first isolated from two Japanese immunocompromised patients with a cutaneous lesion and lung abscess, and was named by the Medical Mycology Research Center, Chiba University [4]. Keratitis caused by this pathogen has also been reported in this country [15]. The patient developed *Nocardia* keratitis after 2 weeks of unsuccessful treatment

Table 1 Drug susceptibility tests

Drug	MIC (susceptibility)	Drug	MIC (susceptibility)
Ampicillin	8	Clarithromycin	<0.5 (S)
Piperacillin	32	Clindamycin	4
Amoxicillin–clavulanate	16 (R)	Minocycline	0.25 (S)
Cefaclor	16	Chloramphenicol	>32
Cefazolin	8	Vancomycin	>32
Cefotiam	2	Ofloxacin	16
Cefditoren	0.12	Fosfomycin	>64
Flomoxef	<0.5	Cefotaxime	0.12 (S)
Imipenem	<0.13 (S)	Ceftriaxone	0.12 (S)
Meropenem	0.5	Cefixime	2
Getamicin	0.5 (S)	Faropenem	2
Arbekacin	<0.25	Levofloxacin	4 (R)
Erythromycin	1	Sulfamethoxazole-trimethoprim (119:6)	0.12 (S)

MIC values in μg/ml

R indicates resistant, *S* sensitive

Susceptibilities to drugs listed in susceptibility tests by the National Committee for Clinical Laboratory Standards are shown

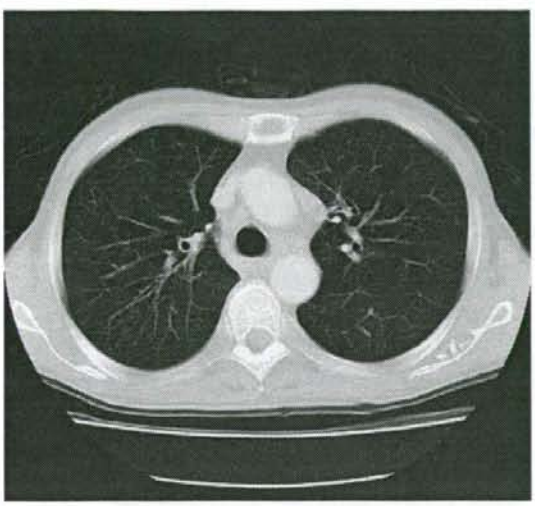

Fig. 5 CT scan of the lung after treatment

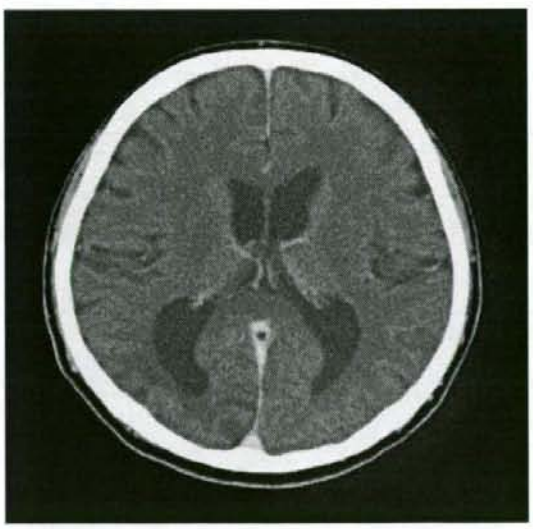

Fig. 6 CT scan of the head after treatment

of a corneal ulcer in her right eye. There have been no reports in other countries. Limited numbers of case reports might suggest that the species is endemic to Japan.

Because of the heterogeneity of the sensitivity to antibiotics among species causing nocardiosis [16], it is important to identify *Nocardia* species. For example, TMP–SMX is effective against *N. brasiliensis*, *N. transvalensis*, and the *N. asteroides* complex, but is not against *N. otitidiscaviarum* [16, 17]. The diagnostic method using 16S rRNA gene sequencing, which is now a more precise method to identify species, is a prerequisite for the selection of therapeutic drugs. We identified that the present case was caused by *N. exalbida*. This species has been reported to be sensitive to TMP–SMX [4], which prompted us to use the regimen on the initial diagnosis of *Nocardia*. In fact, *N. exalbida* in this patient was subsequently found to be sensitive to TMP–SMX.

In this case, we added meropenem to TMP–SMX, which appeared to be successful. TMP–SMX is used widely as the mainstay of therapy for nocardial infection [16, 18]; however, in patients with severe infection or disseminated nocardiosis including CNS involvement and an immunosuppressed status, TMP–SMX monotherapy showed a high mortality rate [18], and combined therapy with amikacin, imipenem, or third-generation cephalosporin is recommended. Considering the significance of these reports, we administered higher doses of TMP–SMX (13 mg/kg TMP and 65 mg/kg SMX) intravenously, combined with meropenem at 4 g/day instead of the commonly used

imipenem. We confirmed that *N. exalbida* was sensitive to carbapenem in this patient by susceptibility testing, and treatment proved to be successful. Since meropenem penetrates the CNS well and causes fewer adverse reactions including seizures compared to imipenem, the agent might be an alternative choice, especially in patients with CNS nocardiosis [19, 20].

The optimal duration of antibiotic therapy is unknown. We are planning to continue antimicrobial therapy for at least 12 months with low-dose maintenance therapy using TMP–SMX, because there are reports of relapse after the discontinuation of therapy, particularly in immunocompromised hosts [17, 18].

We did not opt for surgery with excision or drainage in this case, even with the multiloculated nocardial brain abscess, which is usually considered a surgical indication. As abnormal neurological findings did not exacerbate after the start of antibiotics and a CT scan of the head showed improvement of the brain abscess, we were able to avoid surgery [21]. Mamelak et al. [21] reported that an immunocompromised status or multiple abscesses were significantly poor prognostic factors. They also proposed that all abscesses larger than 2.5 cm in diameter should be aspirated, regardless of the immune status of the patient, and craniotomy should be performed to excise the abscess if any abscess enlarges after 2 weeks of antibiotic therapy or fails to shrink after 4 weeks of therapy. Although this patient had the risk factor of being immunocompromised, he showed improvement by antibiotics alone and the abscess shrunk within 4 weeks of therapy.

We noted the pulmonary nodule before the detection of the brain abscess. Considering the usual clinical course of this condition, the pulmonary nodule may have preceded the brain lesion, although bronchoscopy was non diagnostic. This negative culture could be attributed to the difficulty to test for *Nocardia* [16, 17]: the slow growth is sometimes masked by normal flora. If this is the case, it agrees with the previous reports that concurrent nocardial infection outside the CNS occurs in up to 66% of patients in CNS nocardiosis [21]. We should consider nocardiosis in the differential diagnosis in immunocompromised patients, even using standard chemotherapy for hematological malignancy.

Although he had received primary prophylaxis with oral TMP–SPX (320 mg/day of the TMP component) twice weekly, which prevents not only infection of *P. jiroveci* but also nocardial infection, and compliance with medication was very good, he developed disseminated nocardiosis. This is consistent with previous reports that TMP–SMX administered twice or three times a week did not prevent the development of nocardial infection in patients after bone marrow transplantation [22–24]. The reasons for the ineffectiveness of TMP–SMX in this case remain unknown; however, cladribine therapy might have contributed. One year before the onset of nocardiosis he received cladribine, which causes prolonged lymphocytopenia. Seymour JF et al. [25] previously reported that it took 40 months for CD4 counts to recover to the normal range in patients treated with cladribine for hairy-cell leukemia. In this case, the CD4 count, Ig, and blood culture had not been monitored before manifestation of the CNS disease.

In conclusion, we have reported the first case of an *N. exalbida* brain abscess successfully treated with antibiotics. Such an infection should be considered a differential diagnosis in immunocompromised patients with hematological malignancies whose infectious lesions in the lung and brain are refractory to empirical antibiotic therapy.

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ORIGINAL ARTICLE

Stromal cells in bone marrow play important roles in pro-inflammatory cytokine secretion causing fever following bortezomib administration in patients with multiple myeloma

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Abstract Bortezomib blocks the activation of nuclear factor-κB-mediated pro-inflammatory cytokines, however, systemic inflammatory symptoms following bortezomib administration have been reported, although their mechanisms remain elusive. Serum samples were obtained from five patients, who participated in a phase I/II study of Japanese patients with relapsed or refractory multiple myeloma (MM), and developed cyclic fever following bortezomib administration, to measure cytokine levels. Significant correlations between interleukin (IL)-6 or interferon (IFN)-γ and the body temperature were observed in two patients each. Furthermore, we found that IL-6 elevation was not observed after the addition of bortezomib to any examined MM cells alone, but was noted in a case of bone marrow stromal cells (BMSCs) of macrophage origin alone or co-cultured with MM cells. Similarly, a marked increase in IFN-γ levels was induced by adding bortezomib to BMSCs of fibroblast origin. Although this investigation was a preliminary study with a small number of patients, our results suggested that pro-inflammatory cytokines causing bortezomib-associated fever were secreted from BMSCs rather than MM cells.

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Keywords Multiple myeloma · Bortezomib · Bone marrow stromal cells · Pro-inflammatory cytokine · IL-6 · IFN-γ

1 Introduction

Multiple myeloma (MM) is one of the incurable hematological malignancies that continues to relapse or progresses even with conventional treatment modalities. Since conventional-dose melphalan-predonisone chemotherapy and high-dose therapy of melphalan were established, there have been no available treatment options with durable efficacy for patients with relapsed or refractory MM for the past several decades, and so more efficacious therapies are strongly desired. The first of the class of proteasome inhibitors, bortezomib, is a novel agent selectively targeting the 20S proteasome, which exhibits an anti-myeloma effect in part by the inactivation of nuclear factor (NF)-κB-mediated pro-inflammatory cytokines [1], and, therefore, would be expected to have anti-inflammatory effects [2]. Indeed, promising results using bortezomib have already been reported not only in patients with relapsed or refractory MM [3–5], but also in those with previously untreated MM [6].

On the other hand, there are some reports of systemic inflammatory symptoms including lung injury [7–9] and fever [3, 7, 10] following bortezomib administration, which were not associated with infection, although their mechanisms remain elusive. Although the incidence of fever following bortezomib administration was around 20% [3, 4], fever is one of the adverse events which cannot be ignored because it has a marked influence on the patient's activities of daily living. Moreover, it might lead to the development of lung injury, which sometimes results in a

fatal outcome [7–9]. Therefore, clarification of the mechanism of fever following bortezomib administration is important in order for clinicians who treat patients with proteasome inhibitors for MM to understand the contradictory inflammatory syndromes after bortezomib administration.

In the present study, we examined the relationship between serum cytokines and fever following bortezomib administration in patients with MM using statistical analyses, and elucidated the exact mechanism of fever employing cell lines derived from MM and/or bone marrow stromal cells (BMSCs).

2 Materials and methods

2.1 Clinical data collection

We hypothesized that fever after bortezomib administration was associated with increased serum cytokine levels. To investigate this, we analyzed several serum cytokines such as interleukin (IL)-6, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, the clinical symptoms, and their relationship. Ten patients were enrolled from our institution in a multicenter phase I/II study of bortezomib involving 34 Japanese patients in total with relapsed or refractory MM. Bortezomib of 0.7 mg/m^2 ($n = 2$), 1.0 mg/m^2 ($n = 2$), and 1.3 mg/m^2 ($n = 6$) was administered by bolus intravenous injection on days 1, 4, 8, and 11 every 3 weeks. Serum samples were collected before each predose of cycle 2, and 12–24 h after each administration from five patients who developed fever following bortezomib administration in cycle 1 and gave written informed consent. Three (patients 6, 9, and 10) of the remaining five patients did not develop fever, and the other two patients (patients 7 and 8) did not consent to blood sampling. Serum levels of IL-6, IFN-γ, and TNF-α were examined by chemiluminescent enzyme immunoassay, enzyme-linked immunosorbent assay (ELISA), and enzyme immunoassay, respectively. We defined fever following bortezomib administration as a transient fever $\geq$37.5°C, measured on axillary body surfaces, which occurred within 36 h after administration. The responses were determined according to European Group for Blood and Marrow Transplantation criteria [11].

2.2 Cell lines and reagents

As for human MM cell lines, MM.1S was kindly provided by Dr Steven Rosen (Northwestern University, Chicago, IL, USA), and U266 and RPMI8226 were purchased from the American Type Culture Collection (Manassas, VA, USA). All MM cells were seeded at a density of 1×10^5 cells/well in 24-well plates (Iwaki, Tokyo, Japan) and cultured in RPMI1640 (Sigma Chemicals, St Louis, MO, USA) containing 10% fetal bovine serum (FBS) (Bioserum, VIC, Australia). After MM cells were incubated with 5 nmol/l bortezomib (Millennium Pharmaceuticals, Inc., Cambridge, MA, USA) for 16 h, the plates were centrifuged at 1,500 rev/min for 5 min to collect the supernatant. As for the various cloned stromal cells established from human bone marrow, LP101, HAS303, and AA101 [12, 13] were seeded at a density of 5×10^4 cells/well in 24-well plates and cultured in Iscove's modified Dulbecco's medium (IMDM; Gibco BRL, Grand Island, NY, USA) containing 10% FBS, and incubated with bortezomib for 48 h. Before co-culture with BMSCs, the culture medium of MM cells, RPMI1640, was replaced with IMDM containing 10% FBS and cultured for 2 weeks. Then, the culture medium of BMSCs was discarded and BMSCs were co-cultured with MM cells. After co-culture for 24 h, BMSCs and MM cells were further incubated with 5 nmol/l bortezomib for 16 h.

2.3 Cytokine analyses of supernatants

Interleukin-6 was measured using a Human Interleukin-6 EASIA^{TM} ELISA kit (BioSource International, Inc., Camarillo, CA, USA), and IFN-γ and TNF-α were measured using the Cytometric Bead Array System (BD Biosciences, San Jose, CA, USA).

2.4 Statistical analyses

Wilcoxon's signed rank test and Student's *t*-test were used to analyze patient data and values of cytokines in cultured cells before and after bortezomib administration in statistical comparisons, respectively. Spearman's rank correlation test was used to examine correlations between serum cytokines and body temperature (BT). All statistical analyses were performed with SPSS version 11.0J. $P < 0.05$ was considered significant.

3 Results

3.1 Patient characteristics and analyses of serum cytokine levels

The details of the ten patients who were treated at our institution are summarized in Table 1. The median age was 64 years (range 35–73) with six male and four female patients. Seven of the ten patients developed fever after bortezomib administration: neither of the two patients in the 0.7 mg/m^2 cohort, both in the 1.0 mg/m^2, and five of six in the 1.3 mg/m^2. The maximum grade of fever was grade 2 according to the National Cancer Institute

Table 1 Patient characteristics

Patient number	Gender	Age (y)	Days from disease onset to bortezomib administration	Prior treatment	Type of myeloma	Stage (D–S)	Dosage of bortezomib (mg/m^2)	Fever[b] (grade)	Cytokines correlated with fever	Response to bortezomib
1[a]	F	35	593	VAD, COP–MP	IgA-κ	IIA	1.0	Yes (2)	IL-6	NC
2[a]	M	68	1,375	COP–MP	IgG-κ	IIA	1.3	Yes (2)	IL-6[c]	NC
3[a]	M	73	878	COP–MP	IgA-κ	IIIA	1.3	Yes (2)	IFN-γ	PR
4[a]	M	62	418	MP	IgG-κ	IIIA	1.3	Yes (2)	IFN-γ	CR
5[a]	M	67	615	VAD	IgA-κ	IIIA	1.3	Yes (2)	IL-6	NC
6	M	54	3,022	VAD, VMCP, MP, Thalidomide	IgG-κ	IIIA	1.3	No	ND	NC
7	F	57	1,412	ROAD, Thalidomide, VAD, HD-CPA, Melphalan/ASCT, Thalidomide	IgA-λ	IIIA	1.3	Yes (1)	ND	nCR
8	F	56	1,516	ROAD–IN, MCNU–MP, MCNU–VMP, VAD, Thalidomide–DEX	IgG-κ	IIIA	1.0	Yes (1)	ND	NE
9	M	66	480	VAD	IgA-κ	IIA	0.7	No	ND	PD
10	F	69	1,666	COP–MP, Navelbine, COP–MP	IgG-κ	IIIA	0.7	No	ND	NC

y Years, *D–S* Durie-Salmon, *M* male, *F* female, *VAD* vincristine, doxorubicin, and dexamethasone, *COP* cyclophosphamide, vincristine, and prednisolone, *MP* melphalan and prednisolone, *VMCP* vincristine, melphalan, cyclophosphamide, and prednisolone, *ROAD* ranimustine, vincristine, melphalan, and dexamethasone, *HD-CPA* high-dose cyclophosphamide, *ASCT* autologous stem cell transplantation, *ROAD-IN* ROAD and interferon, *MCNU* ranimustine, *VMP* vincristine, melphalan, and prednisolone, *DEX* dexamethasone, *ND* not done, *NC* no change, *PR* partial response, *CR* complete response, *nCR* immunofixation-positive CR (near CR), *NE* not evaluable, *PD* progressive disease

[a] Serum samples were obtained from patients who gave written informed consent to participate in this study

[b] Fever following bortezomib administration was defined as a transient fever (at least 37.5°C axillary temperature) which occurred within 36 h after drug administration and was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0

[c] Patient number 2 showed a tendency toward a correlation between serum IL-6 and fever

Common Toxicity Criteria Version 2.0 in all patients who developed fever following bortezomib administration. Three of the seven patients who developed fever achieved objective responses (one each of complete response, immunofixation-positive complete response, and partial response). In contrast, none of the three patients (two received bortezomib at a dose of 0.7 mg/m^2 and one at 1.3 mg/m^2) without fever had objective responses (Table 1).

Recurrent fever with each injection started from day 2 in three (patients 1, 2, and 3), from day 5 in one (patient 5), and from day 9 in one (patient 4) of five examined patients. BT ($P < 0.01$) and levels of IL-6 ($P < 0.01$) and IFN-γ ($P < 0.01$) in their serum significantly increased after bortezomib administration (Fig. 1a–c), whereas no one showed significant changes in their serum levels of TNF-α (data not shown). Elevations in their levels of serum IL-6 and IFN-γ following bortezomib administration were transient. Significant correlations were observed between serum IL-6 levels and BT in two patients (patient 1, $r = 0.9$, $P < 0.01$; patient 5, $r = 0.87$, $P < 0.01$), and between serum IFN-γ levels and BT in another two patients (patient 3, $r = 0.7$, $P = 0.05$; patient 4, $r = 0.76$, $P = 0.03$). Another patient showed a tendency toward a correlation between serum IL-6 levels and BT (patient 2, $r = 0.67$, $P = 0.07$) (Table 1). We analyzed serum C-reactive protein (CRP) levels as well as serum cytokines (IL-6, TNF-α, and IFN-γ) in the five patients who developed fever following bortezomib administration. Serum CRP levels significantly increased after bortezomib administration ($P = 0.04$), whereas no significant correlation was observed between serum CRP and serum IL-6 levels (data not shown). In the five patients, objective responses (one each of complete and partial responses) to bortezomib were observed only in patients who demonstrated an elevation of serum IFN-γ levels (Table 1).

3.2 Mechanism of cytokine elevation

Although no elevation of IL-6 levels in the supernatant was observed after the addition of bortezomib to MM cells alone (Fig. 2a), bortezomib increased IL-6 levels in the supernatant of LP101 cells, which originated from macrophages, when co-cultured with MM cells except for U266 (Fig. 2b) or without MM cells (Fig. 2c). Similarly, a marked elevation of IFN-γ levels was induced by adding bortezomib to AA101, which was originated from fibroblasts, without MM cells (Fig. 2d); however, bortezomib did not induce IL-6 secretion from AA101 cells either alone (Fig. 2c) or after co-culture with MM cells (data not shown). No significant elevation of IFN-γ levels in the supernatant was observed after the addition of bortezomib to MM.1S cells alone (data not shown). Bortezomib did not increase IFN-γ levels in the supernatant of U266 or RPMI8226 cells either. In the same combination between MM cells and BMSCs as used in the experiments shown in Fig. 2b, no elevation of IFN-γ levels in the supernatant was observed in any of the examined combinations after the addition of bortezomib (Fig. 2e). Although bortezomib did not increase IL-6 levels in the supernatant of AA101 cells, which originated from fibroblasts, when co-cultured with MM cells (Fig. 2f), an elevation of IFN-γ levels was induced by adding bortezomib to AA101 only when co-cultured with MM.1S (Fig. 2g). Elevation of TNF-α was not observed in any combination between MM cells and BMSCs examined (data not shown). After bortezomib administration, HAS303 cells, which were of endothelial cell origin, either with or without MM cells, did not induce IL-6 (Fig. 2c) or IFN-γ (Fig. 2d) elevation.

Furthermore, we assumed that some substance in the supernatant of MM cell culture media can induce IL-6 secretion, and the elevation of IL-6 levels was observed when only the supernatant obtained from the culture medium of MM.1S cells after bortezomib administration was added to LP101 without MM.1S cells (Fig. 2h).

4 Discussion

Because bortezomib has been shown to inhibit interactions between myeloma and stromal cells by interfering with the NF-κB-dependent induction of pro-inflammatory cytokine

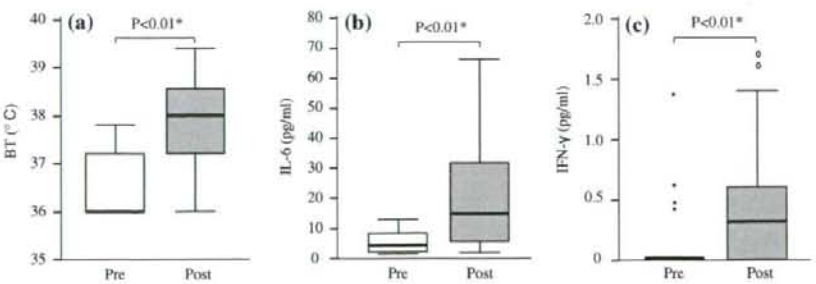

Fig. 1 Analyses of body temperature (BT) and serum cytokine levels. In five patients, their BT (**a**) and serum levels of IL-6 (**b**), and IFN-γ (**c**) significantly increased after bortezomib administration

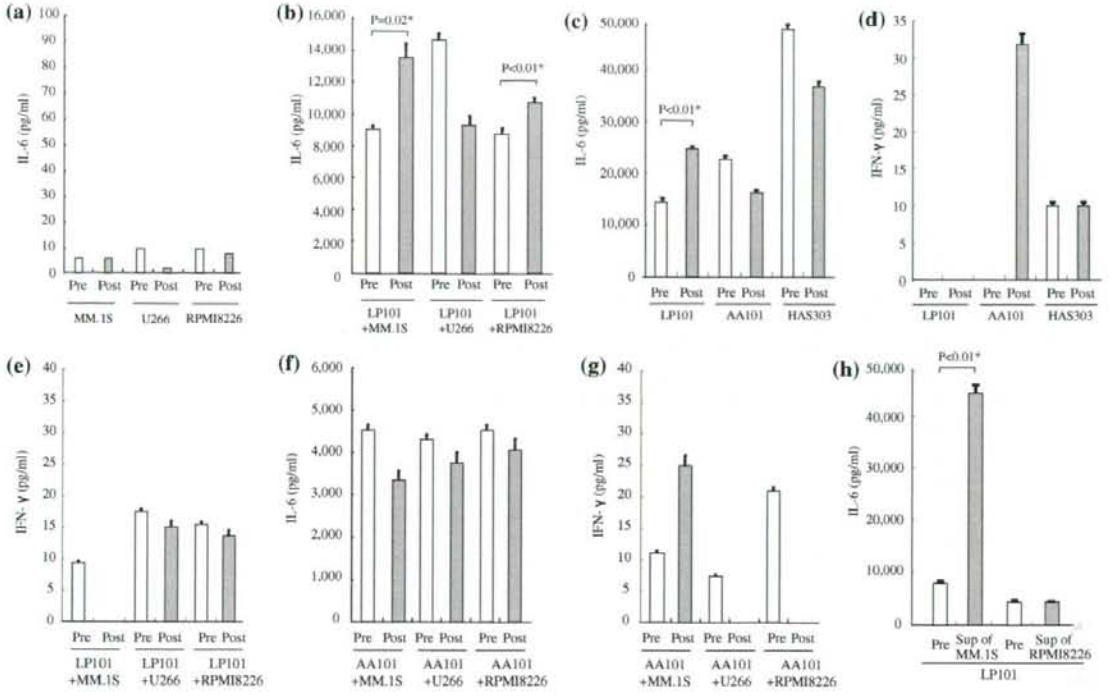

Fig. 2 Levels of cytokines in various combinations between multiple myeloma (MM) and bone marrow stromal cells. The elevation of IL-6 levels in the supernatant was not observed after the addition of bortezomib to any examined MM cells alone (**a**). Bortezomib augmented IL-6 secretion from LP101 cells when co-cultured with MM cells except for U266 (**b**). Bortezomib increased the level of IL-6 secretion from LP101 cells without MM cells (**c**). A marked elevation of IFN-γ levels was induced by adding bortezomib to AA101 cells in the absence of MM cells; however, IFN-γ was not detected at all either before or after bortezomib administration in the supernatant of LP101 cells, and the levels of IFN-γ were not changed after the addition of bortezomib to HAS303 cells (**d**). In the same combination as shown in Fig. 2b, no elevation of IFN-γ levels in the supernatant was observed after the addition of bortezomib (**e**). Although bortezomib did not increase IL-6 levels in the supernatant of AA101 cells, which originated from fibroblasts, when co-cultured with MM cells (**f**), the elevation of IFN-γ levels was induced by adding bortezomib to AA101 after co-culture with MM.1S (**g**). A marked increase in IL-6 levels was observed even when only the supernatant of the MM.1S culture medium after incubation with bortezomib was added to LP101 but not RPMI8226 (**h**). All experiments were performed in triplicate, and *error bars* indicate the mean value $\pm$ SD. *P*-values were considered significant when <0.05. Only significant *P*-values are presented in figures. *Pre* and *Post* The values before and after bortezomib administration, respectively, *Sup* the supernatant obtained from the culture medium of MM.1S or RPMI8226 cells after incubation with bortezomib

secretion from MM cells and exerting an antiangiogenic activity [1], bortezomib is believed to have anti-inflammatory effects [2]. On the contrary, our clinical observations suggested that the BT and serum levels of IL-6 and IFN-γ significantly increased after bortezomib administration, and fever following bortezomib administration was correlated with the increase in levels of serum IL-6 or IFN-γ. Therefore, we postulated that these cytokines causing the bortezomib-associated fever were released from BMSCs rather than MM cells themselves. Indeed, we found that pro-inflammatory cytokines, such as IL-6 and IFN-γ, were secreted from BMSCs, especially macrophages and fibroblasts, respectively, after incubation with bortezomib in vitro.

Some previous reports described that no significant changes [14] or decreases [15] in levels of serum IL-6 were observed after bortezomib administration in patients with various hematological diseases including MM. This discrepancy between these previous reports and our results, in which serum levels of these cytokines increased after bortezomib administration, might be attributable to differences in the timing of serum sample collection. Serum samples were collected before and 12–24 h after bortezomib administration in our study, however, those were collected before and several cycles after administration in the previous study [14]. Furthermore, there were some reports concerning the increase in levels of serum IL-6, which might be associated with inflammatory symptoms

such as lung injury or skin rash [9, 16], although the number of patients was small. These observations are partially consistent with our results.

Our results suggested that pro-inflammatory cytokines were secreted from BMSCs due to the direct effects of bortezomib on BMSCs. On the contrary, Hideshima et al. described that bortezomib decreased IL-6 levels in the supernatant of BMSCs, which were obtained from bone marrow specimens of patients with MM, when co-cultured with or without MM.1S [1]. This discrepancy might result from differences in BMSCs examined in the two studies. The BMSCs used in the previous report were the bulk of BMSCs obtained from patients with MM [1]. On the other hand, each of the BMSCs we used was well-characterized and originated from macrophages, fibroblasts, or endothelial cells [12, 13]. Our results suggested that macrophages played a role in cytokine secretion specific to IL-6 and fibroblasts did specific to IFN-γ after bortezomib administration. The quantity of IL-6 mRNA in LP101 cells before bortezomib administration and after incubation with bortezomib for 48 h was analyzed using the StepOnePlus™ Real Time PCR System (Applied Biosystems, Tokyo, Japan). The cycle threshold values after were lower than those before bortezomib administration (data not shown). This result of real-time PCR was consistent with our experimental results regarding elevated IL-6 following bortezomib administration measured using the ELISA kit.

We further examined whether bortezomib directly affects BMSCs, and found that IL-6 levels were augmented when only the supernatant obtained from the culture medium of MM.1S cells after bortezomib administration was added to LP101 without MM.1S cells (Fig. 2h). Therefore, we suggest that some kind of material [17] secreted from dead MM cells acted on BMSCs. We analyzed IL-1β as well, because IL-1β secreted from MM cells is known to be a potent inducer of IL-6 production [18]. However, IL-1β elevation was not observed in the supernatant of the medium with MM cells examined between pre-dose and after the administration of bortezomib (data not shown).

Not only fever following bortezomib administration but also lung injury has been reported [7–9]. Based on the present results, we speculate that lung injury after bortezomib administration might also be caused through a similar inflammatory process in the bone marrow milieu, because macrophages and fibroblasts are also structural elements of the alveolar septa. Corticosteroids exert anti-inflammatory effects in part by abrogating the proliferation of macrophages and blocking the production of pro-inflammatory cytokines such as IL-6 and IFN-γ. In fact, previous reports showed that combination therapy with corticosteroids decreased the incidence of complications caused by bortezomib [7–9].

In our results, there was a tendency toward a correlation between BT and objective responses, and objective responses were observed only in patients who demonstrated elevated serum IFN-γ levels (Table 1), although the number of patients examined was too small to draw any definite conclusions concerning the predictive factor of the clinical response after bortezomib administration. In the same way, we were not able to draw any conclusions concerning the relationship between serum IL-6 levels and the clinical response after bortezomib administration. Because elevations in the levels of serum IL-6 following bortezomib administration were transient, we suggest that a transient elevation in the levels of IL-6 secreted from BMSCs has little effect on the growth of MM cells.

In this preliminary study, the number of patients was too small to draw any definite conclusions. Nevertheless, our experimental results regarding the elevated cytokine levels following bortezomib administration were consistent with our clinical observations. We believe that our findings provide a valuable clue for clinicians who treat patients with MM with proteasome inhibitors to understand the contradictory inflammatory syndromes after bortezomib administration.

In conclusion, our study revealed that fever following bortezomib administration was correlated with pro-inflammatory cytokines such as IL-6 or IFN-γ, which were secreted from macrophages or fibroblasts, respectively. Our results clarified the validity of using corticosteroids together with bortezomib in addition to its effectiveness in combination [1, 3]. Because this investigation was a preliminary study involving a small number of patients, the causal protein for pro-inflammatory cytokines secretion from BMSCs and the relationship among systemic inflammatory symptoms following bortezomib administration and other cytokines should be further investigated.

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CASE REPORT

Progressive multifocal leukoencephalopathy in a patient with B-cell lymphoma during rituximab-containing chemotherapy: case report and review of the literature

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Abstract Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by the JC polyomavirus. We describe a rare case of PML in a 48-year-old female patient with diffuse large B-cell lymphoma who received rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) therapy. While she was undergoing five cycles of R-CHOP, she noticed gradually progressive neurological symptoms, such as slurred speech and gait disturbance, and she eventually developed high-grade fever. She also developed *Pneumocystis jiroveci* pneumonia. The neurological symptoms deteriorated thereafter, and she developed spastic quadriparesis and bulbar palsy. Magnetic resonance imaging showed hyperintensity within the right cerebellar hemisphere on T2-weighted images. Polymerase chain reaction-based tests of the cerebrospinal fluid revealed the presence of the JC virus. Despite intravenous and intrathecal cytarabine treatment, the patient died of PML 5 months after it was diagnosed. Retrospective analysis of her laboratory data showed that her $CD4^+$ T-cell count before R-CHOP therapy had decreased to 68 μL^{-1}. Thus, when administering rituximab-containing chemotherapy, even to patients with no prior history of opportunistic infections, attention should be paid to the potential occurrence of PML, particularly in patients with low $CD4^+$ T-cell counts.

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Keywords Progressive multifocal leukoencephalopathy · Lymphoma · Rituximab · $CD4^+$ T-cell counts · JC virus

1 Introduction

Progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (CNS), is associated with high rates of morbidity and mortality, and occurs almost exclusively in immunocompromised patients [1]. The JC virus (JCV), a human polyomavirus, is the etiologic agent of PML [2]. It accumulates to high concentrations in oligodendrocytes, causing their destruction by cytolysis [3], and accumulates in astrocytes as well. PML was previously considered a very rare disease; however, its prevalence in association with acquired immunodeficiency syndrome (AIDS) has increased steadily in recent years. However, there have been several cases of PML reported in patients with hematological malignancies not associated with AIDS but related to fludarabine therapy, mainly those diagnosed with chronic lymphocytic leukemia [4, 5]. The newer forms of treatment, such as purine analogs for hematological malignancies, have augmented the incidence of PML. We herein describe an extremely rare case of PML that developed in a patient with diffuse large B-cell lymphoma (DLBCL) during cyclophosphamide (CPA), doxorubicin (DXR), vincristine (VCR), and prednisolone combined with rituximab (R-CHOP) therapy, and review the relevant literature.

2 Case report

In June 2006, a previously healthy 48-year-old Japanese female complained of severe pain in her buttocks and was

referred to our hospital. A computerized tomography (CT) scan revealed an extensive osteolytic mass in her right iliac region and para-aortic lymphadenopathy. An open biopsy and subsequent histological examination of the right iliac mass revealed it to be DLBCL. The patient was diagnosed with stage II disease, and classified as being in a low-risk group by the International Prognostic Index criteria. She was treated with the CHOP regimen (day 1, intravenous: CPA 750 mg m^{-2}, DXR 50 mg m^{-2}, and VCR 1.4 mg m^{-2}; days 1–5, oral: prednisolone 100 mg) and concurrent rituximab at a dose of 375 mg m^{-2}; she tolerated the treatment well.

In October 2006, while she was undergoing five cycles of chemotherapy and achieved a complete response (CR), she noticed gradually progressive neurological symptoms, such as double vision, slurred speech, clumsiness of the right hand, and gait disturbance. Her neurological symptoms continued to worsen, and she eventually developed high-grade fever and was admitted to our hospital. On physical examination, all vital signs except the body temperature were normal. Breath sounds were clear and oxygen saturation was normal. She appeared fully alert and oriented, although neurological examination revealed cerebellar dysmetria by finger-to-nose and heel-to-knee tests, which was worse on the right side. The patient was fully ambulatory, although her gait appeared relatively wide-stanced. There were no signs of sensory disturbance or unilateral pyramidal tract lesions.

Laboratory studies revealed a leukocyte count of 9,200 μL^{-1} (neutrophils, 8,400 μL^{-1}; lymphocytes, 460 μL^{-1}), hemoglobin of 10.6 g μL^{-1}, platelet count of 37.5×10^4 μL^{-1}, LDH of 281 IU L^{-1} [normal range (NR), <229 IU L^{-1}], and C-reactive protein of 12.5 mg dL^{-1} (NR <0.1 mg L^{-1}). Lymphocyte subset analysis revealed an absolute $CD4^+$ T-cell count of 68 cells μL^{-1}. Serum immunoglobulin (Ig) concentrations were 720 mg dL^{-1} IgG (after that, the level of IgG was reduced to 391 mg dL^{-1}), 256 mg dL^{-1} IgA, and 74 mg dL^{-1} IgM. Her serum level of soluble interleukin-2 receptor was elevated to 1,490 U mL^{-1}(NR <531 U mL^{-1}). Her serology was negative for hepatitis B, hepatitis C, syphilis, human immunodeficiency virus (HIV), and human T-cell leukemia virus type I. Routine cultures for bacteria and fungi were negative. The total cell count in the cerebrospinal fluid (CSF) was 1 cell μL^{-1}, with normal levels of glucose and protein, and a negative Gram's stain. Herpes simplex virus DNA was not detected in the patient's CSF by the polymerase chain reaction (PCR).

The patient was suspected to have CNS involvement by lymphoma cells. However, a total body CT scan did not reveal any signs of recurrent or systemic disease, and gadolinium-enhanced cranial magnetic resonance imaging (MRI) did not identify mass lesions. Because she was suspected to have suffered from encephalitis and/or meningitis, treatment with ceftriaxone, ampicillin, and acyclovir was initiated; however, her fever did not subside. A CT scan of the brain showed a nonenhancing hypodense lesion in the right cerebellar hemisphere. Cranial MRI T2-weighted, fluid-attenuated inversion recovery, and diffusion-weighted images (DWI) showed high-intensity lesions in the right cerebellar hemisphere (Fig. 1). The lesions on the CT scan were not accompanied by a mass effect, and gadolinium did not enhance the lesions. The etiology of the neurological disorders could not be determined.

After 7 days the patient's respiratory status suddenly deteriorated. A chest X-ray and CT scan showed bilateral infiltrates expanding diffusely. The DNA sequence specific to *Pneumocystis jiroveci* was detected in the specimen obtained from the bronchoalveolar lavage fluid by PCR. Bacterial culture was negative and microscopic examination did not show any lymphoma cells. Antigens of cytomegalovirus, *Candida*, *Aspergillus*, and *Cryptococcus* were not detected. She was started on sulfamethoxazole and trimethoprim. Prednisolone at a dosage of 40 mg, twice daily, was also prescribed. Her respiratory symptoms improved with the treatment of *Pneumocystis jiroveci* pneumonia (PCP), and the abnormal findings on the chest X-ray and CT scan were normalized. Moreover, she developed adenovirus-, JC virus-, and BK virus-related hemorrhagic cystitis.

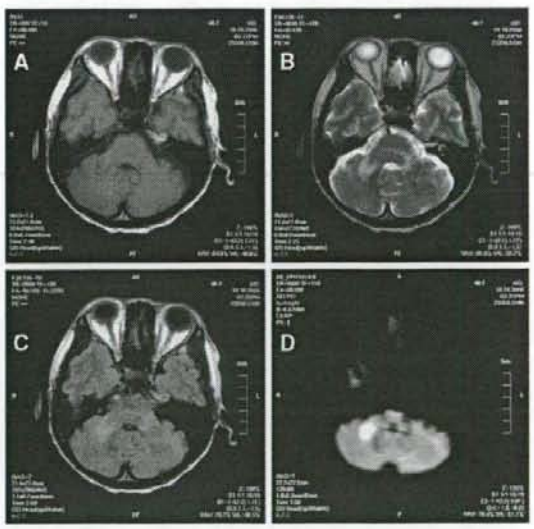

Fig. 1 October 2006: magnetic resonance imaging showing mild cerebellar atrophy in a T1-weighted image (**a**), white matter abnormality in a T2-weighted image (**b**), white matter abnormality in fluid-attenuated inversion recovery (**c**), and intracellular edema in a diffusion-weighted image (**d**)

Her neurologic status deteriorated rapidly with the development of decreased attention, marked gaze-evoked nystagmus, asymmetric spastic quadriparesis, and bulbar paralysis. MRI showed the progression of cerebellar atrophy (Fig. 2) compared with the initial MRI, as well as new scattered areas of signal hyperintensity within the right cerebellar hemisphere and adjacent brainstem. MRI revealed areas of white matter disease consistent with a demyelinating process, and PCR of the CSF showed JCV DNA.

PML was diagnosed, and intravenous injections of cytarabine were given accordingly at a dosage of 2 mg kg^{-1} for five consecutive days. Despite the treatment, the patient's neurological condition continued to worsen. A repeat MRI showed progressive worsening of the radiological findings. The patient subsequently received an intrathecal injection of cytarabine at a dose of 20 mg per day. However, the therapy was unsuccessful, and she died 8 months after the diagnosis of DLBCL, i.e., 5 months after the onset of PML.

3 Discussion

Progressive multifocal leukoencephalopathy is an uncommon demyelinating disease of the CNS caused by lytic infection of oligodendrocytes with JCV [3]. Up to 64% of healthy adults shed JCV in the urine in the absence of any clinical symptoms, which suggests that asymptomatic, active JCV infection is common in immunocompromised persons [6]. The clinical manifestations of PML are hypothesized to occur when B cells infected with JCV are activated under an immunosuppressed condition; subsequently, JCV enters the brain, where astrocytes and oligodendrocytes support JCV replication, resulting in neurological damage [7]. The disease is characterized by the development of disseminated demyelinating plaques in the cerebral white matter and adjacent areas [3]. Death within 6 months is common.

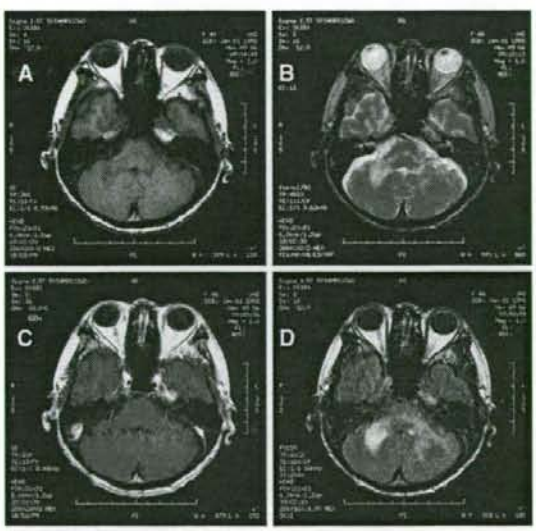

Fig. 2 December 2006: magnetic resonance imaging showing cerebellar atrophy in a T1-weighted image (**a**), white matter abnormality and expansion into the region of the adjacent brainstem in a T2-weighted image (**b**), cerebellar atrophy without areas of enhancement in a T1-weighted image (**c**), and white matter abnormality and expansion of the region of the adjacent brainstem in fluid-attenuated inversion recovery (**d**)

JCV reactivation is typically observed among patients with severe immunodeficiency. The cause of the immunologic deficit is mostly a long-standing hematological disorder, HIV infection, hematological malignancies, immunosuppressive medications, or immunosuppressive treatment after organ transplantation [3]. In the HIV-infected population, PML is strongly correlated with depressed $CD4^+$ T-cell counts [8]. Rare cases of PML have been reported among patients with lymphoma [4]. Our report details the clinical features of a patient with DLBCL who developed both PML and PCP. Because both PML and PCP are opportunistic infections, we suspected a preceding immunosuppressed state, and identified a decrease in absolute $CD4^+$ T-cell counts at the initial presentation of DLBCL. The patient had no underlying diseases that might cause immunodeficiency, such as additional malignancies or AIDS. The definition of idiopathic $CD4^+$ T-lymphocytopenia is consistent with this condition [9], but it is not certain if this is an abnormality directly related to malignant lymphoma. On the other hand, a reduction in the $CD4^+$ T-cell count is noted at the initial examination in some patients with malignant lymphoma, suggesting a possibility for the development of an opportunistic infection. The present patient developed PCP and viral hemorrhagic cystitis in addition to PML. We believe that we should be cautious regarding the development of an opportunistic infection in patients with a low $CD4^+$ T-cell count at the initial examination, and the R-CHOP regimen should be accompanied by supportive care such as trimethoprim–sulfamethoxazole to prevent PCP under close observation. For patients with a low $CD4^+$ T-cell count before the initiation of treatment, both the risk of developing an opportunistic infection following the R-CHOP regimen and the prognosis of malignant lymphoma should be assessed: if the latter is relatively favorable, one should consider the possiblity of omitting either rituximab or glucocorticoids in the drug combination treatment of the R-CHOP regimen. In recipients of bone marrow transplants, PML has also been associated with rituximab treatment [5, 10]. To our knowledge, however, initial R-CHOP immunochemotherapy for B-cell lymphoma has