Successful Treatment of TAFRO Syndrome, a Variant of Multicentric Castleman's Disease, with Cyclosporine A: Possible Pathogenetic Contribution of Interleukin-2

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Multicentric Castleman's disease is a systemic inflammatory disorder characterized by lymphadenopathy and excessive interleukin-6 production. A unique clinicopathologic variant of multicentric Castleman's disease, TAFRO (i.e., thrombocytopenia, anasarca, fever, renal failure or reticulin fibrosis, and organomegaly) syndrome, was recently proposed in Japan. Despite the successful use of anti-interleukin-6 therapy in some patients with TAFRO syndrome, not all patients achieve remission. The pathophysiological etiology of and suitable therapeutic strategies for this variant have not been established. Here, we present our experience of a unique case of TAFRO syndrome in a 78-year-old woman whose symptoms responded differently to several therapies. Tocilizumab, an anti-interleukin-6 receptor antibody, successfully induced remission of fever and lymphadenopathy. However, severe thrombocytopenia persisted and she developed anasarca, ascites, and pleural effusion shortly thereafter. Rituximab, an anti-CD20 antibody, and glucocorticoid therapy provided no symptom relief. In contrast, cyclosporine A, an immunosuppressive agent that blocks T cell function by inhibiting interleukin-2, yielded immediate improvements in systemic fluid retention and a gradual increase in platelet count, with complete resolution of disease symptoms. Excessive serum interleukin-2, when used as an anti-cancer agent, has been reported to cause side effects such as fluid retention, thrombocytopenia, and renal failure. Our case was unique because the anti-interleukin-2 therapy successfully improved symptoms that were not relieved with anti-interleukin-6 therapy. The present report therefore provides insight into the possible role of interleukin-2, in addition to interleukin-6, in TAFRO syndrome. This report will certainly help to clarify the pathogenesis of and optimal treatment strategies for TAFRO syndrome.

Introduction

Castleman's disease (CD), which was first described in 1956 by Dr. Benjamin Castleman and colleagues, comprises a heterogeneous group of rare disorders characterized by polyclonal B lymphocyte proliferation (Castleman et al. 1956). In the 1980s, Frizzera and coworkers reviewed three types of eponymous variants: localized CD, hyaline-vascular type; localized CD, plasma cell type; and multicentric CD (MCD) (Frizzera et al. 1983; Frizzera 1988). MCD presents with episodic systemic inflammatory symptoms, reactive proliferation of morphologically benign lymphocytes, and multiple organ system impairment due to the excessive production of interleukin-6 (IL-6) and other pro-inflammatory cytokines. Moreover, MCD is most commonly accompanied by symptoms and signs of autoimmunity (Muskardin et al. 2012; Fajgenbaum et al. 2014). In Western countries, MCD is usually associated with human herpes virus-8 and human immunodeficiency virus infection. In contrast, Japanese patients with MCD seldom harbor these viruses (Masaki et al. 2013).

Takai et al. (2010) reported three patients who shared a constellation of symptoms, thrombocytopenia, anasarca, fever, reticulin fibrosis, and organomegaly, as well as hyaline-vascular-type histology in the lymph node of one patient. Since then, additional case reports (Inoue et al. 2013; Masaki et al. 2013) involving this constellation of symptoms have been published. These findings collectively suggest a novel clinical entity comprising systemic inflammatory disorders on a background of immunological abnormality that exceeds the ordinary spectrum of MCD.

At first, all reported cases of this systemic inflamma-

Table 1. Proposed criteria for the diagnosis of TAFRO syndrome.

DEFINITON OF TAFRO SYNDROME
1. Blood count abnormalities: low platelet and/or red blood cell counts
(thrombocytopenia, microcytic anemia)
2. Systemic inflammation: polyserositis (pleuritis/peritonitis);
inflammation of the tissue lining the lungs or abdominal cavities (pleural effusion, ascites)
3. Renal dysfunction
4. Myelofibrosis
5. Immunologic disorder: rheumatoid factor, platelet-associated IgG, anti-thyroid antibody,
and positivity on direct Coombs test
6. Antinuclear antibody
7. Rare polyclonal hyper-γ-globulinemia: less than 4,000 mg/dL
8. Laboratory data abnormalities:
elevated alkaline phosphatase level and decreased lactate dehydrogenase level
9. Elevated levels of interleukin-6 and vascular endothelial growth factor in serum or effusions
10. Lymphadenopathy: generally of mild degree (less than 1.5 cm in diameter)
11. Mixed-type and, less frequently, hyaline vascular-type Castleman's disease histology

tory disorder involved Japanese patients. Therefore, a research meeting was organized in Japan to clarify this novel clinical entity as well as its diagnostic criteria and treatment. Accordingly, Kawabata et al. (2013) proposed the clinical term "TAFRO" (i.e., thrombocytopenia, anasarca, fever, renal failure or reticulin fibrosis, and organomegaly) syndrome, for which the proposed diagnostic criteria are shown in Table 1. However, a case of a South Asian adolescent with TAFRO syndrome was recently reported in India (Koduri et al. 2014), indicating that TAFRO syndrome is not endemic only to Japan.

The pathophysiology of TAFRO syndrome is poorly understood, and no therapeutic strategy has been established. Here, we report a case of TAFRO syndrome successfully treated with the immunosuppressant agent cyclosporine A (CyA). CyA reduces immunologic activity by interfering with T cell growth and activity via the inhibition of calcineurin, which, under normal circumstances, is responsible for activating the transcription of interleukin-2 (IL-2) (O'Keefe et al. 1992). The clinical course of the present patient was unique in that her symptoms responded differently to various therapeutic approaches, suggesting that dysregulation of multiple immunological processes might contribute to the pathogenesis of TAFRO syndrome.

Case Presentation

A 77-year-old Japanese woman was admitted to our hospital with a tentative diagnosis of malignant lymphoma. One month before admission, anemia and thrombocytopenia were identified during an annual medical checkup. Three weeks before admission, the patient developed a high-grade fever and was prescribed several series of anti-

biotics. However, her intermittent fever remained, and her general condition gradually deteriorated. She was referred to our hospital with anemia, thrombocytopenia, and a fever of several weeks' duration. Her previous medical history was unremarkable, except for tuberculosis and right hip replacement, and she neither smoked nor drank alcohol.

Upon physical examination, slight pitting edema was present on the dorsa of both hands and both legs. No skin lesions were observed, and her superficial lymph nodes were not palpable. Laboratory test results revealed normocytic anemia, thrombocytopenia, renal dysfunction, and an elevated serum C-reactive protein (CRP) level (Table 2). Her serum immunoglobulin (Ig) G level was 1,318 mg/dl, with no detectable M-protein. Her serum complement level was within normal limits. An antinuclear antibody test yielded a score of ×80 with a speckled and homogenous pattern. Her anti-Sjögren's syndrome antigen (SS)-A antibody test result was positive, but results for double-stranded DNA, ribonucleoprotein, Smith, SS-B, and Jo1 antigen antibodies were negative. Proteinase 3-anti-neutrophil cytoplasmic antibodies (ANCA) and myeloperoxidase-ANCA were not elevated. The platelet-associated IgG (PAIgG) level was 96 ng/10^7 cells. Her serum soluble IL-2 receptor (sIL-2R) and IL-6 concentrations were elevated at 2,490 U/ml and 26.9 pg/ml, respectively, whereas her serum angiotensin-converting enzyme concentration was not elevated; repeated blood cultures were sterile. QuantiFERON-TB and β-D-glucan test results were both negative.

Bone marrow examination revealed hypoplastic marrow with increased numbers of myeloid cells and megakaryocytes without myelofibrosis. No atypical phenotype populations were detected. Moreover, ^{18}F-fluorodeoxy-

Table 2. Laboratory data upon admission.

WBC	6,520/μl	CRP	9.6 mg/dl	sIL-2R	2,490 U/ml
Blast	0%	TP	6.2 g/dl	IL-6	26.9 pg/ml
Promyelo	0%	Albumin	2.9 g/dl	ACE	9.2 U/l
Myelo	1%	T-Bil	0.9 mg/dl	PCT	0.84 ng/ml
Metamyelo	0%	AST	31 U/l	IgG	1,318 m/dl
Stab	6%	ALT	25 U/l	IgA	244 mg/dl
Seg	66%	ALP	387 U/l	IgM	94 mg/dl
Eosi	2%	γ GTP	33 U/l	C3	124 mg/dl
Baso	0%	LDH	130 U/l	C4	26.2 mg/dl
Mono	15%	CK	23 U/l	CH50	56.9 U/l
Lymp	10%	Amylase	53 IU/l	PAIgG	96 ng/10^7cells
RBC	275 x 10^4/μl	UA	7.6 mg/dl	ANA	(+) (index80)
Hb	8.4 g/dl	BUN	19 mg/dl	EBV-VCA IgM	(−)
Hct	24.7%	Cre	0.93 mg/dl	EBV-VCA IgG	(+) (index80)
MCV	89.8 fl	Na	137 mEq/l	EVB-EBNA	(−)
MCH	30.5 pg	K	3.9 mEq/l	EBV-EADR-IgG	(+) (index10)
MCHC	34.0%	Cl	104 mEq/l	EBV-DNA	430 copies
PLT	4.4 x 10^4/μl	Ca	7.7 mg/dl	CMV-IgM	(±)
PT	75%	T-CHO	114 mg/dl	CMV-IgG	(+)
PT-INR	1.11	TSH	3.88 uIU/ml	CMVpp65C7	(−)
APTT	27 sec	F-T4	0.88 ng/dl	β-D-glucan	≤ 5.0 pg/ml
Fibrinogen	520 mg/dl	Ferritin	200.4 ng/ml	Aspergillus Ab	(−)
AT-Ⅲ	66%	BNP	21.1 pg/ml	QuantiFERON-TB	(−)
FDP	14 μg/ml	CEA	< 0.5 ng/ml	Mycoplasma	(−)
D-Dimer	5.8 μg/ml	CA19-9	2.1 U/l	Cryoglobulin	(−)

WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; PT, prothrombin time; PT-INR, prothrombin time-international normalized ratio; APTT, activated partial thromboplastin; FDP, fibrin/fibrinogen degradation products; AT-III, antithrombin III; TP, total protein; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; CK, creatine kinase; UA, uric acid; Cre, creatinine; BUN, blood urea nitrogen; T-CHO, total cholesterol; TSH, thyroid stimulating hormone; F-T4, free thyroxine; BNP, brain natriuretic peptide; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; ACE, angiotensin-converting enzyme; PCT, procalcitonin; C3, complement 3; C4, complement 4; CH50, 50% hemolytic complement; ANA, anti-nuclear antibody; EBV, Epstein Barr virus; VCA, viral-capsid antigen; EBNA, EBV nuclear antigen; EADR, early antigen-diffuse and restrict complex; CMV, cytomegalovirus; Ab, antibody.

glucose positron emission tomography (FDG-PET) revealed weak uptake in the spleen and bilateral cervical, supraclavicular, axillary, abdominal para-aortic, and mesenteric lymph nodes (Fig. 1).

Left cervical lymph node biopsy was performed on day 11 (Fig. 2). Histopathological examination revealed lymphoid follicles with atrophic germinal centers and interfollicular expansion, as well as blood vessel penetration and tight concentric lymphocytic layers. The interfollicular zone was characterized by proliferating endothelial venules and infiltrating mature plasma cells. These findings were consistent with the manifestation of mixed-type CD. Flow cytometric analysis of the lymph node cells revealed a normal kappa/lambda light chain ratio. Based on these findings, the patient was diagnosed with MCD.

Tocilizumab (8 mg/kg), an anti-interleukin-6 receptor antibody, was initiated on day 19, administered one week later, then every two weeks (days 19, 26, 40, and 54). Her fever and lymphadenopathy improved immediately, and her CRP level decreased gradually. In contrast, her thrombocytopenia and anasarca persisted and gradually worsened, and she developed bilateral pleural effusion and massive ascites. In addition, her serum IL-6 level increased to 282 pg/ml, without serum vascular endothelial growth factor elevation. An ascites sample was sterile, and no lymphoma or other malignant cells were detected in the samples. Additional
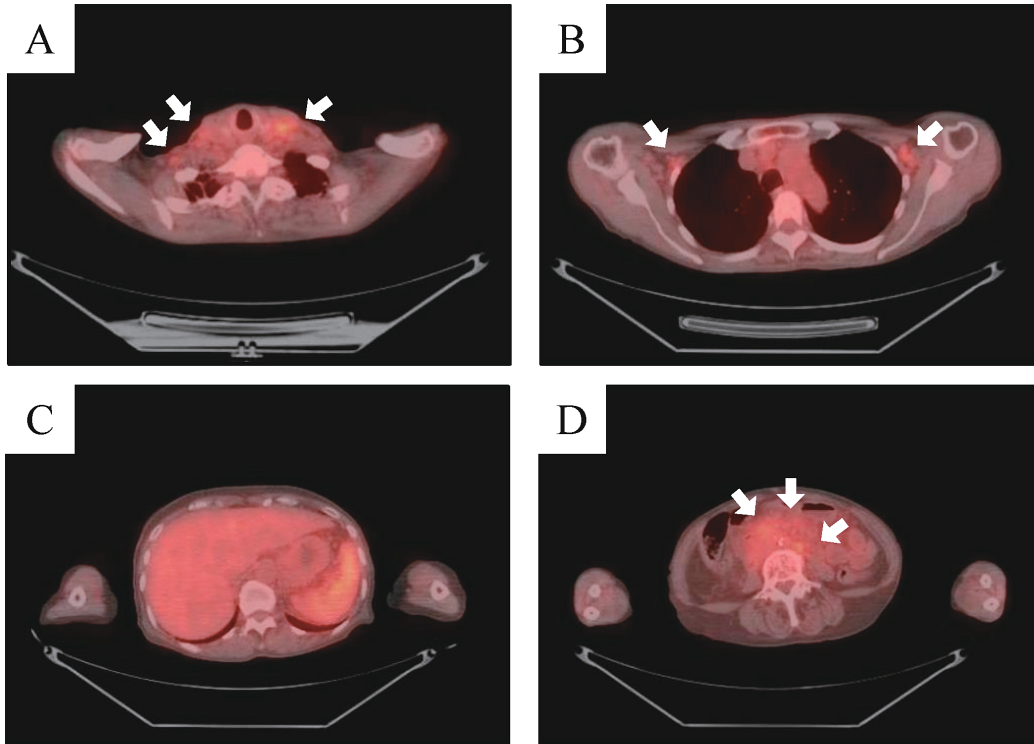
Fig. 1. ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) images at admission.
Multiple enlarged lymph nodes were observed among the cervical, supraclavicular (A), axillary (B), and abdominal para-aortic and mesenteric (D) lymph nodes at admission (white arrows). The patient also exhibited splenomegaly with weak FDG uptake (C).
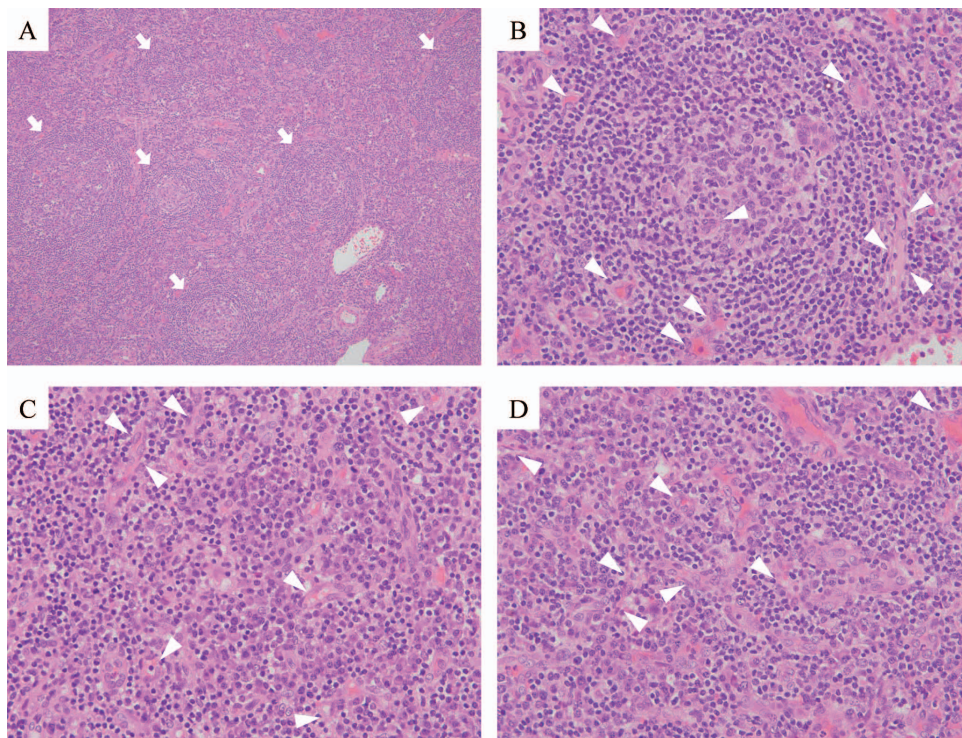

Fig. 2. Histopathological findings in the left cervical lymph node.
Hematoxylin and eosin stain. (A) Numerous lymphoid follicles with atrophic germinal centers (white arrows) and inter-follicular expansion (original magnification, ×100). (B) Tight concentric lymphocytic layers surround the follicle to form an onionskin architecture with blood vessel penetration (white arrowheads) (original magnification, ×400). (C, D) The interfollicular zone is characterized by proliferating endothelial venules (white arrowheads) and a large number of infiltrating mature plasma cells (original magnification, ×400).
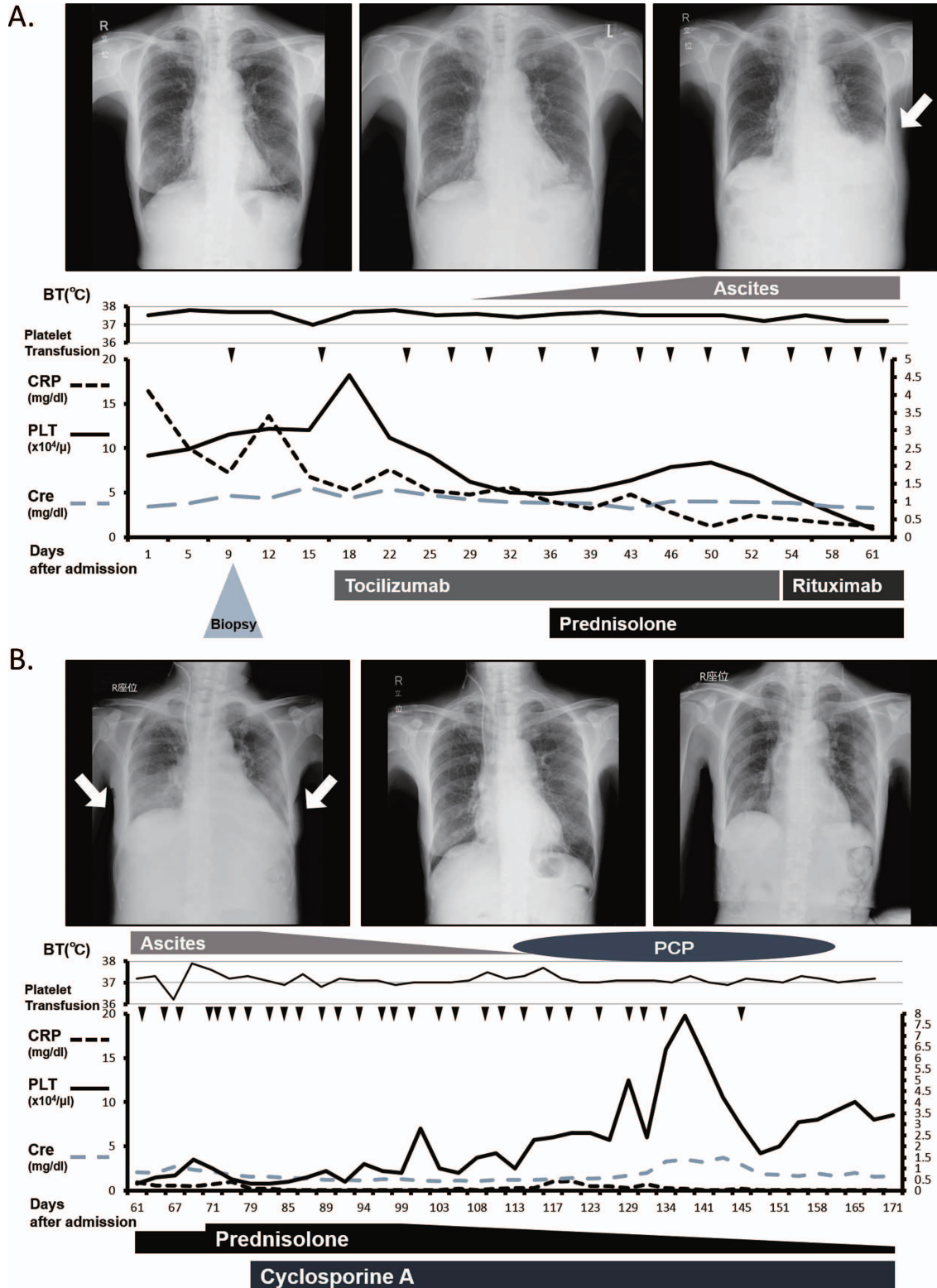
Fig. 3. Disease course of the patient.
(A) Clinical course before cyclosporine A administration. Her fever and C-reactive protein (CRP) level improved grad-
ually. In contrast, her platelet (PLT) count remained low, and she required multiple platelet transfusions. In the upper
line chart, the black solid line represents the body temperature. In the bottom line chart, the black solid line represents
platelet counts (right y-axis), the black dotted line indicates the serum CRP level (left y-axis) and the gray dotted line
represents the serum creatinine level (right y-axis). In addition, she developed pleural effusion (indicated on chest x-ray
with white arrows) and ascites. (B) Clinical course after cyclosporine A administration. Her bilateral pleural effusion
(indicated on chest x-ray with white arrows) disappeared, and her PLT count increased gradually.
BT, body temperature; Cre, creatinine; PCP, pneumocystic pneumonia.

corticosteroid therapy ($0.5 \ mg \cdot kg^{-1} \cdot day^{-1}$) was initiated on day 36 but provided no symptom relief. Based on her clinical course and laboratory data, we made a definitive diagnosis of TAFRO syndrome (Fig. 3A).

Rituximab ($375 \ mg/m^2$), an anti-CD20 antibody, was introduced on day 61 and the therapeutic corticosteroid dosage was increased to $1 \ mg \cdot kg^{-1} \cdot day^{-1}$ on day 72, but neither provided symptom relief. On day 81, she was treated with the immunosuppressive agent CyA at 100 mg/day. Her systemic fluid retention improved immediately, and her platelet count increased gradually. This CyA dose was maintained to achieve a target trough level of 100-150 ng/ml, and corticosteroid therapy was tapered carefully (50, 40, 30, 25, 12.5, 10, 7.5, and 5 mg on days 72, 79, 86, 93, 100, 107, 114, 135, and 171, respectively). On day 130, the patient developed pneumocystic pneumonia, which was successfully treated with sulfamethoxazole-trimethoprim. After undergoing rehabilitation, she was discharged on day 172 (Fig. 3B). One year after discharge, the patient has remained well; her initial symptoms disappeared without any signs of disease recurrence.

Discussion

MCD comprises a heterogeneous group of rare and poorly understood disorders characterized by the proliferation of morphologically benign lymphocytes following proinflammatory hypercytokinemia, most notably IL-6. Diagnosis is based on the presence of lymph node histopathologic features and clinical correlates characteristic of CD after the systematic exclusion of all other infectious, autoimmune, and neoplastic diseases known to exhibit the same features as MCD (Fajgenbaum et al. 2014). The new disease concept of TAFRO syndrome was recently proposed in Japan as a unique clinicopathologic variant of MCD. TAFRO syndrome currently poses serious diagnostic and therapeutic challenges for pathologists and clinicians.

Fajgenbaum et al. (2014) recently proposed a novel model of MCD pathogenesis. In this model, MCD is regarded as a common endpoint reachable through multiple processes each involving immune dysregulation and a common pathway of elevated proinflammatory cytokine release (e.g., IL-6 and vascular endothelial growth factor). At least one of the following three candidate processes is responsible for driving MCD hypercytokinemia: (1) autoimmune/ autoinflammatory mechanisms, (2) ectopic cytokine secretion by tumor cells within lymph nodes or extranodally, and (3) viral signaling. These proinflammatory cytokines subsequently trigger further immune responses to promote the inflammatory process. The perpetuation of proinflammatory hypercytokinemia by this positive feedback loop is considered the key pathway that results in the subsequent clinical and histopathologic features of MCD.

Several features of TAFRO syndrome differ considerably from those of MCD, including severe thrombocytopenia, massive fluid retention, and steroid therapeutic efficacy.

As TAFRO syndrome is currently considered a variant of MCD, it should share some aspects of the pathological pathway of MCD. However, as shown in our case, some clinical features of TAFRO syndrome cannot be ameliorated via IL-6-related pathway blockade, suggesting the existence of an immunological disturbance independent of the above-described proinflammatory cytokine feedback loop. This suggests that TAFRO syndrome may actually not be a variant of MCD, but rather a distinct immunological disorder that shares some clinical features of MCD.

As in our case, some patients with TAFRO syndrome have been reported to achieve remission with CyA treatment (Takai et al. 2010; Inoue et al. 2013). However, in contrast to our patient, patients in previous reports did not receive tocilizumab therapy. As mentioned previously, CyA suppresses the whole immune system, including IL-6-related pathways (Moutabarrik et al. 1994, O'Keefe et al. 1992). CyA interferes in T cell activity via the inhibition of calcineurin, which under normal circumstances, is responsible for activating IL-2-encoding gene transcription. Therefore, findings from previous cases could not sufficiently suggest the possible existence of an IL-6-independent pathway in the pathogenesis of TAFRO syndrome. Our case is the first to suggest that, in addition to IL-6, an IL-2-related immunological abnormality plays a role in the pathogenesis of TAFRO syndrome. Moreover, in support of this hypothesis, IL-2 administration is known to induce capillary leak syndrome, elevated creatinine levels, and thrombocytopenia in some cases when used to treat cancer (Atkins et al. 1999; Atkins 2002; Petrella et al. 2007). As IL-2 has recently received attention as an effective immunotherapy for human cancers, several clinical trials have revealed the abovementioned toxic effects of IL-2 therapy (Rosenberg 2014). These clinical manifestations are common symptoms of TAFRO syndrome, but are rarely observed as clinical manifestations of MCD. Taken together, the involvement of an IL-2-dependent pathway in addition to IL-6 should be considered in the etiology of TAFRO syndrome. Although additional investigation is required to test this hypothesis, we are certain the case reported herein will provide clues to a better understanding of the pathological mechanism of TAFRO syndrome.

In conclusion, the etiology, pathology, and strategies for the optimal management of TAFRO syndrome remain unclear. TAFRO syndrome likely has unique pathogenesis that distinguishes it from the ordinary pathogenesis of MCD. We propose a hypothesis concerning the potential role of IL-2 in TAFRO syndrome on the basis of clinical observations from a limited number of cases. Therefore, multicenter clinical surveys of similar cases are required to better understand this rare disease.

Conflict of Interest

The authors declare no conflict of interest.

References

Atkins, M.B. (2002) Interleukin-2: clinical applications. *Semin. Oncol.*, **29**, 12-17.

Atkins, M.B., Lotze, M.T., Dutcher, J.P., Fisher, R.I., Weiss, G., Margolin, K., Abrams, J., Sznol, M., Parkinson, D., Hawkins, M., Paradise, C., Kunkel, L. & Rosenberg, S.A. (1999) High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J. Clin. Oncol.*, **17**, 2105-2016.

Castleman, B., Iverson, L. & Menendez, V.P. (1956) Localized mediastinal lymphnode hyperplasia resembling thymoma. *Cancer*, **9**, 822-830.

Fajgenbaum, D.C., van Rhee, F. & Nabel, C.S. (2014) HHV-8-negative, idiopathic multicentric Castleman disease: novel insights into biology, pathogenesis, and therapy. *Blood*, **123**, 2924-2933.

Frizzera, G. (1988) Castleman's disease and related disorders. *Semin. Diagn. Pathol.*, **5**, 346-364.

Frizzera, G., Banks, P.M., Massarelli, G. & Rosai, J. (1983) A systemic lymphoproliferative disorder with morphologic features of Castleman's disease. Pathological findings in 15 patients. *Am. J. Surg. Pathol.*, **7**, 211-231.

Inoue, M., Ankou, M., Hua, J., Iwaki, Y., Hagihara, M. & Ota, Y. (2013) Complete resolution of TAFRO syndrome (thrombocytopenia, anasarca, fever, reticulin fibrosis and organomegaly) after immunosuppressive therapies using corticosteroids and cyclosporine A: a case report. *J. Clin. Exp. Hematop.*, **53**, 95-99.

Kawabata, H., Takai, K., Kojima, M., Nakamura, N., Aoki, S., Nakamura, S., Kinoshita, T. & Masaki, Y. (2013) Castleman-Kojima disease (TAFRO syndrome): a novel systemic inflammatory disease characterized by a constellation of symptoms, namely, thrombocytopenia, ascites (anasarca), microcytic anemia, myelofibrosis, renal dysfunction, and organomegaly: a status report and summary of Fukushima (6 June, 2012) and Nagoya meetings (22 September, 2012). *J. Clin. Exp. Hematop.*, **53**, 57-61.

Koduri, P.R., Parvez, M., Kaza, S., Pappu, P. & Anuradha, S. (2014) Castleman-Kojima disease in a south Asian adolescent. *J. Clin. Exp. Hematop.*, **54**, 163-166.

Masaki, Y., Nakajima, A., Iwao, H., Kurose, N., Sato, T., Nakamura, T., Miki, M., Sakai, T., Kawanami, T., Sawaki, T., Fujita, Y., Tanaka, M., Fukushima, T., Okazaki, T. & Umehara, H. (2013) Japanese variant of multicentric Castleman's disease associated with serositis and thrombocytopenia: a report of two cases: is TAFRO syndrome (Castleman-Kojima disease) a distinct clinicopathological entity? *J. Clin. Exp. Hematop.*, **53**, 79-85.

Moutabarrik, A., Nakanishi, I., Takahara, S., Hachim, K., Bengahnem, M.G., Ramadani, B., Ishibashi, M. & Zaid, D. (1994) Effect of immunosuppressive agents FK 506 and cyclosporin and steroids on the expression of IL-6 and its receptor by stimulated lymphocytes and monocytes. *Transpl. Int.*, **7**, S552-S555.

Muskardin, T.W., Peterson, B.A. & Molitor, J.A. (2012) Castleman disease and associated autoimmune disease. *Curr. Opin. Rheumatol.*, **24**, 76-83.

O'Keefe, S.J., Tamura, J., Kincaid, R.L., Tocci, M.J. & O'Neill, E.A. (1992) FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. *Nature*, **357**, 692-694.

Petrella, T., Quirt, I., Verma, S., Haynes, A.E., Charette, M. & Bak, K.; Melanoma Disease Site Group of Cancer Care Ontario's Program in Evidence-based Care (2007) Single-agent interleukin-2 in the treatment of metastatic melanoma: a systematic review. *Cancer Treat. Rev.*, **33**, 484-496.

Rosenberg, S.A. (2014) IL-2: the first effective immunotherapy for human cancer. *J. Immunol.*, **192**, 5451-5458.

Takai, K., Nikkuni, K., Shibuya, H. & Hashidate, H. (2010) Thrombocytopenia with mild bone marrow fibrosis accompanied by fever, pleural effusion, ascites and hepatosplenomegaly. *Rinsho Ketsueki*, **51**, 320-325.