RESEARCH ARTICLE

Clinicopathologic analysis of TAFRO syndrome demonstrates a distinct subtype of HHV-8-negative multicentric Castleman disease

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Multicentric Castleman disease (MCD) describes a heterogeneous group of disorders involving systemic inflammation, characteristic lymph node histopathology, and multi-organ dysfunction because of pathologic hypercytokinemia. Whereas Human Herpes Virus-8 (HHV-8) drives the hypercytokinemia in a cohort of immunocompromised patients, the etiology of HHV-8-negative MCD is idiopathic (iMCD). Recently, a limited series of iMCD cases in Japan sharing a constellation of clinical features, including thrombocytopenia (T), anasarca (A), fever (F), reticulin fibrosis (R), and organomegaly (O) has been described as TAFRO syndrome. Herein, we report clinicopathological findings on 25 patients (14 males and 11 females; 23 Japanese-born and two US-born), the largest TAFRO syndrome case series, including the first report of cases from the USA. The median age of onset was 50 years old (range: 23–72). The frequency of each feature was as follows: thrombocytopenia (21/25), anasarca (24/25), fever (21/25), organomegaly (25/25), and reticulin fibrosis (13/16). These patients frequently demonstrated abdominal pain, elevated serum alkaline phosphatase levels, and acute kidney failure. Surprisingly, none of the cases demonstrated marked hypergammoglobulinemia, which is frequently reported in iMCD. Lymph node biopsies revealed atrophic germinal centers with enlarged nuclei of endothelial cells and proliferation of endothelial venules in interfollicular zone. 23 of 25 cases were treated initially with corticosteroids; 12 patients responded poorly and required further therapy. Three patients died during the observation period (median: 9 months) because of disease progression or infections. TAFRO syndrome is a unique subtype of iMCD that demonstrates characteristic clinicopathological findings. Further study to clarify prognosis, pathophysiology, and appropriate treatment is needed.
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Introduction

Multicentric Castleman disease (MCD) is a rare disorder often characterized by episodes of systemic inflammation, reactive proliferation of morphologically benign lymphocytes, multicentric lymphadenopathy, polyclonal gammaglobulinemia, microcytic anemia, hypoalbuminemia, and elevated serum inflammatory proteins, such as C-reactive protein (CRP) [1–3]. The diagnosis of MCD is established histologically upon lymph node biopsy when characteristic hyaline vascular (HV), plasma cell (PC), or mixed type features are observed [4,5]. These histopathological and clinical abnormalities are believed to stem from pathologic hypercytokinemia, most notably of interleukin-6 (IL-6), though the cytokine responsible for initiating the inflammatory cascade may vary from patient to patient. These clinical features and lymph node changes can be seen in other diseases as well; therefore, MCD represents a diagnosis of exclusion [6–10].

Additional Supporting Information may be found in the online version of this article.

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TABLE I. TAFRO Symptoms and Histological Findings in TAFRO-iMCD

TAFRO symptoms		
Thrombocytopenia	Platelet count	$<100 \times 10^3/\mu L$
Anasarca	Pleural fluids and ascites	on computed tomography
Fever	Body temperature	>38.0°C (100.4F)
Reticulin fibrosis	Evaluated in bone marrow biopsy	
Organomegaly	Lymphadenopathyand/or hepatomegaly/splenomegaly	on computed tomography

*These were evaluated at time of diagnosis.

Histological findings of lymph nodes		
	TAFRO-iMCD	iMCD-NOS
Germinal centers	Atrophic germinal centers with enlarged nuclei of endothelial cells	Hyperplastic germinal centers of varying sizes
Interfollicular zone	Proliferation of endothelial venules, small numbers of mature plasma cells	Sheets of proliferating mature plasma cells
HHV-8	Negative	Negative
Light chain restrictions	None	None

HHV-8; Latency-associated nuclear antigen-1 assess to Human Herpes Virus-8.

Human herpes virus (HHV)-8, which infects B-cells and expresses a viral homolog of IL-6, drives the disease in immunocompromised MCD patients [11,12]. There is also a significant cohort of MCD patients around the world that are HHV-8-negative and the etiology of disease is unknown [13–15]. These cases are referred to as idiopathic MCD (iMCD) [6]. Possible etiologies in these patients include a virus other than HHV-8, paracrine secretion of cytokines by a small population of neoplastic cells, or autoinflammatory mechanisms [6]. The unknown etiology of these iMCD cases presents a significant challenge with regards to designing appropriate treatment regimens.

Recently, Takai et al. [16] reported three patients who shared a constellation of clinical symptoms that have been called TAFRO syndrome: thrombocytopenia (T), anasarca (A), fever (F), reticulin fibrosis (R), and organomegaly (O). Of note, one of these three patients underwent a lymph node biopsy demonstrating hyaline-vascular-like changes consistent with MCD. These patients responded well to immunosuppressive therapy with cyclosporine and prednisolone. Takai and colleagues proposed that this novel clinical entity represents a group of systemic inflammatory disorders rooted in autoimmunity [16]. Since this initial description in 2010, an additional 11 cases of TAFRO syndrome have been reported [14,17–22], and most recently, a Caucasian case was reported in Europe [23]. All of these patients in which a lymph node biopsy was performed have demonstrated histopathology consistent with MCD. Physicians in the United States of America (USA) have reported having iMCD patients with TAFRO features (Personal Communications, van Rhee, F; Personal Communications, Uldrick, T). However, these descriptions of TAFRO in the USA have not been published, and a larger recognition and reporting of TAFRO is needed outside of Japan.

To investigate the clinical entity of TAFRO syndrome and further characterize its histopathological findings, we have analyzed the largest-to-date series of 25 patients diagnosed with HHV-8 negative or iMCD, demonstrating the TAFRO syndrome (TAFRO-iMCD). This is the largest series of patients reported to have TAFRO syndrome, and the first description of TAFRO in the USA.

Methods

Patients. Patients who had been diagnosed with HHV-8-negative iMCD were selected from pathology files in the Department of Pathology, Okayama University from 1999 to 2013 for careful medical record review by a team of physicians. Twenty-three Japanese patients were found to demonstrate at least three out of five TAFRO clinical symptoms and characteristic TAFRO-iMCD lymph node histopathology, such as atrophic germinal centers with enlarged nuclei of endothelial cells, proliferation of endothelial venules with enlarged nuclei in interfollicular zone, and small numbers of mature plasma cells [18]. Twelve of these cases were referred to our institution because their physicians suspected TAFRO syndrome. Two of the cases have been reported previously [17,18]. We also included two patients from the USA, who also were found to have iMCD and demonstrate all five TAFRO clinical features. They were both born in the USA and currently reside there. One is of European descent and has been reported previously [14], while the other patient is of Sri Lankan descent.

As a control, 19 cases of HHV-8 negative MCD with PC-subtype, were selected from the same Okayama University pathological files. These cases are herein referred to as iMCD-NOS (not otherwise specified).

The use of samples and the medical records (clinical history, treatmentm and survival data) in our study was approved by the Institute Review Board (IRB) at Okayama University. Written informed consent was waived by our institutional review board, since our study was limited to the use of excess human tissue samples and medical records.

Latency-associated nuclear antigen-1 (LANA-1) was performed by immunohistochemistry of paraffin-embedded lymph node to assess HHV-8 status. Polymerase chain reaction (PCR) for HHV-8 DNA in the blood was also tested in eight cases of TAFRO-iMCD. For all patients and controls, alternative diagnoses for iMCD were excluded as determined by clinical features, blood cultures, serum immunoelectrophoresis, and autoantibody tests. The differential diagnosis includes infectious diseases, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin pigmentation), lymphoma, and rheumatologic diseases, such as systemic lupus erythematosus (SLE).

Definition of TAFRO symptoms. Thrombocytopenia was defined as a platelet count $< 100 \times 10^3/\mu L$ at the time of diagnosis. Fever was defined as temperature of >38.0° C (100.4F). Anasarca was defined as the presence of pleural fluids and ascites on computed tomography. Organomegaly included lymphadenopathy, hepatomegaly, or splenomegaly, which was also evaluated by radiologists using computed tomography (CT). Reticulin fibrosis was evaluated via bone marrow biopsy (Table I).

Histological and immunohistochemical examination. Paraffin-embedded lymph node and bone marrow biopsy specimens were cut into 4 μm sections and stained with hematoxylin/eosin and reticulin silver impregnation, respectively. The lymph node sections were immunohistochemically stained using an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). Tissue sections were subjected to standardized heating pretreatment for antigen retrieval with antibodies specific for CD20 (L26; 1:200; Novocastra, Newcastle, UK); CD3 epsilon (PS1; 1:50; Novocastra); CD21 (1F8; 1:20; Dako, Carpinteria, CA,USA); CD10 (56C6; 1:50; Novocastra); bcl2 (3.1; 1:200; Novocastra); CD138 (MI15; 1:100; Dako); kappa light chains (kp-53; 1:100; Novocastra); lambda light chains (HP-6054; 1:200; Novocastra) and HHV-8 (13B10; 1:25; Novocastra). *In situ* hybridization (ISH) of kappa and lambda light-chains was performed by an automated Bond Max stainer (Leica Biosystems, Melbourne, Australia).

Statistical analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [24]. Comparisons of the clinical parameters (platelet count, serum level of CRP, immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), lactate dehydrogenase (LD), alkaline phosphatase (ALP), and IL-6) in TAFRO syndrome, and iMCD-NOS were performed with Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. All *P*-values were two sided, and *P*-values of 0.05 or less were considered statistically significant.

Results

Clinical features

TAFRO syndrome group (TAFRO-iMCD). Clinical features are summarized in Table II. The TAFRO-iMCD group included 14 males

TABLE II. Clinical Characteristics and Laboratory Data, TAFRO-iMCD and iMCD-NOS

	TAFRO-iMCD (n=25) Median	(range)	iMCD-NOS (n=19) Median	(range)	*P* value
Age (y.o)	50	(23–72)	47	(28–68)	N.S
Gender (M:F)	14: 11		12: 7		N.S
PS≧2	76.5%	(13/17)	0%	(0/11)	$P<0.01$
Anasarca	96.0%	(24/25)	0%	(0/14)	$P<0.01$
Fever	84.0%	(21/25)	18.2%	(2/11)	$P<0.01$
Reticulin fibrosis	81.3%	(13/16)	N.E		N.E
Abdominal pain	32.0%	(8/25)	N.E		N.E
Plt ($\times 10^3\mu$L)	43	(14–171)	339	(206–500)	$P<0.01$
Hb (g/dL)	9.1	(6.2–16.3)	11.0	(5.8–13.2)	$P=0.12$
Alb (g/dL)	2.3	(1.1–3.5)	2.8	(1.8–4.3)	$P=0.01$
CRP (mg/dL)	14.9	(0.8–30.2)	5.9	(1.5–16.9)	$P=0.01$
IgG (mg/dL)	1,476	(880–2,824)	4,775	(2,176–8,380)	$P<0.01$
IgA (mg/dL)	225.7	(129–432)	621	(258–997)	$P<0.01$
IgM (mg/dL)	74	(37–257)	229	(129–726)	$P<0.01$
LD (IU/L)	210	(110–424)	114	(85–138)	$P<0.01$
ALP (IU/L)	469	(102–2,388)	266	(174–1,240)	$P<0.01$
IL-6 (pg/mL)	16.2	(6.0–67.3)	25.9	(8.6–113)	$P=0.17$
VEGF (pg/mL)	305	(<20–1,410)	N.E		N.E

PS, performance status; Anasarca, pleural fluid and ascites; Fever: >38.0°C (100.4F),.
Plt, Blood platelet count; Hb, Hemoglobin; Alb, Albumin; CRP, C-reactive protein; IgG, Immunoglobulin G; IgA, Immunoglobulin A; IgM, Immunoglobulin M; LD, lactate dehydrogenase; ALP, alkaline phosphatase; IL-6, Interleukin-6 (serum normal value; <5.0 pg/mL); VEGF, Vascular endothelial growth factor (plasma normal value, <115 pg/mL); N.S, not significant; N.E, not evaluated.

and 11 females. The median age of disease onset was 50 years old (range, 23–72). Twenty-three patients were of East Asian origin, whereas one patient was South Asian and one Caucasian. The median duration between disease onset and time to diagnosis by lymph node biopsy was 6 weeks (range, 1–70 weeks). General condition at diagnosis tended to be poor; Eastern Cooperative Oncology Group (ECOG) performance status (PS) was greater than one for 76.5% (13/17) of patients. The median duration between diagnosis and the last follow-up for data collection was 9 months (range: 0–91 mos.).

Since bone marrow biopsy was only performed in 16 cases, all five criteria could only be assessed in those 16 cases. Nine of those TAFRO-iMCD cases fulfilled all five TAFRO criteria. The remaining 16 cases met at least three of four criteria. The frequency of each complication at diagnosis was as follows: thrombocytopenia (21/25), anasarca (24/25), fever (21/25), reticulin fibrosis (13/16), and organomegaly (25/25). Thirty-two percent (8/25) of TAFRO-iMCD cases reported abdominal pain, and three cases experienced painful lymphadenopathy. The median duration between diagnosis and the last follow-up for data collection was 9 months (range: 0–91 mos.).

iMCD control group with plasma cell type (iMCD-NOS). Twelve males and seven females with iMCD-NOS served as controls from the same Okayama University pathological files. The median age of disease onset was 47 years old (range: 28–68). All cases were of Asian origin. The general condition at diagnosis tended to be better than in the TAFRO-iMCD group, as all patients were PS 0 or 1. None of the patients exhibited anasarca. 2/11 cases demonstrated fever and all 19 cases had organomegaly (Table II).

Laboratory features

Laboratory data are presented in Table II. Both groups commonly demonstrated microcytic anemia, hypoalbuminemia, and elevated serum CRP. CRP levels were significantly higher ($P=0.01$) in the TAFRO-iMCD group compared to the MCD-NOS group. Patients in the TAFRO-iMCD group demonstrated severe thrombocytopenia whereas none of the iMCD-NOS cases had thrombocytopenia. The median platelet count was $43 \times 10^3/\mu$L (range $14–171 \times 10^3/\mu$L) for the TAFRO-iMCD group and $339 \times 10^3/\mu$L ($206–500 \times 10^3/\mu$L) for the iMCD-NOS group ($P<0.01$). Serum platelet associated immunoglobulin G were checked in only 10 cases of TAFRO-iMCD, and elevated in nine cases (Median 144, range 12.5–1,340 ng/10^7cells). Median serum LD was not elevated in either group at time of diagnosis. Serum ALP at diagnosis was elevated in 19/24 cases (79.2%) of TAFRO-iMCD cases (median: 469 IU/L, range: 102–2,388 IU/L) without corresponding elevations in transaminases, bilirubin, or LD. The median ALP level was significantly ($P<0.01$) lower yet still elevated in iMCD-NOS group (median: 266 IU/L, range: 174–1,240 IU/L). All five TAFRO-iMCD cases that were tested for ALP isozymes demonstrated hepatogenous-specific isozyme.

Several cases of TAFRO-iMCD showed progressive acute kidney failure and five cases needed temporary hemodialysis because of uremic symptoms during the course of disease. Median serum creatinine level of TAFRO-iMCD cases at diagnosis was 0.96 mg/dL (range 0.52–6.08 mg/dL). None of the TAFRO-iMCD cases demonstrated polyclonal hypergammaglobulinemia (median IgG level 1,476 mg/dL, range 880–2,842 mg/dL). By contrast, 17 of the 19 cases of iMCD-NOS showed marked hypergammaglobulinemia with IgG levels greater than 3,500mg/dL (median IgG level 4,775 mg/dL, range 2,176–8,380 mg/dL) ($P<0.01$).

The median serum IL-6 level at diagnosis was greater for iMCD-NOS (25.9 pg/mL, range: 8.6–113 pg/mL) than for the TAFRO-iMCD group (16.2 pg/mL, range: 6.0–67.3 mg/dL), but these differences were not statistically significant. The median level of plasma vascular endothelial growth factor (VEGF) level was 305 pg/mL in the 16 cases of TAFRO-iMCD that measured VEGF (range <20–1,410 pg/mL), which is approximately three times the upper limit of normal. VEGF was not measured for the iMCD-NOS cases.

Histologic and immunohistochemical findings

Lymph nodes. All 44 patients had one or more lymph nodes samples available for further analysis. All of the 42 Japanese TAFRO-iMCD and iMCD-NOS cases were collected and reviewed at the Department of Pathology, Okayama University. Both cases from the USA were reviewed at National Institutes of Health/National Cancer Institute. All cases of TAFRO-iMCD and iMCD-NOS were tested and found to be negative for HHV-8 by immunohistochemistry for LANA-1. HHV-8 DNA was also not detected by PCR in the blood in all eight cases of TAFRO-iMCD in which this test was performed.

TAFRO-iMCD patients exhibited several common histopathological findings. Nearly all lymph nodes obtained at diagnosis were only slightly enlarged in size with regards to length in greatest diameter

(median: 9 mm, range: 6–14mm). The characteristic histopathological findings of the TAFRO-iMCD group included atrophic germinal centers, expansion of the interfollicular zone, proliferation of highly dense endothelial venules, and relatively few mature plasma cells (Fig. 1a–e). HV features such as penetrating blood vessels were present but not as prominent as usually seen in HV or mixed-type iMCD. Architectural features typical of unicentric hyaline vascular CD were not observed. Also, enlarged nuclei were found in proliferating endothelial cells in the germinal centers and interfollicular zone of TAFRO-iMCD cases (Fig. 1a–d). Immunohistochemical studies showed that the follicular dendritic cell (FDC) networks tended to be expanded or disrupted in the interfollicular zone of TAFRO-iMCD cases (Fig. 1f).

The histological findings in TAFRO-iMCD were quite different from iMCD-NOS. All cases of iMCD-NOS showed classically reported PC-type features, including diffuse marked interfollicular plasmacytosis, prominence of germinal centers, and preservation of overall lymph node architecture [25]. No light chain restrictions were detected by *in situ* hybridization or immunohistochemical staining in either group (Table I).

Bone marrow. Bone marrow biopsy or aspiration samples were available in 22/25 cases with TAFRO-iMCD. Aspirates only were available in six cases. No samples demonstrated infiltration of neoplastic cells. In two cases, biopsy yielded a dry tap and no cellular material was obtained. Of the 22 TAFRO-iMCD bone marrow samples available, 13 were hypercellular (Fig. 1g–i), six were normocellular, and three were hypocellular. Megakaryocytic hyperplasia with slight atypia such as multiple and widely separated nuclei was observed in 12 cases (Fig. 1i). Micromegakaryocytes were not increased obviously. Significant plasmacytosis was not observed.

Myelofibrosis (MF) was scored using a scale from 0 to 3 according to the European consensus on bone marrow fibrosis grading [26]. 13/16 cases were positive for reticulin fibrosis and all 13 cases were classified MF-1 with a very loose network of reticulin fibers (Fig. 1j). Bone marrow samples from iMCD-NOS cases were not tested.

Clinical management and follow-up

Corticosteroids, such as prednisolone, methylprednisolone, and dexamethasone, were used as first-line therapy in 23/25 TAFRO-iMCD cases. 11/23 cases (47.8%) responded well to initial corticosteroids therapy alone. Because of acute deterioration, one case required multidrug therapy at the time of diagnosis with a regimen consisting of rituximab, tocilizumab, cyclophosphamide, and etoposide. One case showed spontaneous remission with only pleural effusion drainage and nonsteroidal anti-inflammatory treatment.

Twelve patients received additional therapy because of poor response to corticosteroids, and nine improved with additional therapies. Some patients received more than one additional treatment regimen. Additional treatments that were used included cyclosporine ($n = 7$); tocilizumab ($n = 6$); rituximab ($n = 4$); siltuximab (anti-IL-6 monoclonal antibody, $n = 1$); thalidomide ($n = 1$); vincristine ($n = 1$); sirolimus ($n = 1$); VDT-ACE-R (bortezomib, dexamethasone, thalidomide, adriamycin, cyclophosphamide, etoposide, and rituximab, $n = 1$); and intravenous injection of immunoglobulin (IVIG) ($n = 1$). One seriously ill patient that was refractory to corticosteroids, rituximab, and siltuximab therapy responded well to a cycle of VDT-ACE-R, but relapsed 15 months later while on siltuximab maintenance administered every 3 weeks. The patient then responded to another cycle of VDT-ACE-R, but relapsed 16 months later while on siltuximab every 3 weeks and weekly VDT maintenance. The patient received a third round of VDT-ACE-R, which induced another complete remission. He is now receiving maintenance IVIG and sirolimus and has been in a complete remission for 21 months. Plasma exchange therapy was used in two cases. Seven cases with severe acute renal failure required temporary dialysis during flares, which was discontinued following multidrug treatment.

The median follow-up period was 9 months (range, 0–91 mos.). Three patients died during the observation period because of progression of disease or sepsis. Three other patients experienced disease flares as they were weaned from immunosuppressive agents (In Supporting Information Table I Treatments and outcomes of TAFRO-iMCD). No patients developed lymphoma, Kaposi sarcoma, or any other malignancies during the follow-up period.

Discussion

We have analyzed the clinical features and histopathological characteristics of 23 Japanese cases and two US cases of HHV-8-negative MCD that demonstrated TAFRO clinical symptoms (TAFRO-iMCD). Relative to iMCD-NOS, TAFRO-iMCD is characterized by a more aggressive clinical course, corticosteroid-refractoriness, thrombocytopenia, higher frequency of anasarca, elevated level of ALP, and normal gammaglobulin levels. These unique clinical and laboratory features suggest that TAFRO-iMCD is a distinct entity within the larger entity of iMCD.

Two great challenges in the diagnosis and treatment of iMCD are the spectrum of nonspecific symptoms and the unclear etiology of the disease. By comparison, HHV-8-positive MCD is easier to diagnose, because positive LANA-1 staining in the presence of characteristic MCD lymph node histopathology is specific for HHV-8-positive MCD. Since B-cells host HHV-8, treatment is targeted at CD20, and B-cell depletion is highly effective [27]. However, iMCD does not have a positive diagnostic biomarker and can demonstrate a wide spectrum of symptoms, which makes the disease extremely difficult to diagnose. Further, once the disease is diagnosed, little is known about the mechanisms of pathogenesis to guide treatment decisions.

Our report advances the understanding of iMCD by addressing the challenges posed by nonspecific symptoms and unclear etiology. With regards to the symptoms of iMCD, we have identified a group of iMCD patients with both TAFRO pathological characteristics and core TAFRO symptoms, which are highly represented in affected patients. Identifying a homogeneous group within iMCD is critical, because it simplifies recognition of disease and diagnosis. Whereas HHV-8-associated MCD is readily recognized through the sensitivity of the LANA-1 staining for the HHV-8 virus and the specificity of "Castleman-like" histopathological features combined with positive LANA-1, so too may TAFRO-iMCD be readily recognized through its easily identifiable clinical symptoms and pathological features. In addition to aiding diagnosis, subclassification of iMCD based on clinical features will help to inform prognosis, appropriate treatments, and new targets for future therapies.

To this end, we propose that MCD can be further classified beyond HHV-8 status. HHV-8 negative iMCD may be further stratified into iMCD with TAFRO features or iMCD-NOS. Based on our series and other iMCD reports, we propose the following diagnostic criteria for iMCD with TAFRO features (Table III). TAFRO-iMCD patients must meet the histopathological criteria, three major criteria and one or more minor criteria. Excluding diseases from the differential diagnosis is necessary. Diseases that should be excluded include rheumatologic diseases such as SLE, infectious diseases such as acute Epstein-Barr Virus, and neoplastic diseases such as lymphoma, POEMS syndrome and cancer. Recently, a newly described hyper inflammatory cytokine syndrome associated with HHV-8 or Kaposi sarcoma-associated herpesvirus (KSHV) infection has been described as KSHV inflammatory cytokine syndrome (KICS) [28]. This clinical condition is characterized by elevated IL-6 because of replicating HHV-8 and presents with clinical inflammatory symptoms similar to MCD. However, these patients do not have characteristic MCD

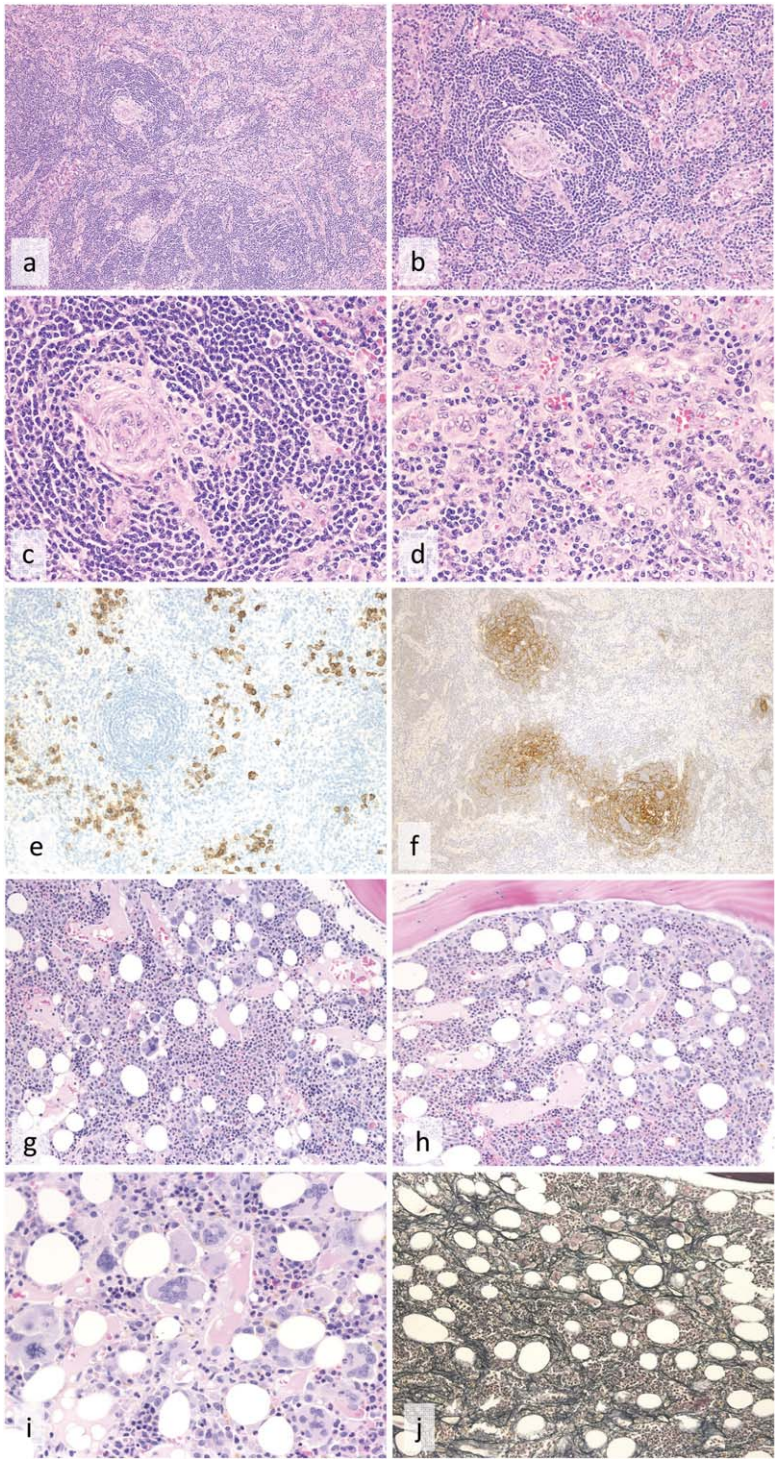

Figure 1. Histological findings of TAFRO-iMCD lymph nodes (a–f) and bone marrow (g–j). a: The biopsy specimen of a mildly enlarged lymph node shows atrophic germinal centers and intact sinuses. (H&E staining); b: Marked proliferation of high endothelial venules were observed in the germinal centers and interfollicular zone; c: Atrophic germinal centers with endothelial cells demonstrating enlarged nuclear proliferation without prominent penetrating hyalinized blood vessels. (H&E staining); d: The interfollicular zone is expanded and there is proliferation of highly dense endothelial venules with enlarged nuclei. (H&E staining); e: There are small numbers of CD138-positive plasma cell in the interfollicular zone. (CD138 immunostaining): f: CD21 immunostaining shows expanded or disrupted patterns of follicular dendritic cell networks. (CD21 immunostaining); g: Fifty-nine percent (13/22) of cases show hypercellular marrow. (H&E staining); h: Megakaryocytes tended to be hyperplastic. (H&E staining); i: Megakaryocytes were slightly atypical, with multiple and widely separated nuclei. Marked plasmacytosis was not observed. (H&E staining); j: Silver stain shows a very loose network of reticulin fibers. (Silver staining).

lymph node histopathology and, like TAFRO-iMCD, often have smaller lymph nodes than HHV-8-associated MCD or iMCD-NOS. Although TAFRO-iMCD and KICS have overlapping symptoms, TAFRO-iMCD can be distinguished from KICS because TAFRO-iMCD patients are HHV-8 negative.

This classification system expands on the previous classification proposed by Kojima et al. that divided Japanese MCD cases into two variants: idiopathic plasmacytic lymphadenopathy with polyclonal hyperimmunoglobulinemia (IPL) type and non-IPL type. Similar to TAFRO-iMCD, non-IPL cases demonstrated HV features, proliferation

TABLE III. Proposed Diagnostic Criteria for TAFRO-iMCD

1. Histopathological Criteria;
   - Compatible with pathological findings of lymph nodes as TAFRO-iMCD[a]
   - Negative LANA-1 for HHV-8
2. Major criteria;
   - Presents 3 of 5 TAFRO symptoms
     - ✓ Thrombocytopenia
     - ✓ Anasarca
     - ✓ Fever
     - ✓ Reticulin fibrosis
     - ✓ Organomegaly
   - Absence of hypergammaglobulinemia
   - Small volume lymphadenopathy
3. Minor criteria need 1 or more;
   - Hyper/normoplasia of megakaryocytes in bone marrow
   - High levels of serum ALP without markedly elevated serum transaminase

Requirements; fulfill histopathological criteria, all major criteria, and 1 or more of minor criteria.
Diseases that should be excluded include rheumatologic diseases such as SLE, infectious diseases such as acute Epstein-Barr Virus, and neoplastic diseases such as lymphoma, POEMS syndrome, and other cancers.
[a] TAFRO characteristic findings of lymph node, ie, atrophic germinal centers with enlarged nuclei of endothelial cells, proliferation of endothelial venules with enlarged nuclear in interfollicular zone, and small numbers of mature plasma cells.
ALP, alkaline phosphatase.

of vascularity in the interfollicular zone, and a greater incidence of pleural effusion and/or ascites, thrombocytopenia, and autoimmune diseases. They also reported that non-IPL cases showed FDC networks with expanded or disrupted follicles [29], which we observed in our TAFRO-iMCD series. IPL type cases exhibited clinical and pathological features more consistent with our iMCD-NOS group.

Kojima et al. also reported that the clinical course of HHV-8-negative MCD in Japan appears to take a more indolent course and progress less frequently to Kaposi sarcoma or B-cell lymphomas [29]. In this series, which included a median follow-up period of 9 months, none of the TAFRO-iMCD patients developed Kaposi sarcoma, lymphoma, or any other malignancies. In contrast, over 20% of HIV-positive, HHV-8-associated MCD patients in another series developed non-Hodgkin lymphoma with a median follow-up time of 20 months [30]. Given the consistencies between Kojima's characterization of non-IPL type and our description of TAFRO-iMCD, we believe that these disease entities are highly similar and may represent a single group. Additionally, we believe the diagnosis of TAFRO-iMCD to be broadly applicable throughout the world.

In addition to providing diagnostic clarity in iMCD, this study identifies TAFRO-iMCD as a critical point of reference for the further elucidation of the etiology of iMCD. The heterogeneity of iMCD may represent many different etiologies, confounding the efforts of investigators trying to uncover a cause of disease. The consistency of symptoms across TAFRO-iMCD patients has a higher likelihood of representing a singular cause of disease, rendering this disease subset an ideal group for further investigation. To this point, one of the hallmarks of MCD is pathologic hypercytokinemia. IL-6 elevation has been frequently associated with MCD. IL-6 is a multifunctional proinflammatory cytokine that stimulates B-cell maturation, increases immunoglobulin production by plasma cells, and stimulates megakaryocyte maturation, which typically results in thrombocytosis [31–34]. In this series of TAFRO-iMCD cases, IL-6 may not be the primary pathological cytokine. IL-6 was only mildly elevated, and clinical features often associated with excess IL-6, such as thrombocytosis and polyclonal hypergammaglobulinemia, were not observed. This contrasts with the control cases of the iMCD-NOS patients. All TAFRO-iMCD cases demonstrated thrombocytopenia with hyper/normoplasia of megakaryocytes in bone marrow at time of diagnosis or with flares, which may be because of increased peripheral thrombocyte consumption that seems to be possibly immunologic in origin. Reticulin fibrosis without plasmacytosis was frequently observed in TAFRO-iMCD cases. Only a small number of cases of HHV-8 associated MCD or iMCD have reported bone marrow findings. HHV-8-associated MCD bone marrow biopsies typically demonstrate reactive plasmacytosis with minimal or patchy grade 1 to 2 fibrosis [35]. Two cases of iMCD have reported bone marrow findings that were very consistent with iMCD lymph node histopathology including lymphoid follicles with regressed, hyalinized germinal centers surrounded by a small mantle zone, and polyclonal, bland plasma cells peripherally. The two cases also demonstrated a reactive increase in megakaryocytes [36]. Also, it has been reported that no significant reticulin fibrosis was present in any cases of POEMS syndrome [37]. While none of the TAFRO-iMCD cases demonstrated hypergammaglobulinemia, almost all cases of iMCD-NOS demonstrated polyclonal hypergammaglobulinemia. Furthermore, of the six cases of TAFRO-iMCD treated with tocilizumab and 1 case of TAFRO-iMCD treated with siltuximab, three responded and the other four were resistant to treatment. Serum IL-6 levels of responders were 20.2, 24.6, and 45.6 pg/mL. Those of nonresponders were 6.0, 8.8, 26.4, and 67.3 pg/mL, suggesting little evidence for an association between serum IL-6 concentration and effectiveness of anti-IL-6 therapy with tocilizumab or siltuximab in TAFRO-iMCD. These findings suggest that elevated IL-6 may not be the primary pathological driver of the proinflammatory hypercytokinemia responsible for TAFRO-iMCD, underlying the importance of further study to identify alternative drivers of disease.

MCD is a heterogeneous disease driven by pathologic hypercytokinemia, which is caused by HHV-8 in some cases and an unknown cause in others. Fajgenbaum et al. proposed that the hypercytokinemia in iMCD may be driven by one or more of the following mechanisms: autoimmune mechanisms, a germline genetic mutation in a gene involved in innate immunity (autoinflammatory), paracrine secretion of cytokines (paraneoplastc syndrome), or a non-HHV-8 virus. This paper contributes evidence that TAFRO-iMCD cases demonstrate unique clinicopathologic characteristics that cluster within iMCD, potentially driven by one of the aforementioned proposed etiologies. Additionally, this report offers the first description of TAFRO-iMCD in the USA, and it is likely that additional cases exist that have gone unrecognized and unreported. We believe that distinguishing between iMCD subtypes will help to clarify diagnosis, treatment and prognosis. Two urgent priorities for the field include: (1) further characterization of MCD laboratory and clinical features via a global patient registry and natural history study, which is currently being planned by the Castleman Disease Collaborative Network and investigators at the University of Pennsylvania, and (2) investigations to discover the etiologies of iMCD in order to improve management and outcomes.

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