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Original article

The potential role of follicular helper T cells in idiopathic multicentric Castleman disease with and without TAFRO syndrome

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ABSTRACT

Idiopathic multicentric Castleman disease (iMCD) is a systemic inflammatory disease of unknown etiology caused by hypercytokinemia. Recently, TAFRO (thrombocytopenia, anasarca, fever, renal failure or reticulin fibrosis, and organomegaly) syndrome has been reported, which shows similar histopathological findings to iMCD and factors associated with a poor prognosis. iMCD shows no plasma cell infiltration in the germinal center (GC), but CD38-positive ($CD38^+$)-plasma cells are observed in the interfollicular area. Our previous report revealed that atrophic change of GC, glomeruloid vascular proliferation, and abnormal proliferation of follicular dendritic cells are more prominent in iMCD with TAFRO ($TAFRO^+$) in comparison to iMCD without TAFRO ($TAFRO^-$). In addition, the numbers of $CD38^+$ and immunoglobulin G4-positive ($IgG4^+$) plasma cells were decreased in the interfollicular area. The roles of T follicular helper cells (Tfh) are well-known to assist B-cell proliferation, maturation, and differentiation?It maintains the formation of GC and is also related in the class switching of IgG isotypes, including IgG4. Thus, we immunohistochemically examined the number of Tfh in GCs in both $TAFRO^-$ and $TAFRO^+$ iMCD. The number of Tfh was significantly decreased in $TAFRO^-$ iMCD (n = 9) and was further decreased in $TAFRO^+$ iMCD (n = 18) in comparison to non-specific lymphadenopathy (n = 6) and IgG4-related disease (n = 4). These results suggest that decreased Tfh may be one etiology of iMCD.

1. Introduction

Naïve $CD4^+$ T cells stimulated by antigen-presenting cells (APC) are induced to differentiate into T follicular helper (Tfh), T helper (Th) 1, Th2, Th9, Th17, Th22, and regulatory T (Treg) cells as a result of exposure to various cytokines [1]. Interleukin (IL) -6 and IL-21 are cytokines that induce naïve T cells to differentiate into Tfh [2]. Germinal center (GC) B-cells are well-known to eventually differentiate into plasma cells or memory B cells, while it has recently been recognized that Tfh localized in the GC assist in the function of GC B-cells [3] (Fig. 1).

B-cell lymphoma 6 protein (Bcl6), the master transcription factor of Tfh, suppresses the expression of several genes which induce Th1, Th2, Th17, and Treg cell proliferation and differentiation [4,5]. Furthermore, inducible co-stimulator (ICOS) is also highly expressed in Tfh and plays an important role in their differentiation and activation [3]. In addition, Tfh express programmed cell death 1 (PD-1), which is an immunosuppressive factor that controls the activation of Tfh in GCs [6] (Fig. 1).

Tfh have critical roles in maturation and activation of GC B-cells, control of antibody production, and formation and maintenance of GC [7]. The stimulation of CD40 ligand (CD40 L) in Tfh is mediated through CD40 on the B-cell surface. CD40 L is a tumor necrosis factor (TNF) family molecule that promotes B-cell differentiation and class switching [8]. The IL-4 produced by Tfh has a function in prompting class switching to immunoglobulin (Ig) G isotypes, including IgG4 anti-

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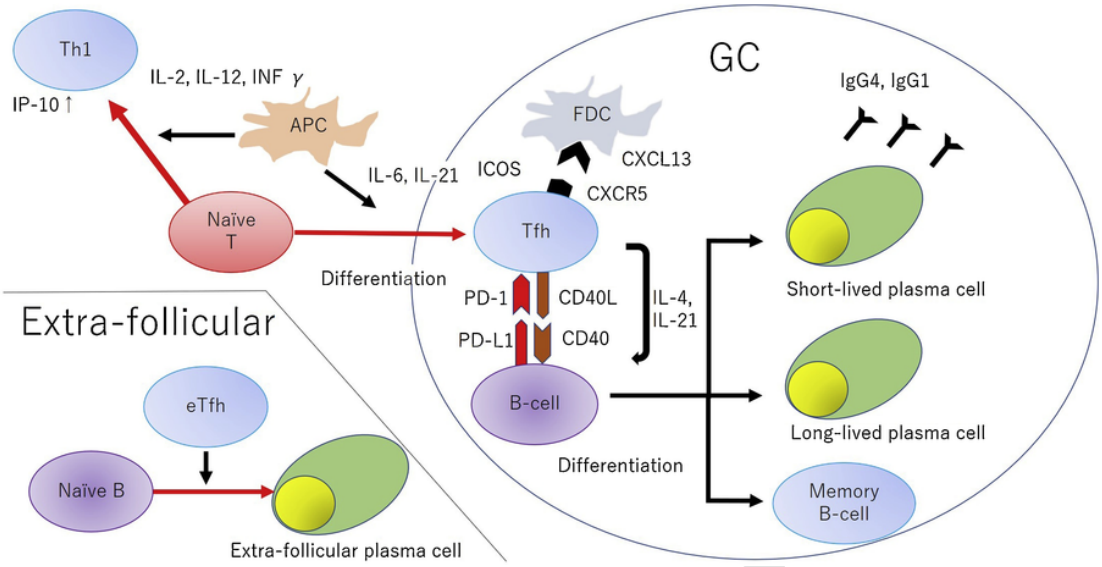

Fig. 1. A schematic model of TAFRO$^+$ iMCD. As a result of the suppression of differentiation to Tfh, differentiation to Th1 is enhanced. In addition to the suppression of plasma cell and memory B-cell differentiation, class switching to IgG4 is suppressed. In addition, the plasma cell differentiation of extra-follicular naïve B-cells is suppressed.

bodies. In addition, some GC B-cells differentiate into short-lived plasma cells by IL-4-secreting Tfh [1]. The interaction of these cytokines stimulates the differentiation and proliferation of GC B-cells and causes differentiation into memory B-cells, short-lived plasma cells, and long-lived plasma cells [1] (Fig. 1).

Furthermore, Tfh plays important roles in the immune function in not only the GCs but also the extra-follicular region. The C-X-C Motif Chemokine Ligand 13 (CXCL13) produced by follicular dendritic cells (FDC) binds to its chemokine receptor, C-X-C chemokine receptor type 5 (CXCR5), which is expressed on the cell surface of Tfh. Thus, extra-follicular Tfh can easily migrate and localize into the GC [9,10]. Furthermore, extra-follicular Tfh induce the differentiation of extra-follicular naïve B-cells into extra-follicular plasma cells [11] (Fig. 1).

Multicentric Castleman's disease (MCD) is a systemic inflammatory disease that is considered to be caused by the overproduction of IL-6. Idiopathic MCD (iMCD) is histopathologically classified into the following types: plasma cell (PC) type, mixed type, and hypervascular (Hyper-V) type [12]. Recently, (thrombocytopenia, anasarca, fever, renal failure or reticulin fibrosis, and organomegaly) TAFRO syndrome, which shows similar histopathological findings to iMCD and factors associated with a poor prognosis, has been reported from Japan [13]. In 2018, Fajgenbaum divided iMCD into iMCD-TAFRO and iMCD- not otherwise specified (NOS) in order to establish diagnostic criteria, develop therapeutic methods, and elucidate the pathogenesis [14]. In our previous report [15], we compared the histopathological findings of nodal lesions of iMCD with TAFRO (TAFRO$^+$) and iMCD without TAFRO (TAFRO$^-$). The most common histological subtype in TAFRO$^+$ iMCD was 'mixed' type (75.7%); the 'Hyper-V' and 'PC' types accounted for 16.2% and 8.1% of cases, respectively. On the other hand, the most common histological subtype in TAFRO$^-$ iMCD was the 'PC' type (72.7%), with the 'mixed' and 'Hyper-V' types accounting for 27.3% and 0% of cases, respectively.

In the majority of TAFRO$^-$ iMCD cases, the PC-type of lymphoid follicles (LFs) showed hyperplastic change without plasma cell infiltration within the GC, while marked plasma cell infiltration was observed in the interfollicular area. Vascular proliferation was not remarkable. In the PC-type of TAFRO$^+$ iMCD, atrophic LF and mild vascular proliferation within GC were seen. In comparison to the mixed-type of TAFRO- iMCD, the incidence of glomeruloid vascular proliferation (GVP) in the GC was significantly increased in the mixed type-of TAFRO$^+$ iMCD. In the interfollicular area, thickened high endothelial venules without hyalinization were densely proliferated, and plasma cells showed perivascular infiltration. Furthermore, in the Hyper-V-type of TAFRO$^+$ iMCD, the GC was more severely atrophic, the distance between the LFs was more extended. In the interfollicular area, prominent high endothelial venules were observed, the numbers of IgG4-positive (IgG4$^+$) plasma cells and CD38-positive (CD38$^+$) plasma cells per high-powered field (HPF) were significantly decreased. In addition, the GVP/LF ratios in TAFRO$^+$ iMCD were significantly higher than those in TAFRO- iMCD.

IgG4-related disease (IgG4-RD) is a systemic fibro-sclerotic disease of unknown etiology characterized by serum IgG4 elevation and IgG4$^+$ plasma cell infiltration. Histologically, IgG-related lymphadenopathy is characterized by hyperplastic GC with IgG4$^+$ plasma cell infiltration. Small lymphocytes and IgG4$^+$ plasma cells are observed in the expanded interfollicular region [16].

In this study, in order to examine the potential role of Tfh in TAFRO$^-$ and TAFRO$^+$ iMCD, the numbers of Tfh in GCs were examined using immunohistochemical staining for CXCR5 and CD40L, which are specific markers of Tfh. In addition, we investigated whether the presence of Tfh in GCs was a useful marker for histopathologically distinguishing between IgG4-RD and iMCD.

2. Materials and methods

2.1. Patients and tissue specimens

Lymph nodes (LNs) from patients with TAFRO$^+$ and TAFRO$^-$ iMCD registered in a retrospective multicenter clinical trial (UMIN000011809) of TAFRO syndrome were collected. Nine TAFRO$^-$ iMCD cases (PC; n = 4, mixed; n = 5) and 18 TAFRO$^+$ iMCD cases (mixed; n = 12, Hyper-V; n = 6) were studied. Based on diagnostic criteria in Japan, certified clinicians (Drs. YM, SF, and HK, co-authors) confirmed the clinical diagnosis of TAFRO$^+$ iMCD or TAFRO- iMCD in each case [17]. All TAFRO$^+$ iMCD cases also corresponded to Iwaki et al.'s diagnostic criteria [18]. The histopathological classification of LNs was performed based on the definition by Fajgenbaum et al. [12]. In addition, 4 cases of IgG4-RD and 6 cases of non-specific lymphadenopathy were used as control cases. This study received approval from the institutional ethics committee of Kanazawa Medical University (No. 2063).

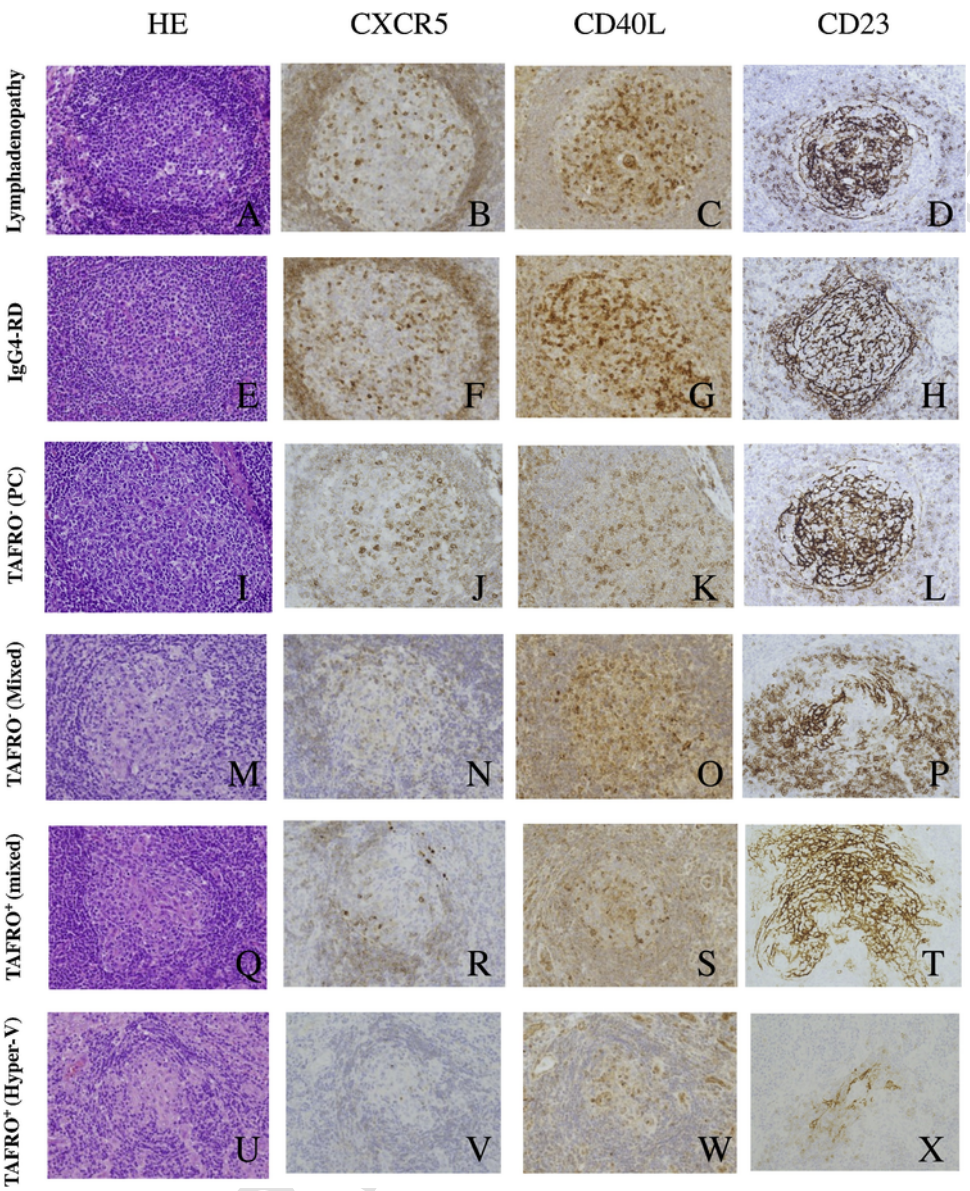

Fig. 2. GC in non-specific lymphadenopathy, IgG4-RD, PC-type TAFRO⁻ iMCD, mixed-type TAFRO- iMCD, mixed-type TAFRO⁺ iMCD and Hyper-V-type TAFRO⁺ iMCD. A: The GC showed normal architecture (HE staining, x200). B C, and D: The Tfh in the GC was preserved. The meshwork of FDCs was also preserved (CXCR5, CD40 L and CD23 immunostaining, x200). E: The GC showed normal architecture (HE staining, x200). F, G, and H: The Tfh in the GC was preserved. The meshwork of FDCs was also preserved (CXCR5, CD40 L, and CD23 immunostaining, x200). I: The GC showed normal architecture and hyperplastic change (HE staining, x200). J, K, and L: The Tfh in the GC was preserved. The meshwork of FDCs was preserved (CXCR5, CD40 L and CD23 immunostaining, x200). M: The GC was mostly preserved, but slight vascular proliferation was observed in the GC (HE staining, x200). N, O and P: The number of Tfh in the GC was decreased. The meshwork of FDCs was destroyed, and abnormal FDC proliferation was seen (CXCR5, CD40 L, and CD23 immunostaining, x200). Q: The GC showed slight atrophy. GVP was observed in the GC (HE staining, x200). R, S, and T: The number of Tfh in the GC was decreased in comparison to Mixed-type TAFRO- iMCD. The meshwork of FDCs was destroyed, and abnormal FDC proliferation was seen (CXCR5, CD40 L, and CD23 immunostaining, x200). U: The GC showed marked atrophy. The GC was occupied by GVP (HE staining, x200). V, W, and X: Tfh were almost absent in the GC. The meshwork of FDCs were completely destroyed, and abnormal FDC was seen (CXCR5, CD40 L, and CD23 immunostaining, x200).

All tissue samples were embedded in paraffin after 10% neutral buffered formalin fixation. The sections were stained with hematoxylin and eosin (H&E). Immunostaining was performed according to previously described methods [19,20]. To evaluate the number of Tfh and the number of Tfh in the GC per 0.01 mm^2, 5 HPFs were selected from prominent areas of aggregation in the GC and the numbers of cells were counted under a light microscope. All immunohistochemical analyses were reviewed by two certified surgical pathologists (N. K. and A. S.) in our department and very high rates of concordance (>95%) were obtained. For the few instances of disagreement, a consensus score was determined by a third board-certified pathologist (S. Y.) in our department.

2.2. Immunohistochemistry

All immunostaining was performed automatically (BenchMark GX, Ventana Medical System, Tucson, AZ, USA), and the antigen-antibody complex was visualized with 3,3′-diaminobenzidine solution. For immunostaining, an anti-human CXCR5 monoclonal antibody (clone 51505, 1:100 dilution; R & D systems, Minneapolis, USA) and anti-human CD40 L (clone 66502-1-Ig, 1:500 dilution; Proteintech, Rosemont, USA) were applied with incubation for 30 min at 37 °C. CXCR5 and CD40 L are specifically expressed on Tfh. However, CD40 L is mainly expressed on activated $CD4^+$ T cells and is expressed on

Table 1
Histopathological findings of CXCR5 + -Tfh.

	Lymphadenopathy (n = 6)	IgG4-RD (n = 4)	TAFRO$^-$ iMCD (n = 9)	TAFRO$^+$ iMCD (n = 18)
Number of Tfh in GC	74.9 ± 57.2	77.3 ± 33.6	22.9 ± 27.4****	4.8 ± 7.5****
Number of Tfh in GC per 0.01 mm^2	9.0 ± 3.9	14.1 ± 15.9*	7.6 ± 4.7	3.1 ± 3.9****

Abbreviations: CXCR5$^+$ C-X-C chemokine receptor type 5-positive; Tfh T follicular helper; Lymphadenopathy Non-specific lymphadenopathy; IgG4-RD IgG4-related disease; n, number; TAFRO$^-$ iMCD, idiopathic multicentric Castleman disease without TAFRO syndrome; TAFRO$^+$ iMCD, idiopathic multicentric Castleman disease with TAFRO syndrome.
GC, germinal center; *, p < 0.05; ****, p < 0.0001;

some CD8$^+$ T cells. The number of cells strongly positive for the cell membrane in the GC was counted.

2.3. Statistical analysis

The PRISM software program, ver. 6 (Graph Pad Software, La Jolla, CA, USA) was used for all statistical analyses. The Mann-Whitney U test was used to compare the histopathological data of TAFRO$^-$ and TAFRO$^+$ iMCD. *P* values of < 0.05 were considered to indicate statistical significance.

3. Results

3.1. The sizes of areas of GCs

The sizes of areas of the GCs in non-specific lymphadenopathy were similar to that in IgG4-RD (0.09 ± 0.07 vs. 0.11 ± 0.08 mm^2, $P = 0.3717$). The area of the GCs in TAFRO$^-$ and TAFRO$^+$ iMCD were significantly smaller than that of the GCs in non-specific lymphadenopathy (0.03 ± 0.03 vs. 0.09 ± 0.07 mm^2, $P < 0.0001$; 0.01 ± 0.01 vs. 0.09 ± 0.07 mm^2, $P < 0.0001$). The area of the GCs in Hyper-V-type TAFRO$^+$ iMCD was significantly smaller than that of the GCs in mixed-type TAFRO$^+$ iMCD (0.005 ± 0.004 mm^2 vs. 0.01 ± 0.01 mm^2, $P = 0.001$).

3.2. The numbers of Tfh in the GCs

As shown in Fig. 2 and Table 1, the numbers of CXCR5-positive (CXCR5$^+$)-Tfh in non-specific lymphadenopathy and IgG4-RD did not differ to a statistically significant extent (74.9 ± 57.2 vs. 77.3 ± 33.6, $P = 0.5828$). The numbers of CXCR5$^+$-Tfh in TAFRO$^-$ and TAFRO$^+$ iMCD were significantly lower in comparison to the number in the GCs in non-specific lymphadenopathy (22.9 ± 27.4 vs. 74.9 ± 57.2, $P = < 0.0001$; 4.8 ± 7.5 vs. 74.9 ± 57.2, $P < 0.0001$). The numbers of CXCR5$^+$-Tfh in TAFRO$^-$ iMCD was significantly higher in TAFRO$^+$ iMCD (22.9 ± 27.4 vs. 4.8 ± 57.2, $P = < 0.0001$). The numbers of CXCR5$^+$-Tfh in PC-type and mixed-type TAFRO- iMCD were significantly decreased in comparison to those in non-specific lymphadenopathy (28.5 ± 33.4 vs. 74.9 ± 57.2, $P < 0.0001$; 20.0 ± 23.2 vs. 74.9 ± 57.2, $P < 0.0001$). The numbers of CXCR5$^+$-Tfh in Hyper-V-type TAFRO$^+$ iMCD were significantly decreased in comparison to those in mixed-type TAFRO$^+$ iMCD (0.5 ± 1.1 vs. 7.0 ± 8.4, $P < 0.0001$).

As shown in Fig. 2 and Table 2, the numbers of CD40L-positive (CD40L$^+$)-Tfh in IgG4-RD were significantly larger than those in the non-specific lymphadenopathy (123.0 ± 66.4 vs. 210.0 ± 105.8, $P = 0.0027$). In contrast, the numbers of CD40L$^+$-Tfh in TAFRO$^-$ and TAFRO$^+$ iMCD were significantly lower in comparison to the those in the GCs of non-specific lymphadenopathy (48.8 ± 52.0 vs. 123.0 ± 66.4, $P < 0.0001$; 7.1 ± 9.0 vs. 123.0 ± 66.4, $P < 0.0001$). The numbers of CD40L$^+$-Tfh in TAFRO$^-$ iMCD was significantly higher in TAFRO$^+$ iMCD (48.8 ± 52.0 vs. 7.1 ± 9.0, $P = < 0.0001$). The numbers of CD40L$^+$-Tfh in PC-type and mixed-type TAFRO- iMCD were significantly decreased in comparison to those in non-specific lymphadenopathy (79.1 ± 58.7 vs. 123.0 ± 66.4, $P = 0.0104$; 24.6 ± 29.2 vs. 123.0 ± 66.4, $P < 0.0001$). In comparison to mixed-type CD40L$^+$-Tfh in TAFRO$^+$ iMCD, the numbers of Hyper-V-type CD40L$^+$-Tfh were significantly decreased (10.1 ± 9.7 vs. 1.0 ± 1.5, $P < 0.0001$).

3.3. The number of Tfh per 0.01 mm2 in the GC

A significant difference was observed in the number of CXCR5$^+$-Tfh per 0.01 mm^2 in the GC between non-specific lymphadenopathy and IgG4-RD (9.0 ± 3.9 vs. 14.1 ± 15.9, $P = 0.0268$). The number of CXCR5$^+$-Tfh in TAFRO$^+$ iMCD was significantly lower than that in non-specific lymphadenopathy (3.1 ± 3.9 vs. 9.0 ± 3.9, $P < 0.0001$); however, there was no significant difference in the number of CXCR5$^+$-Tfh in TAFRO$^-$ iMCD (7.6 ± 4.7 vs. 9.0 ± 3.9, $P = 0.0711$). The numbers of CXCR5$^+$-Tfh in TAFRO- iMCD was significantly higher in TAFRO$^+$ iMCD (7.6 ± 4.7 vs. 3.1 ± 3.9, $P < 0.0001$). The numbers of CXCR5$^+$-Tfh in PC-type TAFRO$^-$ iMCD were not significantly decreased in comparison to those in non-specific lymphadenopathy (9.0 ± 5.1 vs. 9.0 ± 3.9, $P = 0.5241$); however, there was significant difference in the number of CXCR5$^+$-Tfh in mixed-type TAFRO$^-$ iMCD (6.4 ± 4.0 vs. 9.0 ± 3.9, $P = 0.0210$). In comparison to the number of CXCR5$^+$-Tfh in mixed-type TAFRO$^+$ iMCD, the number was significantly decreased in Hyper-V-type TAFRO$^+$ iMCD (7.0 ± 8.4 vs. 0.5 ± 1.1, $P < 0.0001$).

The number of CD40L-positive (CD40L$^+$)-Tfh in non-specific lymphadenopathy and IgG4-RD did not differ to a statistically significant extent (17.2 ± 5.4 vs. 22.8 ± 11.3, $P = 0.1411$). The number of CD40L$^+$-Tfh in TAFRO$^+$ iMCD was significantly lower than that in non-specific lymphadenopathy (5.9 ± 6.1 vs. 17.2 ± 5.4, $P < 0.0001$). The number of CD40L$^+$-Tfh in TAFRO$^-$ iMCD was not significantly different from that in non-specific lymphadenopathy cases (15.4 ± 9.0 vs. 17.2 ± 5.4, $P = 0.1016$). The numbers of CD40L$^+$-Tfh in TAFRO- iMCD was significantly higher in TAFRO$^+$ iMCD (15.4 ± 9.0 vs. 5.9 ± 6.1, $P < 0.0001$). The numbers of CD40L$^+$-Tfh in PC-type

Table 2
Histopathological findings of CD40L + -Tfh.

	Lymphadenopathy (n = 6)	IgG4-RD (n = 4)	TAFRO$^-$ iMCD (n = 9)	TAFRO$^+$ iMCD (n = 18)
Number of Tfh in GC	123.0 ± 66.4	210.0 ± 105.8*	48.8 ± 52.0****	7.1 ± 9.0****
Number of Tfh in GC per 0.01 mm^2	17.2 ± 5.4	22.8 ± 11.3	15.4 ± 9.0	5.9 ± 6.1****

Abbreviations: CD40L$^+$, CD40 ligand-positive; Tfh, T follicular helper; Lymphadenopathy, Non-specific lymphadenopathy; IgG4-RD, IgG4-related disease; n, numberTAFRO$^-$ iMCD, idiopathic multicentric Castleman disease without TAFRO syndrome; TAFRO$^+$ iMCD, idiopathic multicentric Castleman disease with TAFRO syndrome GC, germinal center; *, p < 0.05; ****, p < 0.0001.

TAFRO- iMCD were not significantly decreased in comparison to those in non-specific lymphadenopathy (20.3 ± 9.0 vs. 17.2 ± 5.4, $P = 0.2842$); however, there was significant difference in the number of $CD40L^+$-Tfh in mixed-type TAFRO- iMCD (11.5 ± 46.9 vs. 17.2 ± 5.4, $P = 0.0003$). In comparison to the number of $CD40L^+$-Tfh in mixed-type $TAFRO^+$ iMCD, the number of cells was significantly decreased in Hyper-V-type $TAFRO^+$ iMCD (8.1 ± 6.3 vs. 1.4 ± 2.2, $P < 0.0001$).

3.4. Serum IgG4 and IL-6 levels

In patients with IgG4-RD, serum IgG4 levels were elevated (n = 4, 1543.2 ± 1460.4 mg/dL). Serum IgG4 levels were elevated in $TAFRO^-$ iMCD (n = 3, 367.1 ± 251.9 mg/dL); however, serum IgG4 levels were not elevated in $TAFRO^+$ iMCD (n = 5, 54.0 ± 37.1 mg/dL).

In patients with IgG4-RD, serum IL-6 levels were slightly elevated (n = 4, 4.8 ± 2.5 pg/mL). There was no significant differences in Serum IL-6 levels between $TAFRO^+$ iMCD and $TAFRO^-$ iMCD (n = 7; 42.0 ± 60.4 pg/mL vs. n = 14; 54.0 ± 37.1 pg/mL, $P = 0.7931$).

4. Discussion

In this study, we thoroughly examined the number of Tfh in $TAFRO^-$ and $TAFRO^+$ iMCD. In comparison to TAFRO- iMCD, the number of Tfh in the GC was significantly lower in $TAFRO^+$ iMCD. As a result of decreased number of Tfh in the GC, it is possible for the GCs to turn atrophic. The cause of the absence of plasma cells within the GC in iMCD may be closely related to the suppression of differentiation of GC B-cells to plasma cells and memory B-cells. Class switching to IgG isotype containing an IgG4 antibody is also considered to be suppressed.

In comparison to the number of Tfh in mixed-type $TAFRO^+$ iMCD, the number of Tfh was significantly decreased in Hyper-V-type $TAFRO^+$ iMCD. As shown in our previous report [15], the Hyper-V type is a histological subtype characterized by marked atrophic change of the GCs and reduced $IgG4^+$ and $CD38^+$ plasma cell infiltration. Thus, it is likely that Hyper-V-type $TAFRO^+$ iMCD represents a more advanced histological subtype of $TAFRO^+$ iMCD.

Yajima et al. [21] reported that loss of IL-21 was observed in the atrophic GCs of iMCD by a real-time polymerase chain reaction (PCR). The decreased production of IL-21 that induces naïve T cells to differentiate into Tfh may reduce the number of Tfh in GCs. Iwaki et al. [22] showed that the serum level of interferon gamma-inducible protein 10 kDa (IP-10) was significantly higher in patients with $TAFRO^+$ iMCD. IP-10 is a specific ligand for CXCR3, which is specifically expressed on Th1. IP-10 is known to be elevated in autoimmune diseases, infectious diseases and neoplastic diseases. It is hypothesized that the elevation of serum IP-10 is caused by the suppression of Tfh differentiation and the enhancement of Th1 differentiation (Fig. 1).

Pierson et al. [23] reported that chemokine CXCL13 was significantly upregulated in a plasma proteomics analysis of iMCD. The CXCL13 produced by FDC can bind to the chemokine receptor, CXCR5, which is expressed on the cell surface of Tfh. To the best of our knowledge, there is no report to explain the relationship between Tfh and abnormal FDC proliferation. As a result of the decreased number of extra-follicular Tfh migrating into the GC and the decreased number of Tfh localized in the GC, it appeared that abnormal FDC proliferation was caused. Further examinations are needed to fully clarify the pathogenesis of abnormal FDC proliferation.

We previously reported that the number of $IgG4^+$ plasma cells in $TAFRO^+$ iMCD was significantly lower in comparison to $TAFRO^-$ iMCD cases [15]. The serum IgG4 level of $TAFRO^+$ iMCD were decreased compared to that of $TAFRO^-$ iMCD cases. Therefore, the differentiation of extra-follicular naïve B-cells to extra-follicular plasma cells may also be suppressed (Fig. 1).

It is well-known that many Tfh are present in the GCs in IgG4-RD [24]. IL-4-secreting Tfh contribute to B-cell differentiation and the class switching of IgG isotypes, including IgG4. Thus, Tfh plays an important role in the onset of IgG4-RD. Since the numbers of Tfh in $TAFRO^+$ or $TAFRO^-$ iMCD were rather smaller in comparison to IgG4-RD cases, it seems that class switching to IgG4 was also suppressed. Thus, we suggest that evaluating the presence of Tfh within GCs is useful for histopathological differentiation between IgG4-RD and iMCD.

In conclusion, we found that the number of Tfh was decreased in the GCs of patients with $TAFRO^+$. This histological finding may suggest that Tfh play critical roles in the pathogenesis of $TAFRO^+$ iMCD.

Author contribution

NK and SY participated in the conception of the study and the writing of the manuscript. KM, MK, AS, XG, SF, HK, YM, KT, SA and SN performed clinical imaging and/or the pathological/ /immunohistochemical analyses of the lesion specimens. All of the authors have read and approved the final manuscript.

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Declaration of competing interest

The authors declare no conflicts of interest in association with the present study.

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