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Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease

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IgG4-related disease sometimes involves regional and/or systemic lymph nodes, and often clinically and/or histologically mimics multicentric Castleman's disease or malignant lymphoma. In this study, we examined clinical and pathologic findings of nine patients with systemic IgG4-related lymphadenopathy. None of these cases were associated with human herpes virus-8 or human immunodeficiency virus infection, and there was no T-cell receptor or immunoglobulin gene rearrangement. Histologically, systemic IgG4-related lymphadenopathy was classified into two types by the infiltration pattern of IgG4-positive cells: interfollicular plasmacytosis type and intra-germinal center plasmacytosis type. The interfollicular plasmacytosis type showed either Castleman's disease-like features or atypical lymphoplasmacytic and immunoblastic proliferation-like features. By contrast, the intra-germinal center plasmacytosis type showed marked follicular hyperplasia, and infiltration of IgG4-positive cells mainly into the germinal centers, and some cases exhibited features of progressively transformed germinal centers. Interestingly, eight of our nine (89%) cases showed eosinophil infiltration in the affected lymph nodes, and examined patients showed high elevation of serum IgE. Laboratory examinations revealed elevation of serum IgG4 and soluble interleukin-2 receptors. However, the levels of interleukin-6, C-reactive protein, and lactate dehydrogenase were within normal limits or only slightly elevated in almost all patients. One patient showed a high interleukin-6 level whereas C-reactive protein was within the normal limit. Autoantibodies were examined in five patients and detected in four. Compared with the previously reported cases of multicentric Castleman's disease, our patients with systemic IgG4-related lymphadenopathy were significantly older and had significantly lower C-reactive protein and interleukin-6 levels. In conclusion, in our systemic IgG4-related lymphadenopathy showed pathologic features only partially overlapping those of multicentric Castleman's disease, and serum data (especially C-reactive protein and interleukin-6) are useful for differentiating the two. Our findings of eosinophil infiltration in the affected tissue and elevation of serum IgE may suggest an allergic mechanism in the pathogenesis of systemic IgG4-related lymphadenopathy.
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Keywords: systemic IgG4-related lymphadenopathy; C-reactive protein; interleukin-6; immunoglobulin E; multicentric castleman's disease

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Recently, autoimmune pancreatitis and its related disorders, such as sclerosing cholangitis, sclerosing sialadenitis (Küttner's tumor), retroperitoneal fibrosis, and Mikulicz's disease have been shown to share IgG4-related abnormalities. Such abnormalities include an elevated serum IgG4 level

and numerous IgG4-positive plasma cells infiltrating the affected tissue, and these disorders are classified as IgG4-related diseases.[1–11]

Castleman's disease is a rather rare atypical lymphoproliferative disorder[12] classified according to the histopathologic findings of the affected lymph nodes as plasma cell type, hyaline-vascular type or a mixed-type variant of the two.[13,14] Patients with the plasma cell or the mixed-type variant frequently have systemic manifestations (so-called multicentric Castleman's disease), such as low-grade fever, fatigue, loss of appetite, and weight loss. Abnormal laboratory findings include anemia, hypoalbuminemia, hypocholesterolemia, hypergammaglobulinemia, increased C-reactive protein, and interleukin-6.[13–16] These symptoms are closely related to high interleukin-6 levels, suggesting a cytokine disease. Idiopathic plasmacytic lymphadenopathy with polyclonal hyperimmunoglobulinemia is considered identical to multicentric Castleman's disease in Western countries.[17,18] Idiopathic plasmacytic lymphadenopathy and multicentric Castleman's disease show similar clinicopathology. However, idiopathic plasmacytic lymphadenopathy has a significantly better 5-year survival rate than multicentric Castleman's disease.[17,18] Multicentric Castleman's disease exhibits an aggressive and usually fatal disease course associated with infectious complications and risk of malignant tumors; one-third of such patients develop Kaposi sarcoma or B-cell lymphoma.[17,18]

IgG4-related disease sometimes involves regional and/or systemic lymph nodes,[3,4,11] and is often clinically and/or histologically suspected to be multicentric Castleman's disease and/or malignant lymphoma.[5,6,11] However, systemic IgG4-related lymphadenopathy is either not mentioned or only briefly alluded to in previous reports.[3,11]

In this study, we examined systemic IgG4-related lymphadenopathy in detail, and its differences from multicentric Castleman's disease, with specific reference to clinical and pathologic findings.

Materials and methods

Patients and Materials

Nine Japanese patients with systemic IgG4-related lymphadenopathy were clinicopathologically examined. All cases were retrieved from the surgical pathology consultation files of the Department of Pathology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University in Okayama, Japan. The preliminary diagnostic criteria consisted of an elevated serum IgG4 value (>135 mg/dl) and infiltration of IgG4-positive cells in the affected lymph nodes (IgG4/IgG-positive cell ratio $>40\%$). These diagnostic criteria were suggested by previous reports.[6,11]

Clinical information was obtained from the patient records, referring pathologists, or clinicians. All data and samples from patients were collected with their informed consent.

Histological Examination and Immunohistochemistry

Surgically biopsied lymph node specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial sections (4 μm) were cut from each paraffin-embedded tissue block, and several sections were stained with hematoxylin and eosin.

Immunohistochemistry was performed on paraffin sections using an automated Benchmark XT slide stainer (Ventana Medical Systems Inc., Tucson, AZ, USA). Tissue sections underwent standardized heating pretreatment for antigen retrieval prior to the immunohistochemical procedure. Primary antibodies used were: CD20 (L26; 1:200; Novocastra, Newcastle, UK), CD3 epsilon (PS-1; 1:50; Novocastra), CD5 (4C7; 1:100; Novocastra), CD10 (56C6; 1:50; Novocastra), CD21 (1F8; 1:20; Dako, Carpinteria, CA, USA), CD138 (MI15; 1:100; Dako), Bcl-2 (3.1; 1:200; Novocastra), IgG (Polyclonal; 1:10 000; Dako), IgG4 (HP6025; 1:1000; The Binding Site, Birmingham, UK), Kappa (NCL-KAP; 1:100; Novocastra), Lambda (NCL-LAM; 1:200; Novocastra), and human herpes virus type-8 (137B1; 1:50; Novocastra).

The number of IgG4- or IgG-positive cells was estimated at the areas with the highest density of positive cells. Five different high-power fields (HPF: $\times 10$ eyepiece and $\times 40$ lens) in each section were counted, and the average number of positive cells per HPF was calculated.

Polymerase Chain Reaction for Detecting Immunoglobulin Heavy Chain Gene and T-Cell Receptor Gene Rearrangements

Polymerase chain reaction was used in the analysis of the immunoglobulin heavy chain gene and T-cell receptor gamma gene rearrangement. Polymerase chain reaction was performed according to standard procedures as described previously.[19–22]

The primers used for immunoglobulin heavy chain gene amplification were as follows:[19–21]
5′-TGG[A/G] TCCG[C/A] CAG [G/C] C [T/C][T/C] C [A/C/G/T] GG-3′ as the upstream consensus V region primer; 5′-TGAGGAGACGGTGACC-3′ as the consensus J region primer; and 5′-GTGACCAGGGT [A/C/G/T] CCTTGGCCCCAG-3′ as the consensus J region primer.

The primers used for T-cell receptor gamma gene amplification were as follows.[22]

for tube A:
VgIf: 5′-GGAAGGCCCCACAGCRTCTT-3′
Vg10: 5′-AGCATGGGTAAGACAAGCAA-3′
Jg1.1/2.1: 3′-CGAGTAYCATTGAAGCGGACCATT-5′
Jg1.3/2.3: 3′-GAGAAACCGTCACCTTGTTGTG-5′
for tube B:
Vg9: 5′-CGGCACTGTCAGAAAGGAATC-3′
Vg11: 5′-CTTCCACTTCCACTTTGAAA-3′

Jg1.1/2.1: 3′-CGAGTAYCATTGAAGCGGACCATT-5′
Jg1.3/2.3: 3′-GAGAAACCGTCACCTTGTTGTG-5′

Statistical Analysis

All statistical analyses were carried out using the Mann–Whitney *U*-test with SPSS software (version 14.0; SPSS Inc., Chicago, IL, USA). Values of $P<0.01$ were considered statistically significant.

Results

Clinical Findings

The clinical findings are summarized in Tables 1 and 2. There were seven men and two women with a median age of 72.0 years (range, 45–82 years). All patients showed systemic lymphadenopathy, and clinically, and/or histologically suspected multicentric Castleman's disease, and/or malignant lymphoma. The size of the lymph nodes ranged from 1 to 2 cm in diameter, with an average of 1.7 cm. Analysis of the patient lifestyles did not suggest any risk factors for human immunodeficiency virus infection, and anti-human immunodeficiency virus-1 antibody was negative in the seven patients examined. Various autoantibodies were detected in four of five patients examined (Table 1). Hypergammaglobulinemia was detected in all patients, although serum IgM and IgA was normal in almost all patients. However, the serum IgE value was significantly elevated in the examined patients. The serum IgG4 level was also significantly elevated (average = 818.44 mg/dl; s.d. = 502.94) in all patients. C-reactive protein was normal or only slightly elevated (average = 0.29 mg/dl; s.d. = 0.25). Interleukin-6 was normal or only slightly elevated (average = 8.45 pg/ml; s.d. = 11.61), but one patient showed a high interleukin-6 level whereas C-reactive protein was within the normal limit. Lactate dehydrogenase was normal or only slightly elevated (average = 197.22 IU/l; s.d. = 51.29). In contrast, soluble interleukin-2 receptor was significantly elevated (average = 1875.78 U/ml; s.d. = 835.87). Hemoglobin, albumin and total cholesterol were normal in almost all patients. Compared with previous reports of multicentric Castleman's disease,[16] our patients with systemic IgG4-related lymphadenopathy showed more advanced age distribution, no significant elevation of C-reactive protein or interleukin-6 levels, no anemia, no hypoalbuminemia, and no hypocholesterolemia. These differences between the groups were statistically significant (Table 3).

Pathological and Immunohistological Findings of the Lymph Node

Histologically and immunohistologically, we classified the cases into two types by the infiltration

Table 1 Clinical findings of nine patients with systemic IgG4-related lymphadenopathy

Patient no.	*Age*	*Sex*	*LN swelling*	*Extranodal lesions*	*Autoantibody*	*Treatment and clinical outcome (Follow-up period, months)*
1	73	M	Cervical, supraclavicular, axilla, hilar, mediastinal, para-aortic, retroperitoneal, inguinal, up to 2 cm	Right lung nodule	ANA+	Follow up: stable (15)
2	77	M	Cervical, supraclavicular, axilla, hilar, mediastinal, para-aortic, inguinal, up to 2 cm	Developed parotid gland swelling	Anti-dsDNA antibody+, ANA+	Steroid: good response, LN and parotid gland swelling disappeared (11)
3	67	F	Cervical, submandibular, supraclavicular, axilla, hilar, mediastinal, para-aortic, inguinal, up to 2 cm.	None	ND	Follow up: stable and spontaneous regression of LN (8)
4	72	M	Cervical, submandibular, hilar, para-aortic, retroperitoneal, up to 2 cm	Submandibular gland mass	ANA+	Submandibular gland mass and lymph node excision: stable (25)
5	82	F	Cervical, axilla, hilar, pelvic, para-aortic, inguinal, up to 2 cm	Developed lacrimal and parotid gland swelling	RF−	Follow up: stable (5)
6	81	M	Cervical, axilla, hilar, mediastinal, inguinal, up to 1.5 cm	Skin lesion	ANA+, RF−	Steroid: good response, decrease LN swelling and skin lesion (1)
7	58	M	Cervical, submandibular, hilar, mediastinal, para-aortic, up to 2 cm	None	ND	Steroid: good response, LN swelling disappeared (13)
8	57	M	Cervical, axilla, hilar, mediastinal, para-aortic, up to 1.5 cm	Developed bilateral lacrimal swelling, skin lesion	ND	Steroid: good response, LN and lacrimal swelling and skin lesion disappeared (15)
9	45	M	Cervical, submandibular, para-aortic, inguinal, up to 1.5 cm	Developed bilateral lacrimal swelling, skin lesion	ND	Steroid: Steroid: good response, LN and lacrimal swelling and skin lesion disappeared (35)

M, male; F, female; LN, lymph node; ANA, antinuclear antibody; RF, rheumatoid factor; ND, not determined.

Table 2 Laboratory data of nine patients with systemic IgG4-related lymphadenopathy

Patient no.	*IgG1 (mg/dl; nl = 320–748)*	*IgG2 (mg/dl; nl = 208–754)*	*IgG3 (mg/dl; nl = 6.6–88.3)*	*IgG4 (mg/dl; nl = 4.8–105)*	*IgG4/IgG (%; nl, 3–6%)*	*IgG (mg/dl; nl = 870–1700)*	*IgA (mg/dl; nl = 110–410)*	*IgM (mg/dl; nl = 35–220)*	*IgE (IU/ml; nl, <170)*	*CRP (mg/dl; nl, < 0.3)*	*IL-6 (pg/ml; nl, < 4.0)*	*LDH (IU/l; nl = 119–229)*	*sIL-2R (U/ml; nl = 220–530)*	*Hb (g/dl)*	*Alb (g/dl)*	*T-cho (mg/dl)*
1	1280	1320	169	312	10.1	3081	ND	ND	ND	0.13	4.1	147	1762	11.1	3.6	177
2	ND	ND	ND	1100	20	5800	111	38	686.9	0.6	9	280	2170	13.6	3.4	141
3	1180	694	11	583	23.6	2468	469	133	2920	0.2	1.2	156	3620	12.7	4.4	151
4	[605]	[519]	[40]	[345]	[22.9]	3320 [1509]	293	38	ND	0.8	11.3	268	1760	13	3.6	129
5	1420	845	132	1050	30.36	3447	101	19	ND	0.13	2.8	210	1900	11.2	3.9	221
6	ND	ND	ND	780	18.0	4341	298	ND	ND	0.17	35.7	166	2470	11.3	2.8	138
7	1650	1170	52.3	1280	30.74	4152.3	182	33	ND	0.32	ND	137	1130	14.7	3.7	159
8	1730	1070	47	1700	37.45	4547	72	29	[932]	0.08	1.8	211	1270	13.2	3.7	176
9	[755]	[582]	[21.6]	[216]	[13.72]	1710 [1574.6]	302	78	[521]	0.19	1.7	200	800	15.8	4.3	176

nl, normal; CRP, C-reactive protein; IL-6, interleukin-6; LDH, lactate dehydrogenase; sLL-2R, soluble interleukin-2 receptor; Hb, hemoglobulin; Alb, albumin; T-cho, total cholesterol; ND, not determined. [ ] Determined after therapy.

pattern of IgG4-positive cells; interfollicular plasmacytosis type and intra-germinal center plasmacytosis type (Table 4).

The IgG4/IgG-positive cells ratio ranged from 44.7 to 72.7%, with an average of 57.6%.

Interfollicular Plasmacytosis Type

The lymph nodes of patient no. 1, 2, 3, 4, 5, and 6 were of interfollicular plasmacytosis type. On low-power field, the lymph nodes demonstrated many germinal centers, usually normal to atrophic with a distinct mantle zone and expansion of the interfollicular area. The interfollicular area showed a moderate to marked increase in vascularity, and heavy infiltration with mature plasma cells, plasmacytoid cells, small lymphocytes, and eosinophils. The follicular dendritic cell networks showed a normal or reactive pattern in all cases. Histologically, the lymph nodes of patients no. 1, 3, and 4 showed Castleman's disease-like features (Figure 1). In contrast, the lymph nodes of patients no.2, 5, and 6 showed atypical lymphoplasmacytic and immunoblastic proliferation[23,24] -like features (Figure 2) — ie, the lymph nodes had germinal centers that were usually atrophic to normal, and prominent lymphoplasmacytic infiltration with various numbers of immunoblasts in the interfollicular area. Compared with the histology of the Castleman disease-like features, the histology of atypical lymphoplasmacytic and immunoblastic proliferation-like features was characterized by decreased lymphoid follicles, atrophic germinal centers, a marked increase in vascularity, and more distinct immunoblasts.

Five of these six cases showed eosinophil infiltration in the interfollicular area. In terms of immunohistochemistry, IgG4-positive cells infiltrated the interfollicular areas, and the IgG4/IgG-positive cell ratio ranged from 44.7 to 72.7%, with an average of 56.7%. Three of six patients had exocrine organ lesions, and one patient (patient no. 6) had a skin lesion (not examined histologically).

There were no human herpes virus type-8-positive cells, no immunoglobulin light-chain restriction, and no immunoglobulin heavy chain gene rearrangement in any cases. In addition, there was no T-cell receptor gamma gene rearrangement in the atypical lymphoplasmacytic and immunoblastic proliferation-like cases (patient nos. 2, 5, and 6).

Intra-Germinal Center Plasmacytosis Type

The lymph nodes of patient nos. 7, 8, and 9 were the intra-germinal center plasmacytosis type. On low-power field, the lymph nodes demonstrated numerous lymphoid follicles with hyperplastic germinal centers and a distinct mantle zone, and no expansion of the interfollicular area. The interfollicular area showed numerous eosinophils and a small number of mature plasma cells and plasmacytoid

Table 3 Comparison of clinicopathologic findings between systemic IgG4-related lympahadenopathy and multicentric Castleman's disease

	Systemic IgG4-related lympahadenopathy	*Multicentric Castleman's disease*[a]	P-*value*
Age	68.00 (s.d. = 12.44; $n = 9$)	43.36 (s.d. = 12.35; $n = 28$)	<0.001
Male/female ratio	7/2	17/11	0.358
IgG (mg/dl)	3651.8 (s.d. = 1214.1; $n = 9$)	5220 (s.d. = 1956.5; $n = 28$)	0.026
CRP (mg/dl)	0.29 (s.d. = 0.25; $n = 9$)	8.71 (s.d. = 4.98; $n = 28$)	<0.001
IL-6 (pg/ml)	8.45 (s.d. = 11.61; $n = 8$)	34.82 (s.d. = 34.59; $n = 28$)	0.001
Hb (g/dl)	12.96 (s.d. = 1.61; $n = 9$)	9.23 (s.d. = 2.30; $n = 28$)	<0.001
Alb (g/dl)	3.71 (s.d. = 0.48; $n = 9$)	2.72 (s.d. = 0.53; $n = 28$)	<0.001
T-Cho (mg/dl)	163.1 (s.d. = 28.11; $n = 9$)	113.9 (s.d. = 35.86; $n = 28$)	0.001

Averages are shown for age, IgG, CRP, IL-6, Hb, Alb, and T-Cho.
[a]Data from Nishimoto *et al*.[16]

Table 4 Histological findings of nine patients with systemic IgG4-related lymphadenopathy

Patient no.	*Biopsy site*	*Histological findings*	*IgG4/IgG-positive cells (%)*
1	Axillary LN	**Interfollicular plasmacytosis type: Castleman's disease-like feature** normal germinal center, moderate increase in vascularity, expansion of the interfollicular area, IgG4-positive cells in interfollicular area	44.7
2	Cervical LN	**Interfollicular plasmacytosis type: atypical lymphoplasmacytic and immunoblastic proliferation-like feature** normal-atrophic germinal center, moderate increase in vascularity, expansion of the interfollicular area, IgG4-positive cells and eosinophils in interfollicular area	55.7
3	Cervical LN	**Interfollicular plasmacytosis type: Castleman's disease-like feature** normal germinal center, moderate increase in vascularity, expansion of the interfollicular area, IgG4-positive cells and eosinophils in interfollicular area	53.1
4	Cervical LN	**Interfollicular plasmacytosis type: Castleman's disease-like feature** normal germinal center, moderate increase in vascularity expansion of the interfollicular area IgG4-positive cells and eosinophils in interfollicular area	64.0
5	Inguinal LN	**Interfollicular plasmacytosis type: atypical lymphoplasmacytic and immunoblastic proliferation-like feature** atrophic-normal germinal center, marked increase in vascularity, expansion of the interfollicular area, IgG4-positive cells and eosinophils in interfollicular area	50.1
6	Inguinal LN	**Interfollicular plasmacytosis type: atypical lymphoplasmacytic and immunoblastic proliferation-like feature** atrophic germinal center, marked increase in vascularity, expansion of the interfollicular area, IgG4-positive cells and eosinophils in interfollicular area	72.7
7	Cervical LN	**Intra-germinal center plasmacytosis type** marked follicular hyperplasia, eosinophils in interfollicular area, mild increase in vascularity, IgG4-positive cells in germinal center	56.5
8	Inguinal LN	**Intra-germinal center plasmacytosis type** marked follicular hyperplasia with progressively transformed germinal center, eosinophils in interfollicular area, mild increase in vascularity, IgG4-positive cells in germinal center	58.7
9	Inguinal LN	**Intra-germinal center plasmacytosis type** marked follicular hyperplasia with progressively transformed germinal center, eosinophils in interfollicular area, mild increase in vascularity, IgG4- positive cells in germinal center	63.0

LN, lymph node.

cells. In contrast, the intra-germinal center showed extensive infiltration with mature plasma cells and plasmacytoid cells. Follicular dendritic cell networks showed a normal or reactive pattern in all cases. Two cases (patient nos. 8 and 9) showed a focal progressively transformed germinal center (Figure 3).

Immunohistochemically, IgG4-positive cells mainly infiltrated the intra-germinal center, and the percentage of IgG4/IgG-positive cells ratio ranged from 56.5 to 63.0%, with an average of 59.4%.

Two of the three patients had exocrine organ lesions. In addition, two patients (nos. 8 and 9) had

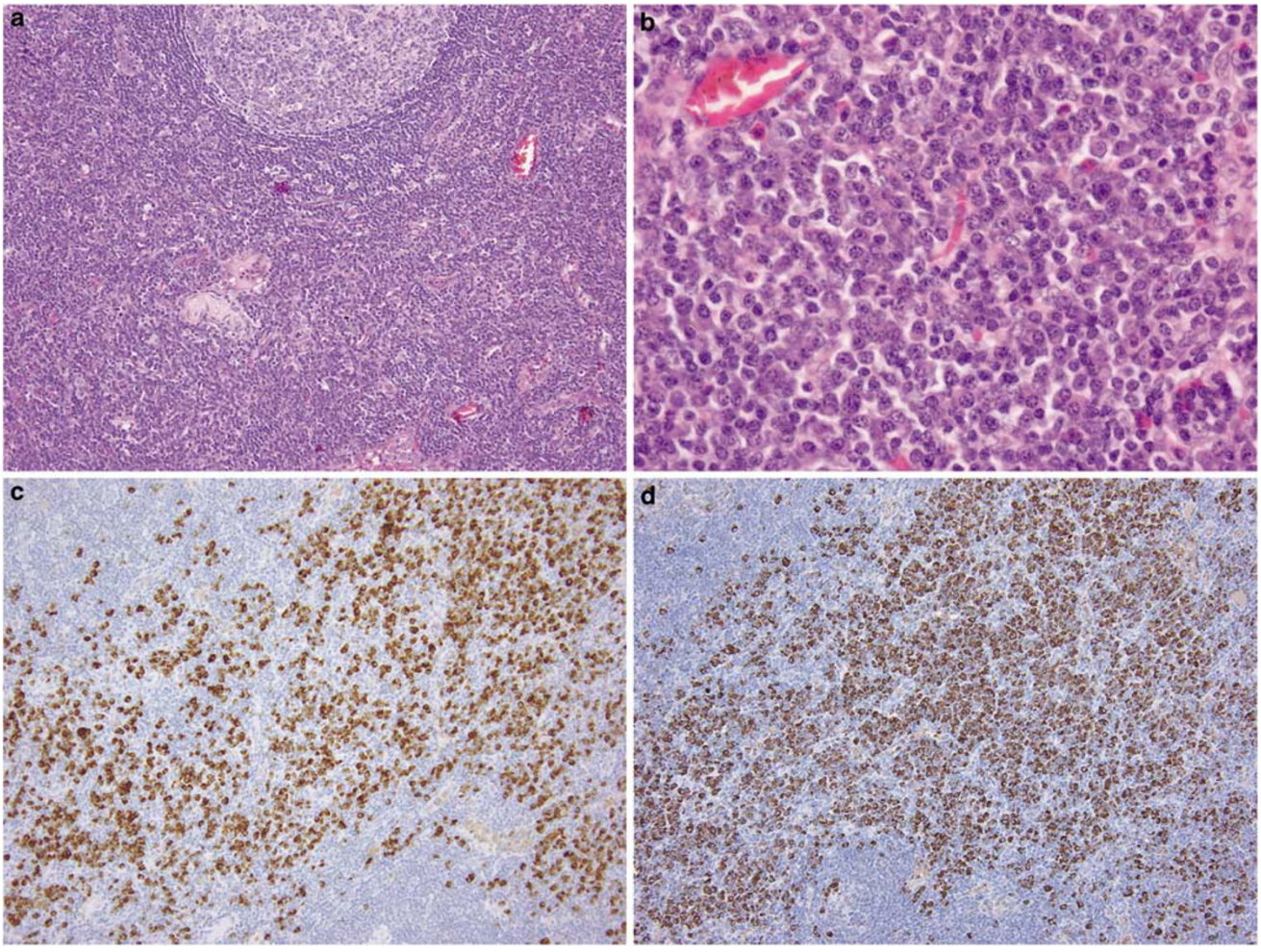

Figure 1 Histopathology of systemic IgG4-related lymphadenopathy (interfollicular plasmacytosis type, Castleman's disease-like features, patient no. 4). (**a**) The lymph node showed normal germinal center with distinct mantle zone, moderate increase in vascularity, and expansion of the interfollicular area. (**b**) The interfollicular area showed heavy infiltration with mature plasma cells, small lymphocytes, and eosinophils. Immunostaining of IgG4 (**c**) and IgG (**d**). A large number of IgG4-positive cells infiltrated the lymph node, and the IgG4/IgG-positive cell ratio was 64.0%. (**a** and **b**) Hematoxylin and eosin staining; (**a**, **c,** and **d**) $\times 100$, and (**b**) $\times 400$.

skin lesions. Histologically, the skin lesions showed lymphoplasmacytic infiltration with abundant IgG4-positive cells and eosinophils (Figure 4).

There were no human herpes virus type-8-positive cells, and no immunoglobulin light-chain restriction or immunoglobulin heavy chain gene rearrangement in any cases.

Discussion

IgG4-related disease is a recently recognized syndrome characterized by mass-forming lesions in mainly exocrine tissue due to lymphoplasmacytic infiltrates and sclerosis, increased serum IgG4 level, and IgG4-positive plasma cells in the affected tissues.[1–10] Clinical manifestations are apparent in the pancreas, bile duct, gallbladder, lacrimal gland, salivary gland, retroperitoneum, kidney, lung, and prostate, in which tissue fibrosis is pathologically induced.[1–10] Recently, many cases have been reported in Western countries, as well as in Japan.[3] However, the precise pathogenesis and pathophysiology of IgG4-related disease remain unclear. As patients presenting with prominent fibrosis are frequently suspected of having malignant tumors, IgG4-related disease should be considered in the differential diagnosis to avoid unnecessary surgery.[1–10]

Recent studies have reported that IgG4-related disease sometimes involves the systemic lymph nodes but clinicopathological characteristics have not been well documented yet.[3,4,11] In this study, we sought to clarify the clinicopathologic features of systemic IgG4-related lymphadenopathy. The clinical findings of systemic lymphadenopathy and hypergammaglobulinemia, which are characteristic of systemic IgG4-related lymphadenopathy, are also highly reminiscent of multicentric Castleman's disease.[15–18] However, none of our patients had anemia,

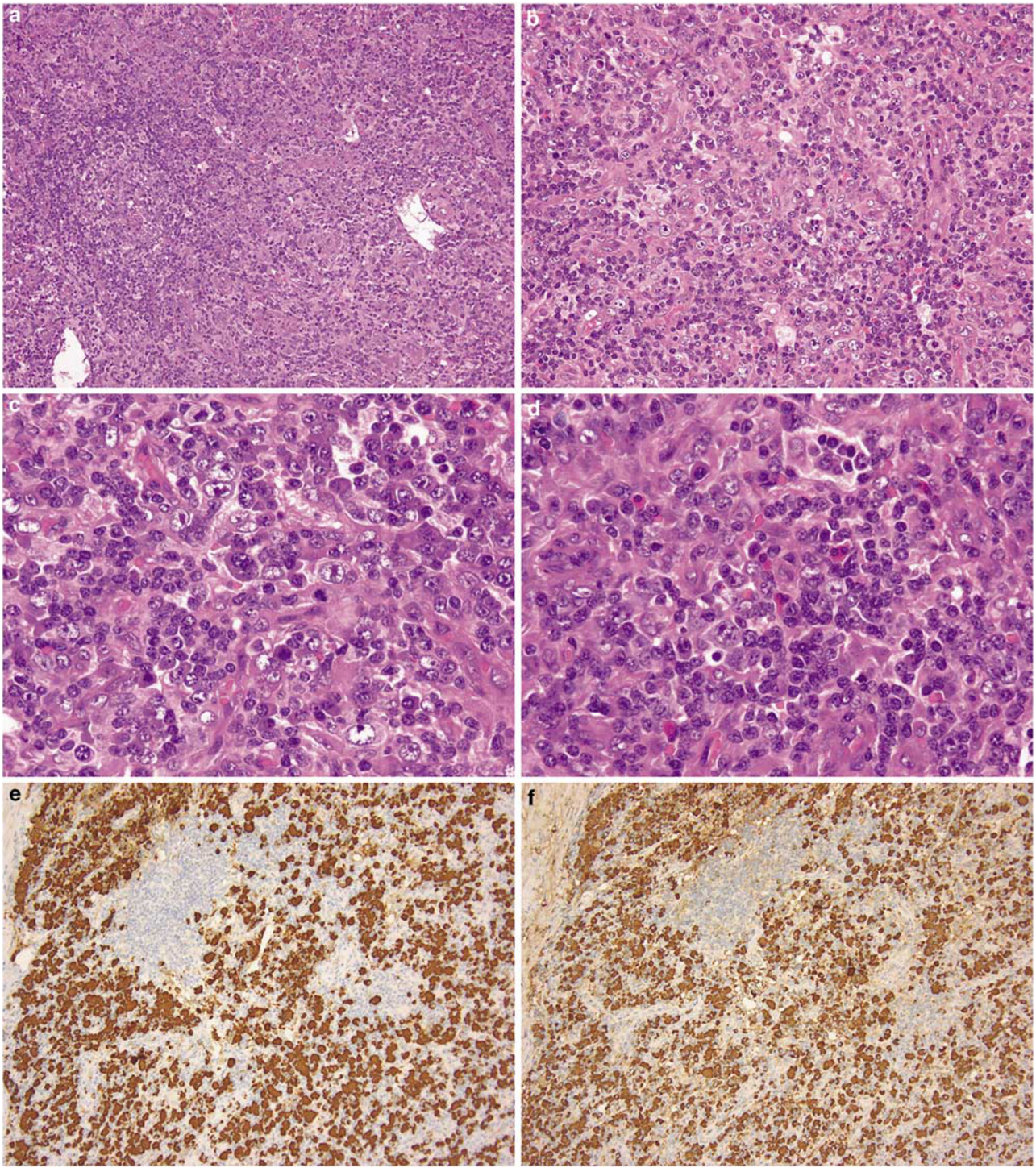

Figure 2 Histopathology of systemic IgG4-related lymphadenopathy (interfollicular plasmacytosis type, atypical lymphoplasmacytic and immunoblastic proliferation-like feature, patient no. 6). (**a**) The lymph node showed atrophic germinal center with discrete mantle zone, and expansion of the interfollicular area. (**b**) High endothelial venules were prominent, and there was polymorphous cellular infiltration; plasma cells and immunoblasts were especially distinct (**c**) The interfollicular area showed heavy infiltration with mature plasma cells, plasmacytoid cells, small lymphocytes, and immunoblasts. (**d**) Eosinophil infiltration was recognized. Immunostaining of IgG4 (**e**) and IgG (**f**). The IgG4/IgG-positive cell ratio was 72.7%. (**a–d**) Hematoxylin and eosin staining; (**a**) $\times$ 40, (**b**) $\times$ 200, (**c** and **d**) $\times$ 400, and (**e** and **f**) $\times$ 100.

hypoalbuminemia, or hypocholesterolemia, and elevated interleukin-6 and C-reactive protein were the exceptions. These findings are quite different from those of multicentric Castleman's disease.[15–18]

Interleukin-6 is a multifunction cytokine that has various biological activities in target cells and regulates immune responses, acute phase reactions, hematopoiesis, and bone metabolism.[25] Dysregulated

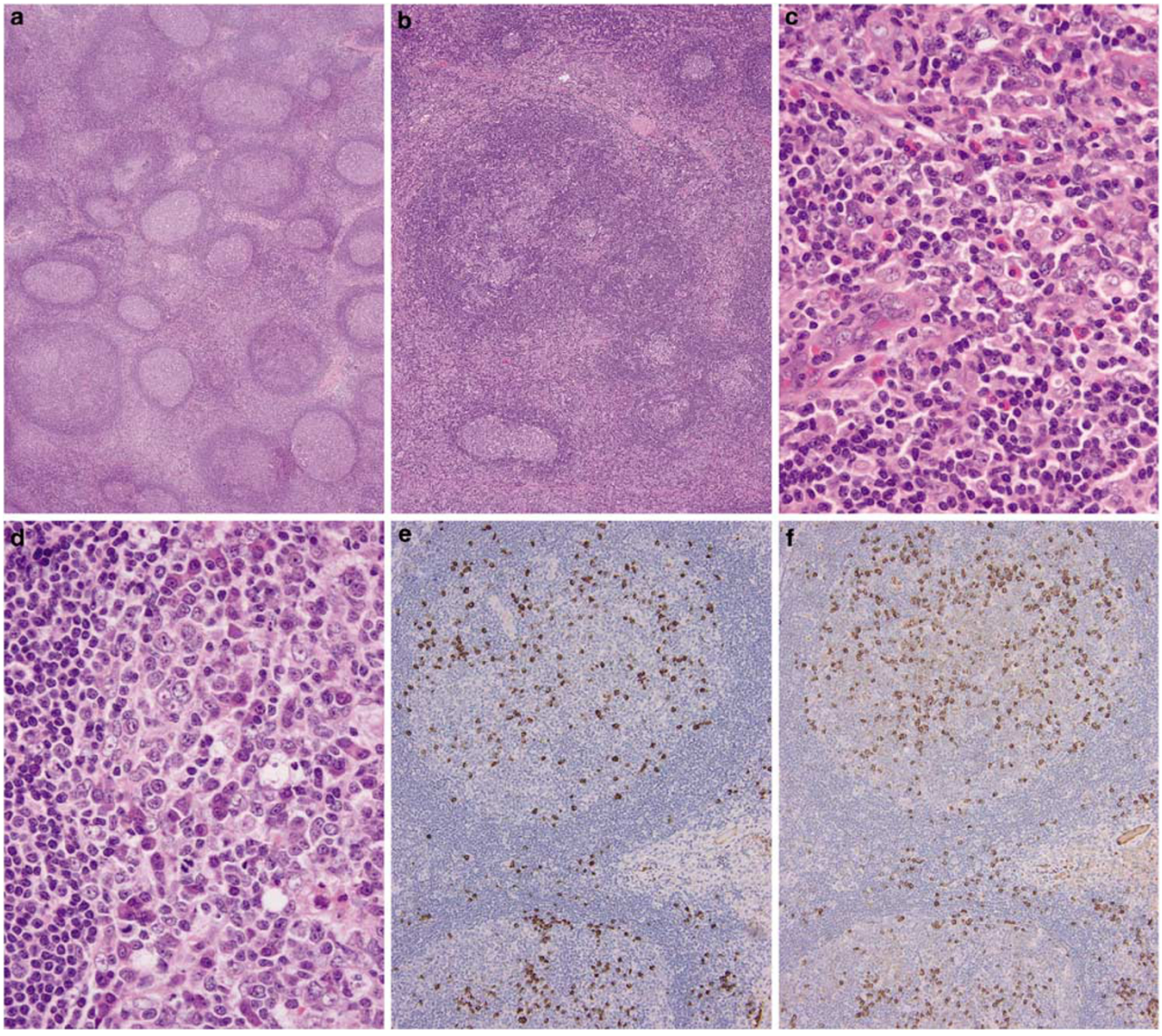

Figure 3 Histopathology of systemic IgG4-related lymphadenopathy (intra-germinal center plasmacytopsis type, patient no. 9). (**a** and **b**) The lymph node showed numerous lymphoid follicles with hyperplastic germinal centers and a distinct mantle zone, with focal progressive transformation of germinal centers. (**c**) Eosinophils infiltrated the interfollicular area. (**d**) Plasma cells and plasmacytoid cells infiltrated the germinal center. Immunostaining of IgG4 (**e**) and IgG (**f**). IgG4-positive cells mainly infiltrated the germinal center, and the IgG4/IgG-positive cell ratio was 63.0%. (**a–d**) Hematoxylin and eosin staining; (**a**) ×20, (**b**) ×40, (**c** and **d**) ×400, and (**e** and **f**) ×100.

overproduction of interleukin-6 is found in autoimmune diseases, such as rheumatoid arthritis, multicentric Castleman's disease, and Crohn's disease.[16–18,26,27] C-reactive protein is a pentamer of 23-kd subunits that is synthesized and secreted by hepatocytes upon stimulation by a variety of inflammatory cytokines, including tumor necrosis factor-α, interleukin-1, and especially interleukin-6. Therefore, interleukin-6 is closely related to the production of C-reactive protein.[25–29] Accordingly, interleukin-6 and C-reactive protein may become considerably important as differential diagnostic markers between systemic IgG4-related lymphadenopathy and multicentric Castleman's disease. Masaki *et al*[6] have reported that IgG4-related disease is not associated with an elevated serum interleukin-6 level, and cited measurement of serum interleukin-6 as an important tool of differential diagnosis. In our present series, two patients (nos. 2 and 4) showed slight, and one patient (no. 6) showed high elevation of serum interleukin-6, but their C-reactive protein levels were not as highly elevated as in multicentric Castleman's disease. Our patients using steroids showed a good response, and the histological findings of patient nos. 2 and 6 showed no similarities to those of Castleman's disease. The reference value of interleukin-6 is generically <4.0 pg/ml. However, Yokayama[30] has reported that the interleukin-6 value was over 25 pg/ml in 7% of the

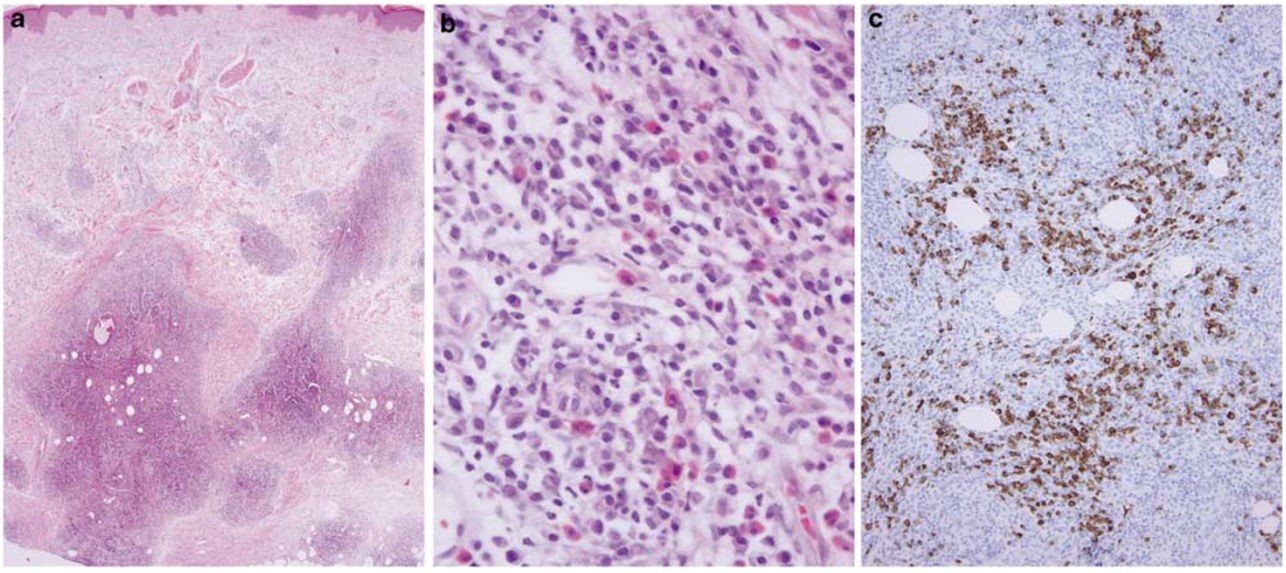

Figure 4 Histopathology of a skin lesion of a patient with systemic IgG4-related lymphadenopathy (patient no. 9). (**a** and **b**) Plasma cells, small lymphocytes and eosinophils showed a nodular-forming infiltration in the intermediate to deep dermis. (**c**) Immunostaining of IgG4; many plasma cells expressed IgG4. (**a** and **b**) Hematoxylin and eosin staining; (**a**) ×20, (**b**) ×400, and (**c**) ×100.

healthy subjects. Therefore, interleukin-6 values might vary widely among individuals.

Our patients with systemic IgG4-related lymphadenopathy all showed hypergammaglobulinemia, but serum IgM and IgA was normal in almost all patients. In contrast, multicentric Castleman's disease is characterized by increased serum IgG, IgM, and IgA levels,[16] which are caused by the increase in serum interleukin-6.[5] In this series, most patients showed cervical, hilar, mediastinal, and para-aortic lymph node swelling, and the lymph nodes were generally not very large (up to 2 cm). These findings were consistent with previous reports of IgG4-related lymphadenopathy.[3,4,11]

Histologically, we could classify the cases into two types based on the infiltration pattern of IgG4-positive cells: interfollicular plasmacytosis type and intra-germinal center plasmacytosis type. Morphologically, the interfollicular plasmacytosis type was characterized by expansion of the interfollicular area, moderate to marked increase in vascularity, and infiltration of IgG4-positive cells mainly in the interfollicular area. Five out of six cases showed eosinophil infiltration in the interfollicular area. These cases demonstrated Castleman disease-like features (patient nos. 1, 3, and 4) or atypical lymphoplasmacytic and immunoblastic proliferation-like features (patient nos. 2, 5, and 6). Koo *et al*[23] reported that atypical lymphoplasmacytic and immunoblastic proliferation is unusual in cases of lymph node lesion associated with various autoimmune diseases, including rheumatoid arthritis. Histopathologically, the lesion is characterized by prominent lymphoplasmacytic infiltration with various number of immunoblasts.[23,24] In this series, three patients showed this pattern, but there was no evidence of rheumatoid arthritis. In addition, there was abundant IgG4-positive cells infiltration in the lesion and the serum IgG4 value was elevated. These findings are consistent with IgG4-related disease. These results suggested that some IgG4-related lymphadenopathy might be confused with multicentric Castleman's disease or atypical lymphoplasmacytic and immunoblastic proliferation. In the case of systemic IgG4-related lymphadenopathy of atypical lymphoplasmacytic and immunoblastic proliferation type, the histology can be confused with that of malignant lymphoma, especially angioimmunoblastic T-cell lymphoma. However, the former has no clear cells, no CD10-positive T-cells, no extrafollicular follicular dendritic cell proliferation, and no T-cell receptor gamma gene rearrangement.

By contrast, the intra-germinal center plasmacytosis type shows marked follicular hyperplasia, a mild increase in vascularity, and IgG4-positive cells mainly infiltrating the germinal centers. In our study, the two patients with this type showed progressively transformed germinal center. The germinal centers are known to be a major site for B-cell selection. In the germinal centers, B cells perform numerous somatic hypermutations and heavy-chain class-switches and only a portion of them are selected through the cooperation of T cells and follicular dendritic cells. The selected B cells exit the germinal centers and become plasma cells.[31] Therefore, the fact that many IgG4-producing plasma cells were found selectively in the germinal centers of our patients was a unique feature. The mechanisms involved in this feature are not clear.

Zen *et al*[10] have reported that the expressions of T helper (Th) 2 cytokines (interleukin-4, interleukin-5, and interleukin-13) and regulatory cytokines (interleukin-10 and transforming growth factor-β) were upregulated in the affected tissues of patients with IgG4-related sclerosing pancreatitis and cholangitis. They have suggested that the prominent Th2 and regulatory immune reactions in this disease might indicate that its pathogenesis involves an allergic mechanism. In addition, they described that interleukin-10 has a major role in directing B-cells to produce IgG4.[10] Therefore, interleukin-10 might induce differentiation of B cells into IgG4-positive plasma cells in the germinal centers. At any rate, this unique histological feature could be a special finding for IgG4-related lymphadenopathy.

Interestingly, eight of our nine cases (89%) of systemic IgG4-related lymphadenopathy showed eosinophil infiltration. Zen *et al*[10] reported that eosinophils infiltrated in the affected tissues in their patients with IgG4-related sclerosing pancreatitis and cholangitis. As previously mentioned, Th2 cytokines (interleukin-4, interleukin-5, and interleukin-13) were upregulated in the affected tissues of patients with IgG4-related disease. Interleukin-5 and interleukin-13 were activated by eosinophil infiltration and IgE production. Masaki *et al*[6] have reported that serum IgE level is elevated in IgG4-related diseases. In our series, the serum IgE value was significantly elevated in the examined patients. So, the findings of eosinophilic infiltration and serum IgE value elevation might be a specific finding of IgG4-related disease.

Little is known about the lymphomagenesis of IgG4-related disease. We recently reported the first case of marginal zone B-cell lymphoma arising from ocular adnexal IgG4-related disease[7] and IgG4-producing marginal zone B-cell lymphoma.[32] However, the present series showed no immunoglobulin light-chain restriction and no immunoglobulin heavy chain gene rearrangement.

In conclusion, in systemic IgG4-related disease, C-reactive protein and interleukin-6 are usually not elevated. Systemic IgG4-related disease and multicentric Castleman's disease showed overlapping but somewhat distinct pathologic findings, and serum data (especially C-reactive protein and interleukin-6) are useful to differentiate between them. Eosinophilic infiltration and serum IgE value elevation might be a specific feature of IgG4-related lymphadenopathy.

Disclosure/conflict of interest

The authors have no potential conflicts of interest.

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