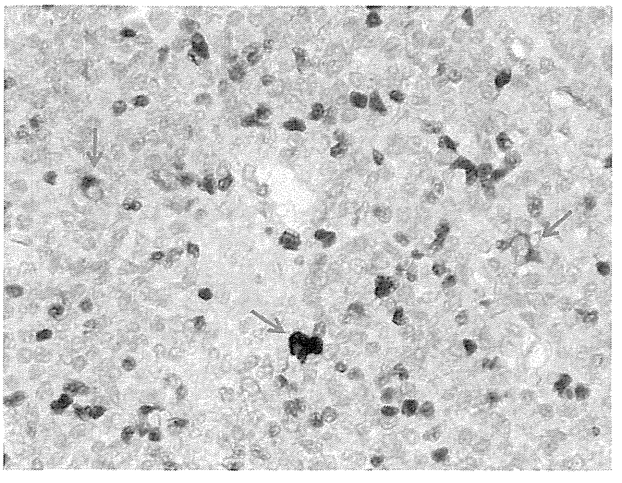

Figure 6 Dual immunostaining of transforming growth factor beta 1 (TGFβ1) and FOXP3. The distribution of TGFβ1- and FOXP3-positive cells were different (TGFβ1/brown, FOXP3/red).

cells, and CD4-positive cell distribution did not correlate with cytokine-positive cell distribution. Moreover, no double-positive cells were observed upon double-immunostaining for each of the cytokines with CD4.

Staining Pattern of IgE in Mast Cells of IgG4-Related Disease

In IgG4-related disease and control groups tested, a high number of mast cells were positive for IgE with varying density and intensity. As is typical for IgE immunostaining, IgE was observed to be weakly-to-moderately expressed on cell surface membranes (Figure 7a). No significant differences were observed in the number of surface membrane IgE-positive cells among the IgG4-related disease and control groups. However, the IgG4-related disease contained a large number of mast cells that were strongly positive for cytoplasmic IgE (Figure 7a). The distribution of the strongly cytoplasmic IgE-positive cells correlated with KIT expression, and the membranous colocalization of IgE and KIT was confirmed by dual immunofluorescence (Figure 7b).

The number of strongly cytoplasmic IgE-positive cells was significantly greater in IgG4-related disease (7.19 ± 5.11 cells/mm^2) than in sialolithiasis (0.068 ± 0.15 cells/mm^2; $P<0.01$) and normal (0.45 ± 0.51 cells/mm^2; $P<0.01$) control tissues (Figure 7c).

Discussion

In this study, we confirmed the upregulation of T helper 2 (IL4) and regulatory T-cell (IL10 and TGFβ1) cytokines in the paraffin-embedded tissue of the submandibular gland IgG4-related disease by real-time PCR. Upregulation of IL5 was not observed in this study, which is similar to previously published data on real-time PCR for paraffin-embedded tissue samples for IgG4-related disease.[11] Interestingly, our immunohistochemical studies detected a large number of mast cells expressing T helper 2 (IL4) and regulatory T-cell (IL10 and TGFβ1) cytokines in the submandibular gland IgG4-related disease, whereas this was not the case for the control groups. Notably, although the overall number of mast cells was similar among the samples of submandibular gland IgG4-related disease, submandibular sialolithiasis, and normal submandibular gland, the IgG4-related disease contained significantly more cytokine-expressing mast cells. Furthermore, interestingly, our immunohistochemical experiments provided no evidence that T cells were producing the upregulated cytokines. In our study, we considered FOXP3-positive cells to be regulatory T cells, as FOXP3 is considered to be a specific marker of regulatory T cells, although a minor amount of effector T cells can exhibit transient expression of FOXP3.[12–14] It has been suggested that T helper 2 and regulatory T cells are most important in the pathogenesis of IgG4-related disease. Although we did find that the number of FOXP3-positive regulatory T cells was significantly increased in IgG4-related disease in our study, we did not find any evidence that the FOXP3-positive regulatory T cells produced regulatory cytokines. Thus, our results do not support the hypothesis that T cells express the cytokines associated with IgG4-related disease; rather, our data indicate that mast cells are the source of these upregulated cytokines. However, a close relationship between regulatory T cells and mast cells has been reported, and TGFβ1 has been shown to promote differentiation of naive T cells to regulatory T cells.[15] Therefore, we hypothesized that the upregulated mast cells produce TGFβ1, which secondarily promotes the differentiation of naïve T cells to regulatory T cells in the tissue affected.

We observed that mast cells in IgG4-related disease were strongly positive for IgE. This result suggests that IgE is associated with the pathogenesis of IgG4-related disease. Consistent with this notion, patients with IgG4-related disease frequently have an allergic background, such as allergic rhinitis, bronchial asthma, and atopic dermatitis. In addition, patients with this disease typically show elevated serum IgE levels as well as elevated IgG4 levels.[16] IgE is a key stimulator of mast cells, and the mechanism underlying IgE stimulation of mast cells to induce various immunological cascades has been extensively studied. Notably, chronic elevation of antigen-independent, non-specific IgE upregulates the high-affinity IgE receptor, FcεRI, on mast cells, which inhibits mast cell apoptosis and promotes cytokine production.[17] Our observation that mast cells in IgG4-related disease were strongly positive for IgE supports the idea that IgE-mediated stimulation of mast cells may have a role in the

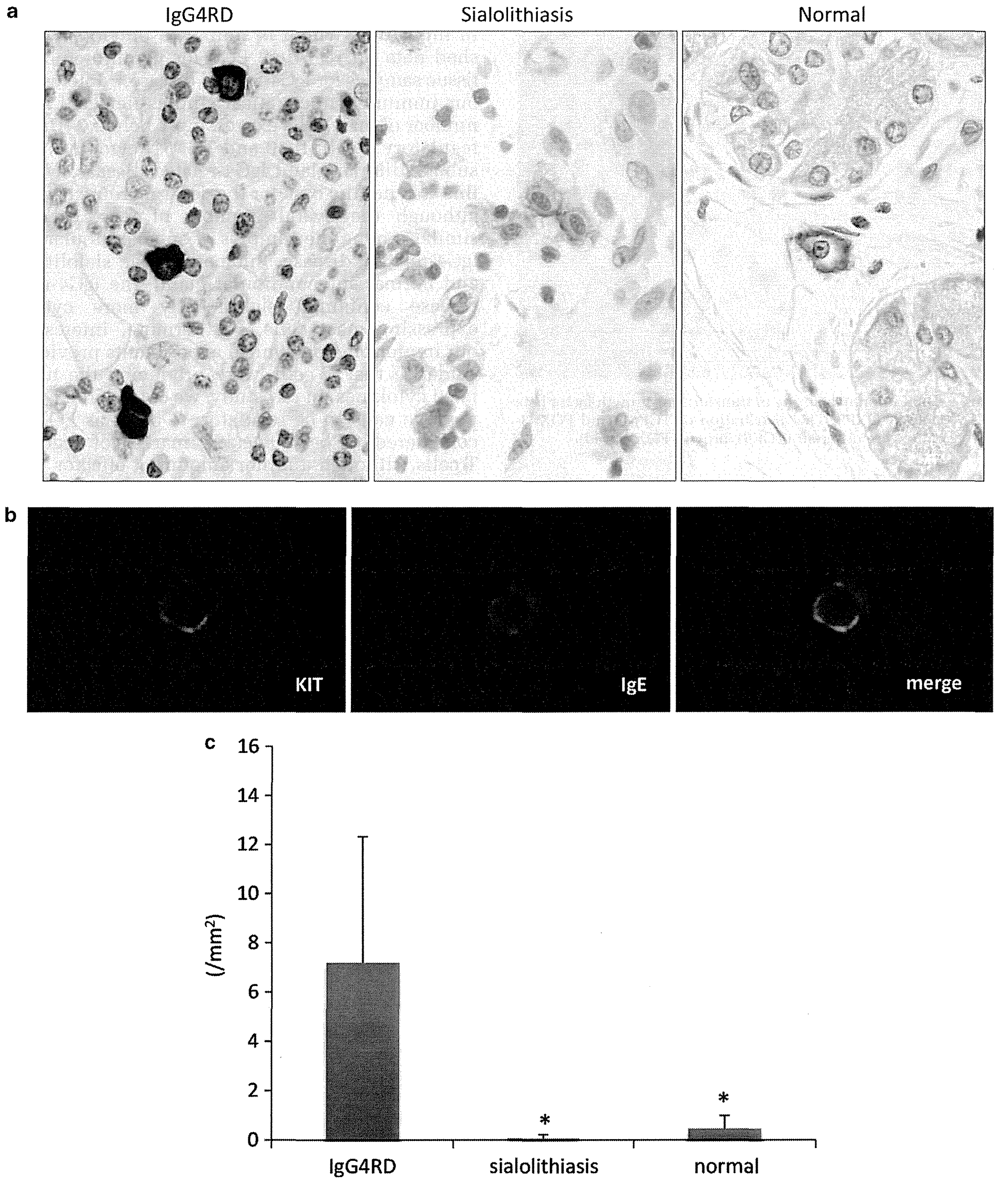

Figure 7 IgE expression in mast cells. (**a**) In IgG4-related disease, mast cells showed strong cytoplasmic positivity for IgE. In contrast, mast cells in sialolithiasis and normal submandibular gland showed only moderate-to-weak IgE positivity and membranous localization. (**b**) Colocalization of IgE- and KIT-positive cells was confirmed by dual immunofluorescence (IgE/red, KIT/green). (**c**) The number of strongly cytoplasmic IgE-positive cells was counted per mm^2 and was significantly different between the IgG4-related disease and control groups. (*$P<0.01$) (IgG4RD, submandibular IgG4-related disease; sialolithiasis, submandibular sialolithiasis; normal; normal submandibular gland).

pathogenesis of IgG4-related disease. However, it remains to be determined why the IgE in these cells was localized to the cytoplasm. Previous studies have shown that the number of FcεRI receptors on mast cells greatly increases in the presence of elevated serum IgE levels.[18] Thus, it is possible that what appeared to be cytoplasmic localization of IgE in our immunostaining experiments may have actually been the result of very high levels of membrane-associated IgE. However, a few reports have suggested that antigen cross-linking could lead to the aggregation and internalization of FcεRI. In particular, IgE was detected in the cytoplasm of mast cells as a result of the internalization and endocytosis of FcεRI.[19] These data suggest that the continuous upregulation of mast cells might result in endocytosis-mediated cytoplasmic accumulation of IgE. However, this hypothesis should be based on the existence of certain antigens, and the role of internalization of FcεRI remains controversial.

Steroid therapy has been the first choice of medication for IgG4-related disease. However, a recent study reported a case of IgG4-related disease that regressed after treatment with an antihistamine agent (epinastine hydrochloride) alone, which indicates an allergic background to the disease and may indirectly suggest the involvement of mast cells.[20] The *in vitro* effects of antihistamines on the inhibition of mediator release from mast cells have been reported.[21] Moreover, an *in vitro* study of human conjunctival mast cells revealed that epinastine inhibited mast cell secretion of cytokines, including IL10.[22] However, it remains unclear why antihistamines inhibit cytokine secretion, although H1 antagonism seems unrelated.

Rituximab is another treatment currently being used for refractory cases of IgG4-related disease. Rituximab therapy has been reported to produce rapid regression in refractory patients.[23] Additionally, rituximab therapy was found to induce regression of symptoms with a decrease in serum IgE levels,[24] and the concentration of IgE showed a steeper decline than serum IgG4 levels. These results provide additional evidence that the allergic reaction mediated by IgE is an important factor in the regulation of IgG4-related disease.

Hyper-IgE syndrome is a complex immunodeficiency characterized by atopic dermatitis associated with extremely high serum IgE levels and susceptibility to infections with extracellular bacteria.[25] Despite the continuously elevated serum IgE level, hyper-IgE syndrome shows clinical features that are quite different from those of IgG4-related disease. Furthermore, hyper-IgE syndrome has been linked to mutations in signal transducer and activator of transcription 3 (STAT3) and tyrosine kinase 2 (TYK2), and to defective signal transduction pathways involving multiple cytokines. In contrast to this downregulation of cytokine signaling, IgG4-related disease is associated with the upregulation of multiple cytokine signals.

To our knowledge, this study provides the first evidence relating mast cells to IgG4-related disease. However, as mast cells are currently known as powerful mediators of the immune response and are closely related with allergic responses, the involvement of mast cells in IgG4-related disease seems logical. Moreover, mast cells have been implicated in other inflammatory diseases. For instance, it was reported that a large number of

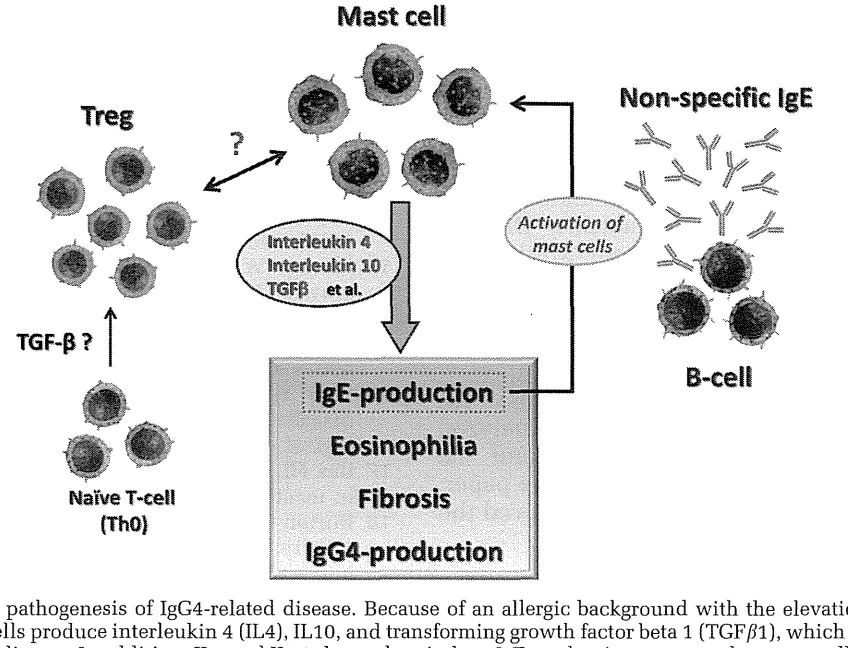

Figure 8 Model for the pathogenesis of IgG4-related disease. Because of an allergic background with the elevation of non-specific IgE levels, activated mast cells produce interleukin 4 (IL4), IL10, and transforming growth factor beta 1 (TGFβ1), which induce the distinctive features of IgG4-related disease. In addition, IL4 and IL10 themselves induce IgE production to upregulate mast cells, and TGFβ1 induces the transformation of naive T cells to regulatory T cells. The relationship between regulatory T cells and mast cells remains unclear.

mast cells was observed in periductal and ductal fibrosis in primary sclerosing cholangitis (PSC) and chronic sclerosing sialadenitis, indicating that mast cells might contribute to fibrosis.[26,27] Mast cell expression of IL10, as observed in our immunohistochemical experiments, may affect mast cell involvement in IgG4-related disease. IL10 is known to be an anti-inflammatory cytokine that can suppress acquired or innate immune responses. Mast cell-derived IL10 has been reported to limit contact dermatitis and chronic irradiation with ultraviolet B.[28] Similarly, in IgG4-related disease, mast cell production of IL10 may prevent allergic inflammation in the affected organs.

On the basis of results of our study and those of previously published reports, we established a hypothesis for the pathogenesis of IgG4-related disease, which is summarized in Figure 8. Patients with IgG4-related disease usually have an allergic background with elevation of non-specific IgE levels. This antigen-independent non-specific IgE binds to FcεRI on mast cells to promote the production of T helper 2 (IL4) and regulatory T-cell (IL10 and TGFβ1) cytokines, which induce the distinctive features of IgG4-related disease such as lymphoplasmacytic infiltration, interstitial fibrosis, and IgG4 production. In addition, IL4 and IL10 induce IgE production, which promotes the upregulation of mast cells. Although it is possible that regulatory T cells interact with mast cells, their involvement in this process is uncertain and they might be secondarily recruited to the affected tissue. According to this scenario, rituximab might exert its effects through the inhibition of IgE-production of B-cells (Figure 8). This model is based on the major finding of this study, which indicates that mast cells have a key role in IgG4-related disease.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (C) (no. 24591447) from the Japan Society for the Promotion of Science and 'Research on Measures for Intractable Disease' Project: matching fund subsidy from Ministry of Health Labour and Welfare, Japan.

Author contributions

Conceived and designed the experiments: YS. Performed the experiments: MT and YS. Analyzed the data: YS, MT, ST, KO, KT, YG, and TI. Contributed materials: YO and TT. Wrote the paper: MT, YS, and TY. All authors read and approved the final manuscript.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Umehara H, Okazaki K, Masaki Y, *et al.* A novel clinical entity IgG4-related disease (IgG4RD): general concept and details. Mod Rheumatol 2012;22:1–14.
2 Deshpande V, Zen Y, Chan JK, *et al.* Consensus statement on the pathology of IgG4-related disease. Mod Pathol 2012;25:1181–1192.
3 Sato Y, Inoue D, Asano N, *et al.* Association between IgG4-related disease and progressively transformed germinal centers of lymph nodes. Mod Pathol 2012;25:956–967.
4 Sato Y, Takeuchi M, Takata K, *et al.* Clinicopathologic analysis of IgG4-related skin disease. Mod Pathol 2012;26:523–532.
5 Zen Y, Fujii T, Harada K, *et al.* Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology 2007;45:1538–1546.
6 Tanaka A, Moriyama M, Nakashima H, *et al.* Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. Arthritis Rheum 2012;64:254–263.
7 Khosroshahi A, Bloch DB, Deshpande V, *et al.* Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. Arthritis Rheum 2010; 62:1755–1762.
8 Gri G, Frossi B, D'Inca F, *et al.* Mast cell: an emerging partner in immune interaction. Front Immunol 2012;3:120.
9 Amin K. The role of mast cells in allergic inflammation. Respir Med 2012;106:9–14.
10 Kashiwakura J, Kawakami Y, Yuki K, *et al.* Polyclonal IgE induces mast cell survival and cytokine production. Allergol Int 2009;58:411–419.
11 Nakashima H, Miyake K, Moriyama M, *et al.* An amplification of IL-10 and TGF-beta in patients with IgG4-related tubulointerstitial nephritis. Clin Nephrol 2010;73:385–391.
12 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. Nat Immunol 2003;4:330–336.
13 Miyao T, Floess S, Setoguchi R, *et al.* Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. Immunity 2012;36:262–275.
14 Da Silva Martins M, Piccirillo CA. Functional stability of Foxp3 + regulatory T cells. Trends Mol Med 2012; 18:454–462.
15 Zhang W, Wu K, He W, *et al.* Transforming growth factor beta 1 plays an important role in inducing CD4(+) CD25(+) forhrad box P3(+) regulatory t cells by mast cells. Clin Exp Immunol 2010;161: 490–496.
16 Sato Y, Notohara K, Kojima M, *et al.* IgG4-related disease: historical overview and pathology of hematological disorders. Pathol Int 2010;60:247–258.
17 Bax HJ, Keeble AH, Gould HJ. Cytokinergic IgE action in mast cell activation. Front Immunol 2012;3:229.
18 Burton OT, Oettgen HC. Beyond immediate hypersensitivity: evolving roles for IgE antibodies in immune homeostasis and allergic diseases. Immunol Rev 2011;242:128–143.
19 Fattakhova GV, Masilamani M, Nrayanan S, *et al.* Endosomal trafficking of the ligated FCεRI receptor. Mol Immunol 2009;46:793–802.

20 Ohshima K, Sato Y, Yoshino T. A case of IgG4-related dacryoadenitis regressed without systemic steroid administration. J Clin Exp Hematol 2013;53:53–56.
21 Cuss FM. Beyond the histamine receptor: effect of antihistamines on mast cells. Clin Exp Allergy 1999; 29:4–59.
22 Galatowicz G, Ajayi Y, Stern ME, *et al.* Ocular anti-allergic compounds selectively inhibit human mast cell cytokines *in vitro* and conjunctival cell infiltration *in vivo*. Clin Exp Allergy 2007;37:1648–1656.
23 Khosroshahi A, Carruthers MN, Deshpande V, *et al.* Rituximab for the treatment of IgG4-related disease: lessons from 10 consecutive patients. Med Baltimore 2012;91:57–66.
24 Wong PC, Fung AT, Gerrie AS, *et al.* IgG4-related disease with hypergammaglobulinemic hyperviscosity and retinopathy. Eur J Haematol 2012;90:250–256.
25 Minegishi Y. Hyper-IgE syndrome. Curr Opin Immunol 2009;21:487–492.
26 Tsuneyama K, Kono N, Yamashiro M, *et al.* Aberrant expression of stem cell factor on biliary epithelial cells and peribiliary infiltration of c-kit-expressing mast cells in hepatolithiasis and primary sclerosing cholangitis: a possible contribution to bile duct fibrosis. J Pathol 1999;189:609–614.
27 Tsuneyama K, Saito K, Ruebner BH, *et al.* Immunological similarities between primary sclerosing cholangitis and chronic sclerosing sialadenitis. Dig Dis Sci 2000;45:366–372.
28 Grimbaldeston MA, Nakae S, Kalesnikoff J, *et al.* Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet. B Nat Immunol 2007;8: 1095–1104.

Modern Pathology (2012) 25, 956–967

Association between IgG4-related disease and progressively transformed germinal centers of lymph nodes

Yasuharu Sato[1], Dai Inoue[2], Naoko Asano[3], Katsuyoshi Takata[1], Hideki Asaoku[4], Yoshinobu Maeda[5], Toshiaki Morito[6], Hirokazu Okumura[7], Shin Ishizawa[8], Shoko Matsui[9], Takayoshi Miyazono[10], Tamotsu Takeuchi[11], Naoto Kuroda[12], Yorihisa Orita[13], Kiyoshi Takagawa[14], Masaru Kojima[15] and Tadashi Yoshino[1]

[1]*Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan;* [2]*Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan;* [3]*Department of Clinical Laboratory, Nagoya University Hospital, Nagoya, Japan;* [4]*Department of Clinical Laboratory, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima, Japan;* [5]*Department of Pathology, Toyama Red Cross Hospital, Toyama, Japan;* [6]*Department of Anatomic Pathology, Kagawa Rosai Hospital, Marugame, Japan;* [7]*Department of Internal Medicine, Toyama Prefectural Central Hospital, Toyama, Japan;* [8]*Department of Pathology, Toyama Prefectural Central Hospital, Toyama, Japan;* [9]*Department of First Internal Medicine, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, Toyama, Japan;* [10]*Department of Gastroenterology and Hematology, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, Toyama, Japan;* [11]*Department of Pathology, Kochi Medical School, Kochi, Japan;* [12]*Department of Diagnostic Pathology, Kochi Red Cross Hospital, Kochi, Japan;* [13]*Department of Otolaryngology, Head and Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan;* [14]*Department of Clinical Laboratory, Kurobe City Hospital, Kurobe, Japan and* [15]*Department of Anatomic and Diagnostic Pathology, Dokkyo University School of Medicine, Mibu, Japan*

Progressively transformed germinal centers is a benign condition of unknown pathogenesis characterized by a distinctive variant form of reactive follicular hyperplasia in lymph nodes. We recently reported Ig G4-related disease in progressively transformed germinal centers. However, no large case series has been reported and clinicopathologic findings remain unclear. Here, we report 40 Japanese patients (28 men, 12 women; median age, 56 years) with progressively transformed germinal centers of the lymph nodes who fulfilled the histological diagnostic criteria for IgG4-related disease (IgG4$^+$ progressively transformed germinal centers), with asymptomatic localized lymphadenopathy involving the submandibular nodes in 24, submandibular and cervical nodes in 14, cervical nodes only in 1, and cervical and supraclavicular nodes in 1. In all, 16 (52%) of 31 examined patients had allergic disease. Histologically, the lymph nodes demonstrated uniform histological findings, namely marked follicular hyperplasia with progressively transformed germinal centers, and localization of the majority of IgG4$^+$ plasma cells in the germinal centers. Serum IgG4, serum IgE and peripheral blood eosinophils were elevated in 87%, 92% and 53% of examined patients, respectively. Eighteen patients subsequently developed extranodal lesions (including five who developed systemic disease), which on histological examination were consistent with IgG4-related disease. IgG4$^+$ progressively transformed germinal centers presents with uniform clinicopathological features of asymptomatic localized submandibular lymphadenopathy, which persists and/or relapses, and sometimes progresses to extranodal lesions or systemic disease. Nine patients were administered steroid therapy when the lesions progressed, to which all

Correspondence: Dr Y Sato, MD, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho Kita-ku, Okayama 700-8558, Japan.
E-mail: satou-y@cc.okayama-u.ac.jp

Received 23 November 2011; revised 22 January 2012; accepted 22 January 2012; published online 6 April 2012

responded well. We suggest that IgG4$^+$ progressively transformed germinal centers should be included in the IgG4-related disease spectrum.
Modern Pathology (2012) **25,** 956–967; doi:10.1038/modpathol.2012.54; published online 6 April 2012

Keywords: IgG4-related disease; IgG4-related lymphadenopathy; PTGC

The term progressively transformed germinal centers was first used by Lennert and Müller–Hermelink to describe reactive follicular hyperplasia in the lymph nodes,[1] and progressively transformed germinal centers is observed in approximately 4% of patients with unspecific lymphadenopathy.[1–6] Germinal centers in affected lymph nodes are usually larger than regular germinal centers and composed mainly of mantle zone lymphocytes and remnants of germinal center cells. Although relapse is frequent, affecting about 20% of patients, progressively transformed germinal centers is considered a non-malignant condition.[2–6]

IgG4-related disease is a recently recognized syndrome characterized by mass-forming lesions with lymphoplasmacytic infiltration, accumulation of IgG4$^+$ plasma cells in affected tissues and increased serum IgG4 levels.[7–14] IgG4-related disease generally involves either localized or systemic lymph nodes,[8,13,14] and five histological subtypes of IgG4-related lymphadenopathy have been recognized.[8] In 2009, we were the first to report patients with IgG4-related disease in progressively transformed germinal centers of the lymph nodes (progressively transformed germinal centers-type IgG4-related lymphadenopathy).[14] Recently, while no large series has yet been reported, Grimm *et al*[15] included 14 cases with progressively transformed germinal centers in their series. However, clinicopathologic findings remain unclear.

Here, we report the clinicopathological characteristics of 40 cases of progressively transformed germinal centers of the lymph nodes that fulfill the histological diagnostic criteria for IgG4-related disease.

Materials and methods

Case Selection

Two of us (YS and TY) reviewed the Pathology Department database of our institution using the search terms 'progressively transformed germinal centers,' 'follicular hyperplasia' and 'lymph node' for the 13-year period from 1998 to 2011. In all, 62 cases of progressively transformed germinal centers were identified, 40 of which fulfilled the histological diagnostic criteria of IgG4-related disease, namely the presence of IgG4$^+$ plasma cells >100/high-power fields (HPFs) and IgG4$^+$/IgG$^+$ plasma cell ratio >40% (IgG4$^+$ progressively transformed germinal centers) (Table 1), whereas 22 did not (IgG4$^-$ progressively transformed germinal centers). The histological diagnostic criteria were outlined by the International Symposium on IgG4-RD (Boston, MA, USA, on 4–7 October 2011; http://www2.massgeneral.org/pathology/symposium/IgG4_related_-systemic_dis.asp).

The clinical records and pathology materials of all cases were reviewed, and cases of multicentric Castleman's disease, malignant lymphoma or other lymphoproliferative disorders (including rheumatoid arthritis-related lymphadenopathy and other immune-mediated conditions, and so on) were histologically and clinically excluded.

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 Bond Max stainer (Leica Biosystems, Melbourne, Australia). The primary antibodies used were as follows: IgG (polyclonal (1:10 000); Dako), IgG4 (HP6025 (1:400); The Binding Site), Kappa (NCL-KAP (1:100); Novocastra) and Lambda (NCL-LAM (1:200); Novocastra). The number of IgG4$^+$ or IgG$^+$ plasma cells was estimated for areas with the highest density of positive cells. Three different HPF ($\times 10$ in the eyepiece and $\times 40$ in the lens) in each section were counted, and the average number of positive cells per HPF was calculated.

Polymerase Chain Reaction for the Detection of Ig Heavy-Chain Gene Rearrangement

Ig heavy-chain gene rearrangement was analyzed by polymerase chain reaction performed according to standard procedures as described previously.[14] The primers used for Ig heavy-chain gene amplification were 5′-TGG[A/G]TCCG[C/A]CAG[G/C]C[T/C][T/C]C[A/C/G/T]GG-3′ as an upstream consensus V-region primer; 5′-TGAGGAGACGGTGACC-3′ as a consensus J-region primer; and 5′-GTGACCAGGGT[A/C/G/T]CCTTGGCCCCAG-3′ as a consensus J-region primer.[14]

Statistical Analysis

Differences in characteristics between the two groups were determined by the χ^2 test, Fisher's

Table 1 Clinical features of 40 patients with IgG4⁺ PTGC

No.	*Age/gender*	*Biopsy site (LN size, cm)*	*Initial presentation*	*Disease progression*	*Treatment (follow-up period, months)*	*Allergic disease*	*Eosinophil count in PB (%;nl<5%)*	*IgG4 (mg/dl; nl = 4.8–105)*	*IgG4/IgG (%; nl = 3–6)*	*IgE (IU/ml; nl) (IgE ratio)*[a]
1	36/M	Submandibular LN (1.5)	Bil. submandibular lymphadenopathy and lt. submandibular gland swelling	None (but residual lymph node lesions persisted)	Follow-up and stable (18)	Drug allergy	6.8	110	10.9	NA
2	75/M	Submandibular LN (3)	Bil. submandibular and cervical lymphadenopathy	NA	NA	NA	NA	NA	NA	NA
3	50/M	Submandibular LN (2)	Bil. submandibular and cervical lymphadenopathy	Residual lymph node lesions persisted and patient developed bil. axillary lymphadenopathy 3 years later	Follow-up (18)	Allergic rhinitis	21.1	183	6.74	NA
4	50/F	Submandibular LN (1.5)	Bil. submandibular lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (4)	Allergic rhinitis	2	24	2	NA
5	66/M	Submandibular LN (2)	Lt. submandibular lymphadenopathy	Relapsed lt. submandibular lymphadenopathy 10 months later[b]	Follow-up (10)	None	5.9	314[c]	19.2[c]	505 (2.9)
6	46/M	Submandibular LN (3.5)	Lt. submandibular lymphadenopathy, lt. submandibular gland swelling and thickened rt. pleura	None (but residual lymph node lesions persisted)	Follow-up and stable (10)	NA	3	NA	NA	NA
7	71/M	Submandibular LN (1.5)	Lt. submandibular and cervical lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (5)	None	2.5	275	12.8	259 (1.5)
8	62/F	Submandibular LN (1.5)	Lt. submandibular lymphadenopathy	None	Follow-up and stable (6)	Contact dermatitis	0	NA	NA	NA
9	75/F	Cervical and supraclavicular LN (2)	Rt. cervical and supraclavicular lymphadenopathy	None (the cervical LN was biopsied 9 months ago but a residual supraclavicular lymph node lesion persisted)	Follow-up and stable (10)	Food allergy	8	36.2	2.3	47.0 (0.27)
10	45/F	Cervical LN (3)	Lt. cervical lymphadenopathy	Residual lymph node lesions persisted; LN size increased in 3 years	Follow-up (36)	Asthma and drug allergy	7	NA	NA	NA
11	64/M	Submandibular LN (3)	Rt. submandibular lymphadenopathy	Relapsed rt. submandibular lymphadenopathy[b] 2 years later; patient developed bil. lacrimal,[b] parotid, and submandibular gland swelling, mediastinal lymphadenopathy, and kidney lesion[b] 11 years later	Steroid therapy was performed when disease progressed, with good response (144)	Asthma, drug allergy and allergic rhinitis	10.5	2550[c]	42.3[c]	NA
12	60/M	Submandibular LN (2)	Lt. submandibular and cervical lymphadenopathy	NA	NA	None	2.2	NA	NA	NA

Table 1 Continued

No.	*Age/gender*	*Biopsy site (LN size, cm)*	*Initial presentation*	*Disease progression*	*Treatment (follow-up period, months)*	*Allergic disease*	*Eosinophil count in PB (%;nl<5%)*	*IgG4 (mg/dl; nl = 4.8–105)*	*IgG4/IgG (%; nl = 3–6)*	*IgE (IU/ml; nl) (IgE ratio)[a]*
13	61/M	Submandibular LN (2)	Lt. submandibular lymphadenopathy	Developed bil. submandibular gland swelling, mediastinum, lung, pancreas kidney and aortic lesions 3 years later	Steroid therapy was performed when disease progressed, with good response (101)	None	4	2240[c]	77.5[c]	NA
14	43/F	Submandibular LN (1)	Rt. submandibular and cervical lymphadenopathy	NA	NA	NA	NA	NA	NA	NA
15	46/M	Submandibular LN (3)	Lt. submandibular lymphadenopathy	Relapsed lt. submandibular LN 5 years later[b] Relapsed rt. submandibular LN 8 years later.[b]	Follow-up (100)	None	7.7	40	2.6	681 (1.9)
16	58/F	Submandibular LN (1.5)	Rt. submandibular lymphadenopathy	Developed bil. submandibular gland swelling 1 year later[b] and bil. lacrimal gland swelling 4 years later	Follow-up (48)	Contact dermatitis	2.2	241[c]	18.4[c]	280[c] (1.6)
17	55/M	Submandibular LN (3)	Rt. submandibular and cervical lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (6)	None	4.1	NA	NA	NA
18	52/F	Submandibular LN (2.5)	Lt. submandibular and cervical lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (3)	NA	4	NA	NA	NA
19	51/F	Submandibular LN (2)	Rt. submandibular lymphadenopathy	Developed bil. submandibular lymphadenopathy and rt. parotid gland swelling 1 year later[b]	Follow-up (18)	None	NA	224	NA	NA
20	52/M	Submandibular LN (2.5)	Lt. submandibular lymphadenopathy	Developed bil. lacrimal gland swelling, skin lesion and systemic lymphadenopathy 5 years later[b]	Steroid therapy was performed when disease progressed, with good response. However, the lesion relapsed 3 months later (96)	None	14[c]	1700[c]	37.5[c]	904[c] (2.5)
21	43/M	Submandibular LN (2)	Lt. submandibular lymphadenopathy	Developed bil. lacrimal gland swelling, skin lesion and systemic lymphadenopathy 3 years later[b]	Steroid therapy was performed when disease progressed, with good response. However, the lesion relapsed 3 years later (78)	Atopic dermatitis	9	216[c]	13.72[c]	1550[c] (4.3)

Table 1 Continued

No.	*Age/gender*	*Biopsy site (LN size, cm)*	*Initial presentation*	*Disease progression*	*Treatment (follow-up period, months)*	*Allergic disease*	*Eosinophil count in PB (%;nl<5%)*	*IgG4 (mg/dl; nl = 4.8–105)*	*IgG4/IgG (%; nl = 3–6)*	*IgE (IU/ml; nl) (IgE ratio)*[a]
22	58/M	Submandibular LN (2)	Bil. submandibular and cervical lymphadenopathy	Developed prostatic lesion and systemic lymphadenopathy 2 years later[b]	Steroid therapy was performed when disease progressed, with good response. However, the lesion relapsed 10 months later (39)	Drug allergy	8	1280[c]	30.74[c]	641[c] (1.8)
23	50/M	Submandibular LN (1)	Bil. submandibular lymphadenopathy	None	Follow-up and stable (36)	None	4	NA	NA	NA
24	45/F	Submandibular LN (2.5)	Rt. submandibular and cervical lymphadenopathy	Developed bil. lacrimal gland swelling 1 year later	Steroid therapy was performed when disease progressed, with good response (16)	None	4	NA	NA	800 (2.2)
25	67/M	Submandibular LN (3)	Bil. submandibular lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (72)	NA	8.2	NA	NA	NA
26	51/F	Submandibular LN (3.5)	Rt. submandibular lymphadenopathy and rt. submandibular gland swelling	None	Follow-up and stable (12)	None	2.8	NA	NA	NA
27	42/M	Submandibular LN (2)	Lt. submandibular and cervical lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (60)	None	6.1	NA	NA	241 (1.4)
28	49/M	Submandibular LN (2.5)	Lt. submandibular, cervical lymphadenopathy, and lt. submandibular gland swelling	NA	NA	NA	1	NA	NA	NA
29	58/M	Submandibular LN (2.5)	Rt. submandibular lymphadenopathy	Developed bil. lacrimal gland swelling and rt. maxillary sinus tumor 3 years later[b]	Steroid therapy was performed when disease progressed, with good response (39)	Allergic rhinitis	9	921[c]	47.5[c]	1090[c] (3.0)
30	60/M	Submandibular LN (2.5)	Rt. submandibular lymphadenopathy	None	Follow-up and stable (26)	Asthma	3	NA	NA	NA
31	46/F	Submandibular LN (2)	Rt. submandibular lymphadenopathy and rt. submandibular gland swelling	NA	NA	Allergic rhinitis	NA	NA	NA	NA
32	72/M	Submandibular LN (2.5)	Bil. submandibular lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (12)	NA	0.4	NA	NA	NA

—521—

Table 1 Continued

No.	*Age/gender*	*Biopsy site (LN size, cm)*	*Initial presentation*	*Disease progression*	*Treatment (follow-up period, months)*	*Allergic disease*	*Eosinophil count in PB (%;nl<5%)*	*IgG4 (mg/dl; nl = 4.8–105)*	*IgG4/IgG (%; nl = 3–6)*	*IgE (IU/ml; nl) (IgE ratio)*[a]
33	51/M	Submandibular LN (3.5)	Rt. submandibular and cervical lymphadenopathy	None (but residual lymph node lesions persisted)	Follow-up and stable (26)	Allergic rhinitis	6.7	169	7	3250 (18.8)
34	68/F	Submandibular LN (1.5)	Rt. submandibular lymphadenopathy and rt. lacrimal gland swelling	None (but residual lymph node lesions persisted)	Follow-up and stable (25)	Food and drug allergy	5.6	NA	NA	NA
35	67/M	Submandibular LN (2)	Lt. submandibular lymphadenopathy	None	Follow-up and stable (27)	Allergic rhinitis and asthma	NA	NA	NA	NA
36	70/M	Submandibular LN (2)	Lt. submandibular lymphadenopathy and lt. parotid gland[b] tumor	Developed rt. submandibular gland swelling and pancreatic lesion[b] 2 years later	Steroid therapy was performed when disease progressed, with good response (43)	NA	10.9	483[c]	27.7[c]	NA
37	51/M	Submandibular LN (1.5)	Rt. submandibular, cervical lymphadenopathy, and bil. submandibular gland swelling	None (but residual lymph node lesions persisted)	Follow-up and stable (18)	None	6.3	NA	NA	NA
38	61/M	Submandibular LN (2)	Bil. submandibular and cervical lymphadenopathy	NA	NA	NA	2.6	NA	NA	NA
39	76/M	Submandibular LN (1.5)	Rt. submandibular lymphadenopathy	Developed skin lesion[b] and bil. lacrimal gland swelling 2 years later	Steroid therapy was performed on bil. swelling of the lacrimal gland, with good response (63)	Asthma	11.6	NA	NA	875 (2.4)
40	57/M	Submandibular LN (3)	Rt. submandibular lymphadenopathy	Relapsed rt. submandibular lymphadenopathy 2 and 8 years later[b]	Follow-up (97)	None	NA	NA	NA	NA

Abbreviations: Bil., bilateral; LN, lymph node; lt., left; NA, not available; nl, normal; PB, peripheral blood; PTGC, progressively transformed germinal centers; rt., right.
[a]IgE ratio: measured value/normal value.
[b]The lesion was histologically diagnosed as IgG4-related disease.
[c]The data was obtained at relapse or disease progression time.

Table 2 Summary of clinical features of IgG4+ PTGC

Number	40
Gender	
Male/female	28/12
Age	
Median (range)	56 (36–76)
≥60	16 (40%)
Allergic disease history	16/31 (51.6%)
Laboratory findings	
Increased eosinophil count in peripheral blood	18/34 (52.9%)
Elevated serum IgG4 level	14/17 (82.4%)
Elevated serum IgE level	12/13 (92.3%)
Initial lymphadenopathy	
Submandibular lymphadenopathy	24 (bilateral; 5)
Submandibular and cervical lymphadenopathy	14 (bilateral; 4)
Cervical lymphadenopathy	1 (unilateral)
Cervical and supraclavicular lymphadenopathy	1 (unilateral)
Number of available follow-up reports	34
Follow-up period	
Median (range)	26 (3–144)
Persistence or relapse of lymph node lesions	23/34 (67.6%)
Progression to extranodal lesions	18/34 (52.9%)
Submandibular gland	5
Lacrimal gland	2
Lacrimal gland and submandibular gland	1
Lacrimal gland and skin	3
Submandibular gland and pleura	1
Lacrimal gland and maxillary sinus	1
Lacrimal gland, submandibular gland, parotid gland, mediastinum, kidney	1
Submandibular gland, mediastinum, lung, pancreas, aorta, kidney	1
Submandibular gland and parotid gland	1
Parotid gland	1
Prostate	1
Progression to systemic lymphadenopathy	3/34 (8.8%)

Abbreviation: PTGC, progressively transformed germinal centers.

exact test, Student's *t*-test or Mann–Whitney *U*-test, as appropriate. All data were analyzed with the STATA software (version 10.0; Stata, College Station, TX, USA).

Results

Clinical Features of IgG4+ Progressively Transformed Germinal Centers

Clinical findings are summarized in Tables 1 and 2. There were 28 men and 12 women with a median age of 56 years (range, 36–76 years). On initial clinical examination, all patients presented with localized submandibular and/or cervical, or cervical and supraclavicular lymphadenopathy. Twenty-four patients showed submandibular lymphadenopathy, which was bilateral in five. Fourteen patients showed submandibular and cervical lymphadenopathy, which was bilateral in four. Only two patients showed cervical, or cervical and supraclavicular lymphadenopathy. In total, 38 (95%) of 40 patients showed submandibular lymphadenopathy. Lymph node biopsy revealed that the size of the biopsied lymph nodes ranged from 1 to 3.5 cm in diameter, with an average of 2.2 cm. In addition, ^{18}F-fluorodeoxy glucose positron emission tomography showed significantly elevated uptake in examined patients (Figure 1). The lesions were therefore all suspected to be malignant lymphomas at initial clinical diagnosis.

Among patients examined for each respective factor, allergic disease was identified in 16 (52%) of 31 patients; peripheral blood eosinophil count was increased in 18 (53%) of 34; serum IgG4 levels were elevated in 14 (82%) of 17; and serum IgE levels were elevated in 12 (92%) of 13.

Figure 1 Ragiological images of IgG4+ progressively transformed germinal centers. Patient no. 12 had localized left submandibular lymphadenopathy and cervical lymphadenopathy (left panel). ^{18}F-fluorodeoxy glucose positron emission tomography showed significantly elevated uptake in the left submandibular lymph node (right panel). The lesion was radiologically and clinically suspected to be malignant lymphoma.

Table 3 Clinicopathological characteristics of patients with IgG4+PTGC and IgG4−PTGC

	IgG4+PTGC (n = 40)	*IgG4−PTGC (n = 22)*	P[a]
Gender			
(Male/female)	28/12	13/9	0.39
Age (years)			
Median	56	47	0.060
Mean (range)	56.5 (36–76)	46.6 (18–78)	
>40 years old	39 (98%)	14 (64%)	<0.0001
>50 years old	30 (75%)	10 (45%)	0.02
Lymphadenopathy area			
Submandibular LN	38 (95%)	1 (4.5%)	<0.0001
Cervical LN	16 (40%)	11 (50%)	0.45
Supraclavicular LN	1 (2.5%)	1 (4.6%)	0.66
Axillary LN	0 (0%)	6 (27%)	0.001
Paraaortic LN	0 (0%)	2 (9%)	0.053
Inguinal LN	0 (0%)	5 (23%)	0.002
IgG4+/IgG+ plasma cell ratio			
Mean	57.4	5.0	
Median (range)	57.8 (44.2–78.1)	3.7 (0–16.7)	<0.0001

Abbreviations: LN, lymph node; PTGC, progressively transformed germinal centers.
[a]IgG4+PTGC *vs* IgG4−PTGC.

Clinicopathological Differences Between IgG4+ Progressively Transformed Germinal Centers and IgG4− Progressively Transformed Germinal Centers

The clinicopathological findings associated with 40 cases of IgG4+ progressively transformed germinal centers and 22 of IgG4− progressively transformed germinal centers (IgG4+/IgG+ plasma cell ratio ≤40%) are summarized in Table 3, Supplementary Figure 1 and Supplementary Table 1. Lymph nodes affected by IgG4+ progressively transformed germinal centers showed a markedly elevated IgG4+/IgG+ plasma cell ratio compared with those with IgG4− progressively transformed germinal centers (mean 57% *vs* 5%, $P<0.0001$). Patients with IgG4+ progressively transformed germinal centers showed an older age distribution and a higher incidence of submandibular lymph node involvement than those with IgG4− progressively transformed germinal centers ($P<0.0001$).

Pathological Findings in IgG4+ Progressively Transformed Germinal Centers

In patients with IgG4+ progressively transformed germinal centers, the lymph nodes demonstrated numerous lymphoid follicles with hyperplastic germinal centers and a distinct mantle zone, but no expansion of the interfollicular zone. Progressively transformed germinal centers were observed in all cases, appearing as round to oval structures 2–3 times the diameter of the other reactive follicles and composed predominantly of small lymphocytes, centrocytes, centroblasts, and numerous mature plasma cells and plasmacytoid cells. The interfollicular zone showed infiltration of a significant number of eosinophils, and T-zones were indistinct (Figure 2).

Interestingly, a unique feature of IgG4+ progressively transformed germinal centers on immunohistochemistry was the localization of the majority of IgG4+ plasma cells in the germinal centers, with only a small number present in the interfollicular zone (Figure 2), except in case no. 3, where they were detected in both the germinal centers and interfollicular zone. The IgG4+ plasma cells were >100/HPF and IgG4+/IgG+ plasma cell ratio was >40% in all cases (Table 3 and Supplementary Figure 1B). Immunoglobulin light-chain restriction was not detected in any case. These histological and immunohistochemical findings were all compatible with progressively transformed germinal centers-type IgG4-related lymphadenopathy.[8,14]

In contrast, the lymph nodes of patients with IgG4− progressively transformed germinal centers showed heterogenous histological findings, demonstrating a small number or numerous lymphoid follicles with or without hyperplastic germinal centers, and expansion or no expansion of the interfollicular zone. The interfollicular zone did not show a significant number of eosinophils, and T-zones were distinct. IgG4+ plasma cells were absent or few, and the IgG4+/IgG+ plasma cell ratio was <40% in all cases.

Disease Progression and Extranodal Lesions in IgG4+ Progressively Transformed Germinal Centers

Thirty-four patients were followed by regular imaging, laboratory findings and clinical evaluation over 3 to 144 months (median, 26 months). During the follow-up period, 23 (68%) patients showed persistence or relapse (or both) of these residual lymph nodes. In all, 18 patients progressed to the development of extranodal lesions, of whom 16 (89%) interestingly showed the involvement of lacrimal and/or submandibular glands. Moreover, 5 of these 18 patients showed progression to systemic disease (Table 2 and Figure 3; patient nos. 11, 13, 20, 21 and 22). Histologically examined extranodal lesions were consistent with IgG4-related disease.

Clinical Management of IgG4+ Progressively Transformed Germinal Centers

In all, 18 of the 34 patients showed stable disease, despite the presence of persistent residual lymph node lesions in almost all. Ten patients showed the localized or systemic relapse of lymphadenopathy, and were re-biopsied. Nine patients were administered steroid therapy when the lesions progressed, to which all responded well (Table 1).

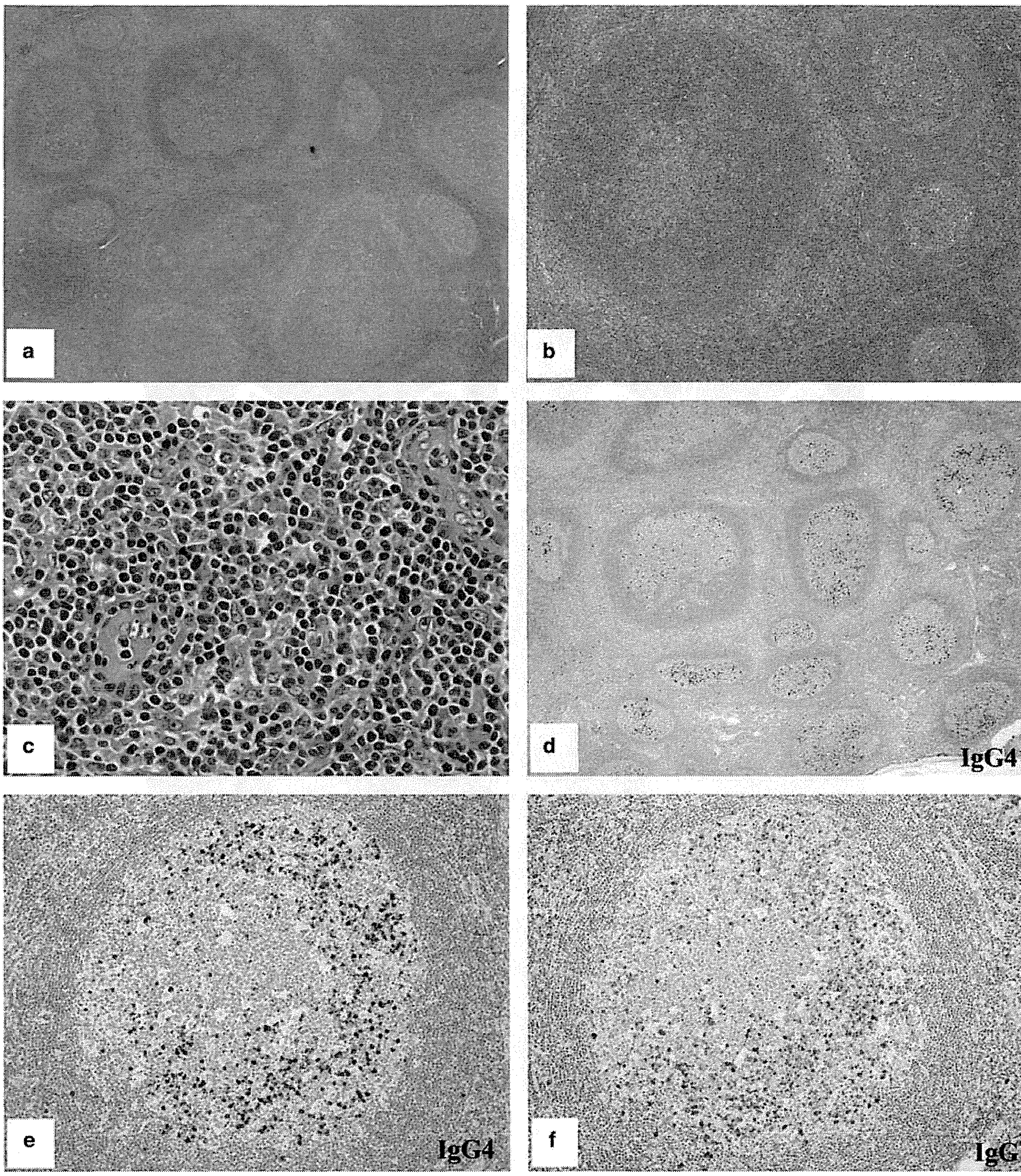

Figure 2 Histological and immunohistochemical features of IgG4^{+} progressively transformed germinal centers. (**a**, **b**) Lymph nodes from patient no. 26 showed numerous lymphoid follicles with hyperplasia and progressively transformed germinal centers (hematoxylin and eosin, **a**: $\times 20$). (**b**) The progressively transformed germinal centers were appearing as round to oval structures 2–3 times the diameter of the other reactive follicles (hematoxylin and eosin, $\times 40$). (**c**) Abundant eosinophil infiltration in the interfollicular zone (hematoxylin and eosin, $\times 200$). (**d**) Localization of the majority of IgG4^{+} plasma cells in the germinal centers (IgG4-immunostaining, $\times 20$). (**e**, **f**) The IgG4^{+}/IgG^{+} plasma cell ratio was >60% (IgG4 and IgG-immunostaining, $\times 100$).

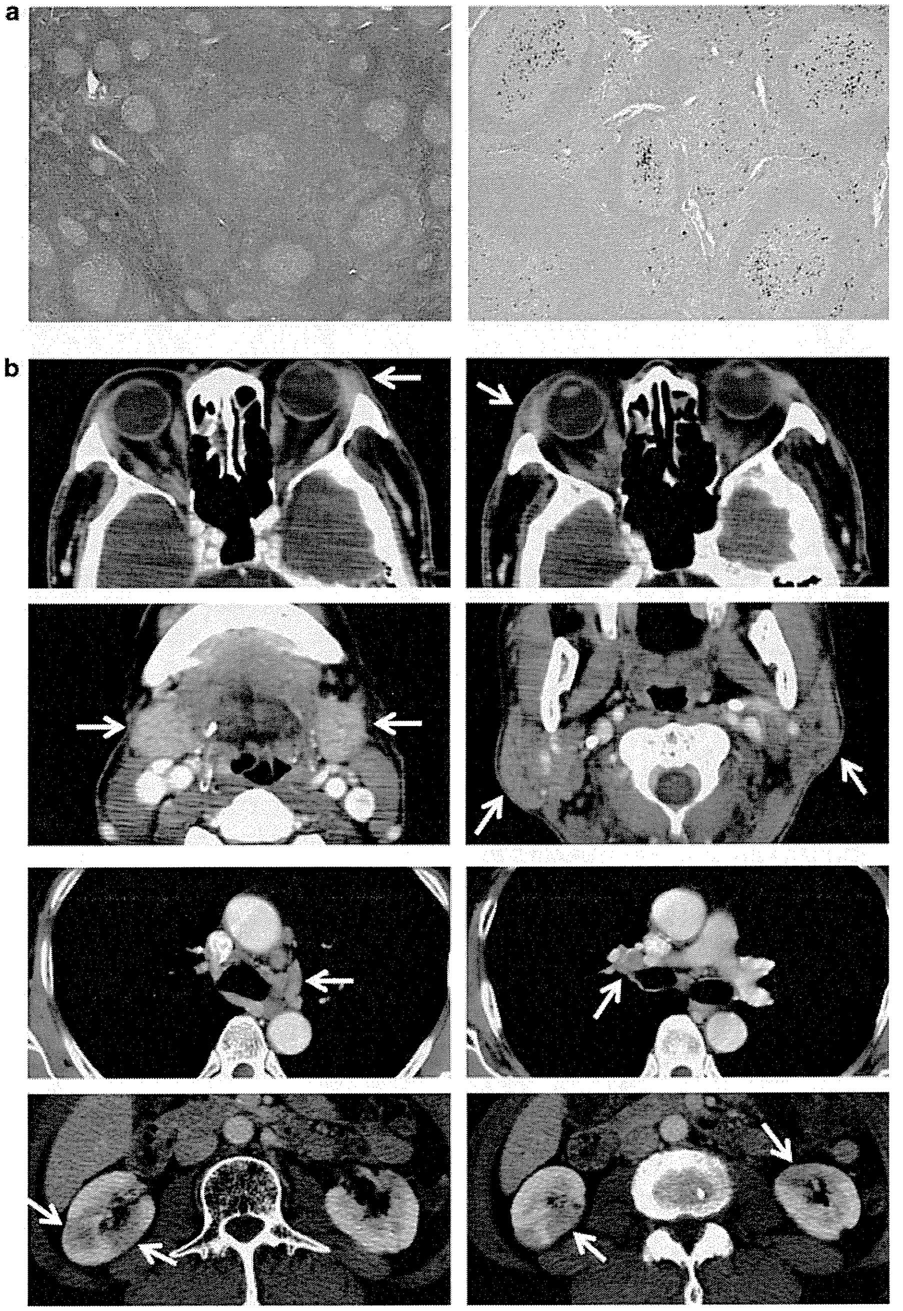

Figure 3 Histological features and radiological images of a patient with IgG4$^+$ progressively transformed germinal centers with progression to systemic disease. (**a**) The affected lymph node from patient no. 11 (initial lymphadenopathy of the submandibular node had been detected 10 years before) showed marked follicular hyperplasia with progressively transformed germinal centers (hematoxylin and eosin, ×20). IgG4$^+$ plasma cells were detected in the germinal centers by immunohistochemistry, and the IgG4$^+$/IgG$^+$ plasma cell ratio was >40% (IgG4 immunostaining, ×40). (**b**) The patient showed progression to systemic disease at 10 years after the initial diagnosis. Computed tomography revealed lesions of the lacrimal glands, submandibular glands, parotid glands and kidney, all of which were bilateral, as well as the mediastinum and paraaortic lymph nodes. The lacrimal gland and kidney lesions were histologically consistent with IgG4-related disease, and serum IgG4 levels and IgG4/IgG ratio were highly elevated (serum IgG4, 2260 mg/dl; serum IgG4/IgG ratio, 46%).

Immunoglobulin Heavy-Chain Gene Rearrangement in IgG4^{+} Progressively Transformed Germinal Centers

No immunoglobulin heavy-chain gene rearrangement was observed in any of the cases examined.

Discussion

In this study, we describe a unique series of 40 patients with progressively transformed germinal centers of the lymph nodes who fulfilled the histological diagnostic criteria of IgG4-related disease.[8,10,11–14] The disease presented with uniform clinicopathology, namely asymptomatic localized submandibular lymphadenopathy and progression to extranodal lesions, particularly the lacrimal and submandibular glands. Patients were predominantly middle-aged to older males, and about half of those examined had concomitant allergic disease. Microscopic observation of the affected lymph nodes revealed marked follicular hyperplasia with progressively transformed germinal centers, eosinophil infiltration in the interfollicular zone and IgG4^{+}plasmacytosis in the germinal centers. Eighteen patients developed extranodal lesions, of which those which were histologically examined were consistent with IgG4-related disease. Moreover, all of the examined patients had elevated serum IgG4 and IgE levels, with the exception of three serum IgG4- and one serum IgE-negative patients. These clinicopathological findings of IgG4^{+} progressively transformed germinal centers are compatible with IgG4-related disease.[8,10,11–14]

IgG4-related disease frequently involves the lacrimal glands, submandibular glands, pancreas, hepatobiliary tract and lymph nodes.[7–14] Nevertheless, virtually any organ can be affected, including the lungs, mediastinum, skin, retroperitoneum, aorta, kidneys and prostate.[7–14] The general condition of patients at presentation is usually good, with no fever or constitutional symptoms. Common laboratory findings include increased serum IgG4 and IgE levels, whereas lactate dehydrogenase level remains unchanged. Patients often show an excellent response to steroid therapy.[7–14]

Progressively transformed germinal centers is a benign condition of unknown pathogenesis, which presents either as a solitary asymptomatic enlarged lymph node, most commonly in the neck, or in multiple anatomical sites, usually in the form of mass lesions.[1–6]

Progressively transformed germinal centers carries with it an increased long-term risk for the development of nodular lymphocyte predominant Hodgkin lymphoma.[3–5] However, no case of progression to nodular lymphocyte predominant Hodgkin lymphoma were seen in our available cases.

In the United States and Germany, progressively transformed germinal centers occurs more commonly in young males.[1–6] Our patients were similar to these previously reported patients in that they were predominantly male and largely presented with solitary asymptomatic lymphadenopathy in the neck, but differed in that they were middle-aged to older. Kojima *et al*[6] also reported that their Japanese progressively transformed germinal centers cases were more frequently middle-aged to older patients, and interestingly that about 30% had chronic sialadenitis or allergic disease.[6] These cases are similar to our series of IgG4^{+} progressively transformed germinal centers, suggesting that this clinical picture might be suitably categorized as progressively transformed germinal centers-type IgG4-related lymphadenopathy. In fact, many cases of IgG4-related disease have been reported in Asia, particularly in Japan.[8–11]

Although the mechanism underlying IgG4-related disease remains unclear,[8–11] a recent study suggested the possible involvement of T helper 2 cells and regulatory immune reactions, indicating a possible allergic mechanism.[16,17] In fact, we found elevated serum IgE levels in almost all patients examined. Furthermore, about half of our patients showed eosinophilia, with marked eosinophil infiltration in the affected tissue, in addition to concomitant allergic disease.

Interestingly, our series of IgG4^{+} progressively transformed germinal centers of the lymph nodes appeared to specifically involve the submandibular lymph nodes, but the reason for this is unclear. These nodes receive lymph from a wide area, including the ocular region, nose and adjacent cheek, paranasal sinus, oral cavity, and salivary glands.[18] This area, particularly the ocular adnexa and salivary glands, is very frequently affected in IgG4-related disease. Indeed, the extranodal lesions detected in our patients frequently involved the area covered by the submandibular lymph nodes. The mechanism might therefore be related to anatomical lymphatic flow.

Three of our patients had normal serum IgG4 levels. This might have been because the measurement of serum IgG4 in these three patients occurred after biopsy, at which time there were no residual main lesions. In this regard, about 20% of patients with IgG4-related pancreatitis are negative for serum IgG4.[8,19,20]

Residual lymph node lesions in our series of IgG4^{+} progressively transformed germinal centers patients showed frequent persistence or relapse (or both), and the disease progressed to either or both extranodal lesions or systemic disease. This explains why, although progressively transformed germinal centers was eventually diagnosed based on histological findings, this pattern of disease progression suggested malignant lymphoma.

In conclusion, we describe here a unique case series characterized by progressively transformed germinal centers with intra-germinal center IgG4^{+} plasmacytosis involving the submandibular lymph node in middle-aged to older patients who clinically presented with asymptomatic localized lymphadenopathy. About half of these patients progressed during the follow-up period to extranodal lesions, systemic disease or both. We suggest that the patients with IgG4$^+$ progressively transformed germinal centers of the lymph nodes may phenotypically present with incipient lesions associated with IgG4-related disease. Moreover, almost all cases described here were suspected to be malignant lymphomas at initial diagnosis or when the disease progressed. Prevention of potentially harmful misdiagnosis requires the recognition of this lesion as a distinct clinicopathological entity, based on careful analysis of clinical and pathological findings through the close collaboration of pathologist and clinician.

Acknowledgement

This work was supported in part by grants from the Okayama Medical Foundation.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Lennert K, Müller–Hermelink HK. Lymphocyten and ihre funktionsformen morphologie, organization und immunologische bedeutung. Verh Anat Ges 1975;69: 19–62.
2 Osborne BM, Butler JJ. Clinical implications of progressive transformation of germinal centers. Am J Surg Pathol 1984;8:725–733.
3 Hansmann ML, Fellbaum C, Hui PK, *et al.* Progressive transformation of germinal centers with and without association to Hodgkin's disease. Am J Clin Pathol 1990;93:219–226.
4 Osborne BM, Butler JJ, Gresik MV. Progressive transformation of germinal centers: comparison of 23 pediatric patients to the adult population. Mod Pathol 1992;5:135–140.
5 Jones D. Dismantling the germinal center: comparing the processes of transformation, regression, and fragmentation of the lymphoid follicle. Adv Anat Pathol 2002;9:129–138.
6 Kojima M, Nakamura S, Motoori T, *et al.* Progressive transformation of germinal centers: a clinicopathological study of 42 Japanese patients. Int J Surg Pathol 2003;11:101–107.
7 Sato Y, Ohshima K, Ichimura K, *et al.* Ocular adnexal IgG4-related disease has uniform clinicopathology. Pathol Int 2008;58:465–470.
8 Sato Y, Notohara K, Kojima M, *et al.* IgG4-related diseases: historical overview and pathology of hematological disorders. Pathol Int 2010;60:247–258.
9 Masaki Y, Dong L, Kurose N, *et al.* Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: Analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis 2009;68:1310–1315.
10 Masaki Y, Kurose N, Umehara H. IgG4-related disease: a novel lymphoproliferative disorder discovered and established in Japan in the 21st century. J Clini Exp Hematopathol 2011;51:13–20.
11 Cheuk W, Chan JKC. IgG4-related sclerosing disease, a critical appraisal of an evolving clinicopathologic entity. Adv Anat Pathol 2010;17:303–332.
12 Stone JH, Khosroshahi A, Hilgenberg A, *et al.* IgG4-related systemic disease and lymphoplasmacytic aortitis. Arthritis Rheumatism 2009;60:3139–3145.
13 Cheuk W, Yuen HKL, Chu SYY, *et al.* Lymphadenopathy of IgG4-related sclerosing disease. Am J Surg Pathol 2008;32:671–681.
14 Sato Y, Kojima M, Takata K, *et al.* Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease. Mod Pathol 2009;22:589–599.
15 Grimm KE, Barry TS, Chizhevsky V, *et al.* Histopathological findings in 29 lymph node biopsies with increased IgG4 plasma cells. Mod Pathol 2012;25: 480–491.
16 Zen Y, Fujii T, Harada K, *et al.* Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. Hepatology 2007;45:1538–1546.
17 Nakashima H, Miyake K, Moriyama M, *et al.* An amplification of IL-10 and TGF-β in patients with IgG4-related tubulointerstitial nephritis. Clin Nephrol 2010;73:385–391.
18 Snell RS. Clinical Anatomy for Medical Students. 6th edn. Lippincott Williams & Wilkins: Philadelphia, 2000, pp 659.
19 Hamano H, Kawa S, Horiuchi A, *et al.* High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 2001;344:732–738.
20 Matsubayashi H, Sawai H, Kimura H, *et al.* Characteristics of autoimmune pancreatitis based on serum IgG4 level. Dig Liver Dis 2011;43:731–735.

Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)

THYROID
Volume X, Number X, 201X

DOI: 10.1089/thy.2013.0448

Elevated Serum Immunoglobulin G4 Levels in Patients with Graves' Disease and Their Clinical Implications

Ken Takeshima, Hidefumi Inaba, Yasushi Furukawa, Masahiro Nishi, Hiroyuki Yamaoka, Waka Miyamoto, Takayuki Ota, Asako Doi, Hiromichi Kawashima, Hiroyuki Ariyasu, Hisao Wakasaki, Hiroto Furuta, Taisei Nakao, Hideyuki Sasaki, and Takashi Akamizu

Background: Immunoglobulin G4-related disease (IgG4-RD) is a new clinical entity that affects various organs with increased IgG4 positive plasmacytes and progressive fibrosis. While IgG4-RDs in association with Hashimoto's thyroiditis or Riedel's thyroiditis have been reported, the relationship between IgG4-RD and Graves' disease (GD) is yet unknown. To elucidate the relation of GD to IgG4-RD, serum IgG4 levels and their clinical implications in patients with GD were investigated.
Methods: In this prospective study, serum IgG4 levels were measured in 109 patients with GD and classified into two groups according to the comprehensive diagnostic criteria of IgG4-RD previously established: (i) GD with elevated-IgG4 levels ($\geq$135 mg/dL), and (ii) GD with nonelevated IgG4 ($<$135 mg/dL).
Results: Seven out of 109 patients with GD (6.4%) had elevated serum IgG4 levels (mean $\pm$ *SD* (range): 175.0 $\pm$ 44.5 (136–266) mg/dL) and elevated ratios of IgG4/IgG (12.7 $\pm$ 4.5% (7.6%–21.2%)). The remaining patients with GD had serum IgG4 levels and IgG4/IgG ratios of 39.6 $\pm$ 27.6 (3–132) mg/dL and 3.2 $\pm$ 2.2% (0.3%–11.5%) respectively. Ages in the elevated IgG4 group were significantly higher than those of the nonelevated IgG4 group: 54.7 $\pm$ 6.2 versus 43.4 $\pm$ 15.4 years respectively. Ultrasound examinations revealed that the elevated IgG4 group had significantly increased hypoechogenic areas in the thyroid in comparison to the nonelevated IgG4 group (low echo scoring: 1.66 $\pm$ 0.81 vs. 0.61 $\pm$ 0.89 respectively). In the correlation analysis, TSAb ($r_s = 0.385$, $n = 42$) titers were significantly correlated with serum IgG4 levels, while they were not significantly different between the two groups. In the elevated IgG4 group, symptoms were controllable with a small dose of antithyroidal drug (ATD; $n = 4$), a combination treatment with ATD and L-T4 ($n = 1$), or L-T4 administration only one year after the first visit ($n = 2$).
Conclusions: A small portion of GD patients harbored elevated serum IgG4 levels. They were older, had increased hypoechoic areas in the thyroid, and appeared to be responsive or prone to be hypothyroid after ATD treatment. Thus, the present study suggests the presence of a novel subtype of GD. Measuring serum IgG4 levels may help to distinguish this new entity and provide potential therapeutic options for GD.

Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a recently proposed clinical entity, first reported in 2001 as a novel subtype of autoimmune pancreatitis (1). It is characterized by elevated serum IgG4 levels, IgG4-positive plasmacytes, and lymphocyte infiltration into multiple organs, inducing tissue fibrosis and organ dysfunction. In addition to the involvement of the pancreas, the lacrimal gland, salivary gland, biliary duct, and retroperitoneal tissue can also be involved in this disease (2).

The relationship between IgG4-RD and thyroid diseases has been previously investigated. Li *et al.* described a novel type of IgG4 thyroiditis on the basis of its clinical and histopathological features (3). The authors found a close relationship between the fibrous variant of Hashimoto's thyroiditis (HT) and IgG4-RD. Riedel's thyroiditis (RT) has been proposed to be an organ manifestation of IgG4-RD (4,5). In cases of RT, elevated serum IgG4 levels and/or an increased number of IgG4-positive plasmacytes with dense fibrous tissue were observed in the thyroid gland, which would respond to steroid therapy, suggesting characteristics of IgG4-RD (4). In addition, Watanabe *et al.* reported that 19% of patients with IgG4-RD who had hypothyroidism showed an increased thyroid volume and TgAb and/or TPOAb positivity. The thyroid function of these patients

The First Department of Medicine, Wakayama Medical University, Wakayama, Japan.

normalized after prednisolone treatment. Furthermore, histology revealed IgG4-bearing plasma cells and loss of thyroid follicles, was the condition was thus termed IgG4-related thyroiditis (6). However, the relationship between IgG4-RD and GD remains unknown.

To elucidate the relationship between IgG4-RD and GD, serum IgG4 levels in 109 patients with GD were measured and compared according to clinical characteristics. Herein, we describe the clinical features of patients with GD and elevated serum IgG4 levels, and discuss diagnostic and therapeutic approaches.

Patients and Methods

Patients

A total of 109 patients with GD at the Wakayama Medical University Hospital, Japan, from January 2011 to October 2012 were prospectively recruited to this study. The diagnosis of GD was based on the presence of hyperthyroidism, positive thyroid stimulating hormone receptor antibody/thyroid stimulating antibody (TRAb/TSAb), and/or increased ^{123}I thyroid uptake. Patients with malignancies or pregnancy were excluded. None of the patients underwent surgery or radioiodine treatment. Patients were divided into two groups: those with elevated serum IgG4 levels (≥ 135 mg/dL) and those with nonelevated serum IgG4 levels (< 135 mg/dL) according to the comprehensive diagnostic criteria of IgG4-RD, the currently established criteria (7). Masaki *et al.* proposed that another criterion for IgG4-RD consists of a ratio of IgG4/IgG of $\geq 8.0\%$ (8); this criterion was therefore also considered in our study. Patients were analyzed for age, sex, smoking, familial history of autoimmune thyroid disease (AITD), presence of Graves' ophthalmopathy (GO) on the basis of the clinical activity score (CAS) (9) and NOSPECS (10), serum IgG4 and IgG levels, thyroid function, thyroid autoantibodies, and ultrasound examination (Table 1). Patients were classified according to intractability in the control of hyperthyroidism: group 1 (intractable patients) who required a moderate or large dosage of antithyroidal drug (ATD) (thiamazole (MMI) ≥ 10 mg/day; propylthiouracil (PTU) ≥ 150 mg/day) to control thyroid function; and group 2 (tractable patients) who could be treated with a small dosage no ATD (MMI ≤ 5 mg/day; PTU ≤ 100 mg/day). Written informed consent was obtained from all patients, and the study protocol was approved by the Wakayama Medical University Hospital Ethics Committee.

Thyroid function tests and thyroid autoantibodies

Serum thyrotropin (TSH), free thyroxine (fT4), and free triiodothyronine (fT3) levels were measured by chemiluminescent immunoassay (Abbott Diagnostics, Tokyo, Japan). Reference ranges were defined as follows: TSH 0.35–4.94 mIU/L; fT4 0.70–1.48 ng/dL; and fT3 1.71–3.71 pg/mL. TRAb was determined by enzyme-linked immunosorbent assay (Cosmic, Tokyo, Japan). Thyroglobulin autoantibodies (TgAb) and thyroid peroxidase antibodies (TPOAb) were measured with an electrochemiluminescent immunoassay (SRL, Tokyo, Japan). Normal values were defined as follows: TRAb < 1 IU/L; TgAb < 28 IU/mL; TPOAb < 16 IU/mL. TSAb activities were determined using the Yamasa's TSAb bioassay kit (Yamasa Ltd., Choshi, Japan). Normal values for TSAb were defined as $< 180\%$.

Serum IgG4 and IgG levels

Serum IgG4 and IgG levels were measured by a nephelometric immunoassay (BML, Osaka, Japan). Reference ranges for IgG4 and IgG were defined as 4–108 mg/dL and 870–1700 mg/dL respectively. Since comprehensive diagnostic criteria for IgG4-RD include a serum IgG4 level ≥ 135 mg/dL, we defined this as the cutoff level in this study.

Ultrasonographic evaluation

Ultrasonographic examinations were performed by conventional gray scale and color Doppler, and by 10 MHz linear transducer (Toshiba Medical, Osaka, Japan). Low echogenicity in the thyroid gland was classified into four categories and scored as previously described (11): Grade 0, diffuse high-amplitude echoes throughout the whole lobe of the thyroid; Grade 1, low-amplitude and nonuniform echoes in the whole or several regions of the thyroid; Grade 2, several sonolucent regions in the thyroid; and Grade 3, no apparent echoes or very low amplitude echoes throughout the whole thyroid. Increase of color Doppler flow in the thyroid gland was determined as follows: 0, none; 1, mild; 2, moderate; and 3, severe. Thyroid size was measured as the sum of both lobes according to the following equation: anteroposterior $\times$ transversal diameters (mm^2) at the maximum position.

Statistical analysis

Fisher's exact test was used to assess data in the two-dimensional contingency tables for comparison with sex, presence of GO, family history of AITD, and smoking. Mann–Whitney *U*-test and Kruskal–Wallis test were used to compare two or three individual groups respectively. Two-tailed Spearman's rank correlation coefficient (r_s) was determined to assess the correlation between two variables. Data for TSH, TRAb, TgAb, and TPOAb were analyzed with log-transformed values. p-Values < 0.05 were accepted as statistically significant (SPSS v15, Chicago, IL). Data are expressed as mean $\pm$ standard deviation (*SD*).

Results

Serum IgG4 and IgG levels in patients with GD

Overall, the serum IgG4 level in patients with GD was 48.3 ± 44.0 mg/dL (range 3–266), and the ratio of IgG4/IgG was 3.8 ± 3.4 mg/dL (range 0.3–21.2; Table 1). Seven (6.4%) of the 109 patients with GD had elevated serum IgG4 levels at 175.0 ± 44.5 mg/dL (range 136–266) and elevated ratios of IgG4/IgG at $12.7 \pm 4.5\%$ (range 7.6–21.2). The remaining patients with GD (93.6%) had serum IgG4 levels and IgG4/IgG ratios of 39.6 ± 27.6 mg/dL (range 3–132) and $3.2 \pm 2.2\%$ (range 0.3–11.5) respectively. Three cases of GD in patients with serum IgG4 levels < 135 mg/dL had serum ratios of IgG4/IgG $\geq 8\%$. There was a significant difference in the ratios of IgG4/IgG between the two groups ($p < 0.001$). However, no significant difference was observed in serum IgG levels between groups.

The elevated-IgG4 group consisted of one man and six women, while the nonelevated-IgG4 group included 14 men and 88 women. No significant difference was observed in sex distribution between groups.

Table 1. Comparisons of Serum IgG4 Levels and Clinical Characteristics of Patients with Graves' Disease (GD)

	Overall (n = *109*)		*Non elevated IgG4* (< *135 mg/dL;* n = *102, 93.6%*)		*Elevated-IgG4* (≥ *135 mg/dL;* n = *7, 6.4%*)		p-*Value*[a]
Sex (M/F)	15/94		14/88		1/6		0.967
Presence of Graves' ophthalmopathy	29 (26.6%)		26 (25.5%)		3 (42.3%)		0.379
Family history of AITD	30 (27.5%)		28 (27.5%)		2 (28.6%)		0.948
Smoking history	33 (30.2%)		31 (30.4%)		2 (28.6%)		0.919
	Mean ± SD *(range)*	n	*Mean* ± SD *(range)*	n	*Mean* ± SD *(range)*	n	p-*Value*[b]
Age (years)	44.1 ± 15.2 (13–79)	109	43.4 ± 15.4 (13–79)	102	54.7 ± 6.2 (49–68)	7	**0.026**
IgG4 (mg/dL)	48.3 ± 44.0 (3–266)	109	39.6 ± 27.6 (3–132)	102	175.0 ± 44.5 (136–266)	7	NA
IgG (mg/dL)	1275.7 ± 298.0 (774–2928)	104	1262.4 ± 288.0 (774–2928)	97	1459.7 ± 392.6 (910–2012)	7	0.179
IgG4/IgG (%)	3.8 ± 3.4 (0.3–21.2)	104	3.2 ± 2.2 (0.3–11.5)	97	12.7 ± 4.5 (7.6–21.2)	7	< **0.001**
Thyroid size on ultrasound (mm^2)[c]	961.3 ± 771.6 (279–4358)	59	962.7 ± 788.9 (279–4358)	54	946.1 ± 622.3 (315–1689)	5	0.957
Degree of hypoechogenicity[d]	0.71 ± 0.93 (0–3)	62	0.61 ± 0.89 (0–3)	56	1.66 ± 0.81 (1–3)	6	**0.005**
Increase of color Doppler flow[e]	1.30 ± 0.90 (0–3)	62	1.33 ± 0.88 (0–3)	56	1.00 ± 1.09 (0–3)	6	0.293
TSH (mIU/L)	0.73 ± 3.03 (< 0.003–25.07)	107	0.68 ± 3.10 (< 0.003–25.07)	100	1.38 ± 1.74 (< 0.003–4.39)	7	ND[f]
fT3 (pg/mL)	9.09 ± 7.59 (1.72–30.0)	107	9.12 ± 7.41 (1.72–30.0)	100	8.63 ± 10.51 (2.52–30.0)	7	0.231
fT4 (ng/dL)	2.25 ± 1.20 (0.44–6.29)	108	2.27 ± 1.19 (0.44–6.29)	101	1.91 ± 1.52 (0.81–4.97)	7	0.212
TSAb (%)	670.8 ± 721.1 (92–3024)	42	648.1 ± 648.8 (92–2320)	38	843.6 ± 1234.3 (214–3024)	4	0.493
TRAb (IU/L)	15.7 ± 26.5 (< 1.0–187.8)	107	16.1 ± 27.2 (< 1.0–187.8)	101	9.6 ± 8.3 (1.3–19.8)	6	0.498
TgAb (IU/mL)	619.3 ± 1190.9 (< 10–4000)	77	536.7 ± 1096.1 (< 10–4000)	70	1445.2 ± 1808.9 (< 10–4000)	7	0.338
TPOAb (IU/mL)	225.5 ± 224.1 (< 5–600)	75	221.0 ± 216.1 (< 5–600)	68	269.9 ± 309.0 (< 5–600)	7	0.812

p-Values were obtained using [a]Fisher's exact test, or [b]Mann–Whitney *U*-test, and *p*-values < 0.05 were accepted as significant, shown in bold.

[c]Thyroid size was measured as the sum of both lobes according to the following equation: anteroposterior × transversal diameters (mm^2) at the maximum position.

[d]Degree of low echogenicity in the thyroid gland was determined by ultrasonography as follows; Grade 0, diffuse high-amplitude echoes throughout the whole lobe of the thyroid; Grade 1, low-amplitude and ununiformed echoes in the whole or several regions of the thyroid; Grade 2, several sonolucent regions in the thyroid; and Grade 3, no apparent echoes or very low-amplitude echoes throughout the whole thyroid.

[e]Increase of color Doppler flow in the thyroid gland was determined as follows: 0, none; 1, mild; 2, moderate; and 3, severe.

[f]Since most TSH levels were undetectable, comparison of TSH levels in the two groups was not determined.

Data for TSH, TRAb, TgAb, and TPOAb were analyzed with log-transformed values.

Values of < 0.003, < 1.0, < 5, < 10, > 30.0, > 600, and > 4000 were calculated as 0, 1.0, 5, 10, 30.0, 600, and 4000 respectively.

AITD, autoimmune thyroid disease; fT3, free triiodothyronine; fT4, free thyroxine; NA, not applicable; ND, not determined; SD, standard deviation; TgAb, thyroglobulin autoantibodies; TPOAb, thyroid peroxidase antibodies; TRAb, thyroid stimulating hormone receptor antibody; TSAb, thyroid stimulating antibody; TSH, thyrotropin.