TABLE 2. CLINICAL PROFILES OF GD PATIENTS WHO HAD ELEVATED SERUM IGG4 LEVELS

Patient	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Age (years)	54	52	49	68	51	53	56
Sex	F	F	F	M	F	F	F
Proptosis	R17, L15	None	R17, L18	R17, L20	None	None	None
Diplopia	None	None	None	Present	None	None	None
NOSPECS	3a	2b	3a	4b	2a	2b	1
CAS	1	2	0	2	0	0	0
Orbital MRI	ND	Muscle, fat	ND	Muscle, fat	ND	ND	ND
TSH (mIU/L)	< 0.003	3.053	0.614	0.007	< 0.003	4.397	1.601
fT3 (pg/mL)	15.48	2.87	2.52	3.51	> 30	2.87	3.19
fT4 (ng/dL)	2.94	0.81	1.11	1.24	4.97	1.35	0.97
TRAb (IU/L)	19.8	3.7	1183	1.4	13.3	1.6	17.5
TSAb (%)	ND	3024	86	214	614	280	ND
TgAb (IU/mL)	169.6	< 10	> 4000	< 10	1393	> 4000	533.5
TPOAb (IU/mL)	7.7	< 5	> 600	33.4	43.5	> 600	> 600
IgG4 (mg/dL)	136	142	153	159	179	190	266
IgG (mg/dL)	910	1324	2012	1395	1954	1369	1254
IgG4/IgG (%)	14.9	10.7	7.6	11.4	9.2	13.9	21.2
Thyroid size in US (mm^2)	ND	315.4	1495	ND	815	416	1689.1
Hypoechogenicity on US	1	1	3	ND	2	2	1
Requirement of ATD doses to control hyperthyroidism after normalization of thyroid function tests	MMI 5 mg/day	MMI 5 mg/day	None (became hypothyroid and supplemented with L-T4 25 μg/day)	MMI 2.5 mg/day	PTU 50 mg/day	None (became hypothyroid and supplemented with L-T4 100 μg/day)	MMI 5 mg/day + L-T4 25 μg/day

NOSPECS and CAS are the severity and activity classifications of Graves' ophthalmopathy respectively. Exophthalmoses in the right and left eyes measured with the Hertel exophthalmometer are represented as (R, mm) and (L, mm) respectively. In the orbital MRI, the presence of extraocular muscles enlargement and the increase of orbital fat are indicated.

ATD, antithyroid drugs; MMI, thiamazole; ND, not determined; PTU, propylthiouracil.

The mean age of the elevated IgG4 group was significantly higher than that of the nonelevated IgG4 group: 54.7±6.2 years (range 49–68) versus 43.4±15.4 years (range 13–79) respectively ($p=0.026$). The number of patients with GO (CAS ≥1) who had a family history of AITD or who were smokers was not significantly different between the two groups (Table 1). Serum TSH, fT3, fT4, TRAb, TgAb, and TPOAb levels did not significantly differ between the elevated and nonelevated IgG4 groups (Table 1). Ultrasound examinations revealed that the elevated IgG4 group had significantly more hypoechogenic areas in the thyroid compared to the nonelevated IgG4 group (low echo scoring: 1.66±0.81 (range 1–3) vs. 0.61±0.89 (range 0–3)) respectively ($p=0.005$). No significant differences were observed in thyroid size or increase of color Doppler flow between the two groups. We also analyzed the patients based on the IgG4/IgG ratio (ratio ≥8%). The elevated IgG4/IgG ratio group had significantly increased hypoechogenic areas in the thyroid in comparison to the nonelevated IgG4 group ($p=0.031$), although the ages of the elevated IgG4/IgG ratio group were not significantly higher than those of the nonelevated IgG4/IgG ratio.

Clinical features of patients with elevated IgG4

The clinical characteristics of the seven GD patients with elevated IgG4 are summarized in Table 2. Of these, six patients had an IgG4/IgG ratio of ≥8%, and five had GO (patients 1, 2, 3, 4, and 6). Patient 4 had diplopia; patients 1, 3, and 4 had mild proptosis (17–20 mm); patients 2 and 4 had swollen extraocular muscles and increased orbital fat; patient 6 showed palpebral swelling. TRAb levels were elevated in all seven cases. In patient 3, who spontaneously became hypothyroid in the follow-up of Graves' hyperthyroid patients treated with a maintenance dose (5–15 mg) of methimazole for more than 10 years, TRAb levels were rapidly and remarkably increased in spite of negative TSAb activity, suggesting the presence of blocking-type TRAb. Nonetheless, this patient showed persistent thyroid enlargement with broad hypoechoic areas in both lobes (Fig. 1). The TRAb activity of patient 6 was initially 35.1% as determined by a 1st generation TRAb assay (normal value <10%) when she was thyrotoxic (fT4 4.2 ng/dL). TgAb and/or TPOAb levels were elevated in most cases except for patient 2. In the elevated IgG4 group, patients were treated with a small dose of ATD (patients 1, 2, 4, and 5), MMI and combined L-T4 (patient 7), or L-T4 alone (patients 3 and 6) one year after ATD treatment (Table 2). None of the patients had pretibial myxedema.

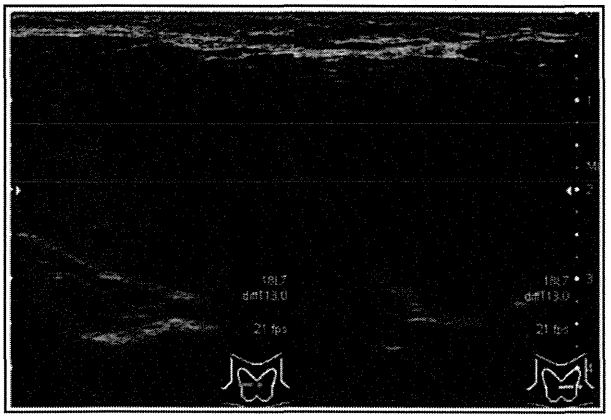

FIG. 1. An ultrasonographic image of patient 3. Note that hypoechoic areas are seen throughout both lobes of the thyroid gland.

Correlation between serum IgG4 and IgG levels and ratios and other clinical parameters

The TSAb titer correlated significantly with both serum IgG4 levels ($r_s=0.385$, $p=0.012$, $n=42$) and IgG4/IgG ratios ($r_s=0.346$, $p=0.027$, $n=41$). In addition, in patients with untreated hyperthyroidism ($n=42$), TSAb titers also significantly correlated with both serum IgG4 levels ($r_s=0.519$, $p=0.039$, $n=16$) and IgG4/IgG ratios ($r_s=0.568$, $p=0.022$, $n=16$). The TPOAb titer correlated significantly with serum IgG levels ($r_s=0.2495$, $p=0.037$, $n=70$), but not with IgG4 levels or IgG4/IgG ratios. No significant correlation of serum IgG4, IgG levels, and the ratios of IgG4/IgG were observed with age, ultrasound findings (thyroid size, low echogenicity, Doppler flow), thyroid hormone levels (fT3, fT4), or TgAb titer. IgG4 levels or IgG4/IgG ratios were not correlated with IgG levels either.

Comparisons of clinical profiles between patients with intractable and tractable GD

Since patients with elevated serum IgG4 appeared to be responsive to ATD or prone to be hypothyroid, patients with GD were divided into two subgroups: group 1 (intractable patients, $n=39$) and group 2 (tractable patients, $n=18$). Although not significant, serum IgG4 (70.5±75.1 mg/dL (range 4–266)) and the ratio of IgG4/IgG (5.8±6.1 mg/dL (range 0.4–21.2)) in group 2 tended to be slightly higher than those in group 1 (IgG4 levels 47.2±29.6 mg/dL (range 3–132); IgG4/IgG ratios 3.7±2.4 (0.3–11.5)). In contrast, serum IgG levels were almost identical within the two groups (group 1 1284.6±237.8 (782–1735); group 2 1219.6±308.9 (774–2928)).

We also classified patients by CAS: patients with CAS=0 ($n=80$), patients with CAS=1 or 2 ($n=17$), and patients with CAS ≥3 ($n=12$). Serum IgG4, IgG levels, and the ratio of IgG4/IgG were not significantly different between the three groups. We also examined orbital magnetic resonance imaging (MRI) scans and NOSPECS in patients with GD, but no clear associations between serum IgG4 levels with findings of orbital MRI or NOSPECS (9) were observed (data not shown).

Next, correlations between serum IgG4, IgG levels, or ratios of IgG4/IgG and other clinical parameters were separately examined in two groups. No obvious correlations were observed in group 1. In group 2, low echoic scores were significantly positively correlated with serum IgG levels ($r_s=0.851$, $p=0.001$, $n=12$) but not IgG4. TPOAb titer was significantly correlated with both serum IgG4 levels ($r_s=0.576$, $p=0.031$, $n=14$) and IgG levels ($r_s=0.637$, $p=0.019$, $n=13$), but not with IgG4/IgG ratios.

Discussion

In the current study, a novel subgroup of patients with GD and elevated serum IgG4 level was identified (6.4% of overall

GD patients; Table 1). Yamamoto *et al.* reported that in healthy controls ($n=21$), the average serum IgG4 level was 43 ± 31 mg/dL and the ratio of IgG4/IgG was $2.9 \pm 1.8\%$ (12). On the basis of these values, in this report, patients with GD demonstrated a higher serum IgG4 level and IgG4/IgG ratios than healthy controls. Moreover, 6.4% of patients had elevated serum IgG4 levels (≥ 135 mg/dL), which meets the comprehensive diagnostic criteria of IgG4-RD.

The elevated IgG4 group did not demonstrate any male predominance (Table 1). This finding is neither consistent with previous reports on IgG4 thyroidits (3), nor with those on IgG4-RD (2). The average age of patients in the elevated IgG4 group was significantly higher than that in the nonelevated IgG4 group, which is similar to that observed in IgG4-RD (2). In contrast, IgG4 thyroiditis is associated with a younger age (3), although the prevalence of HT increases with age (13). Since serum IgG4 levels do not increase with age (14) and ages were not found to be correlated with IgG4 levels, unknown factors other than aging may contribute to the elevation in IgG4 levels.

TgAb elevation is associated not only with IgG4 thyroiditis (3), but also with IgG4-related thyroiditis (6). In the present study, serum TgAb levels tended to be higher, albeit not significantly, in the elevated IgG4 group than in the nonelevated IgG4 group (Table 1). Given that IgG4 is a dominant subtype of TgAb in patients with GD (15–17), TgAb may be at least partly a source of IgG4 in patients 1, 3, and 5–7 with TgAb elevation (Table 2). The presence of TPOAb is also associated with GD. In fact, a significant positive correlation between TPOAb and IgG4 or IgG levels in tractable patients.

The TSH receptor (TSH-R) is a crucial antigen for GD (13). Weetman *et al.* reported that the IgG subclass in TRAb is restricted to IgG1 (18). In contrast, Latrofa *et al.* reported that TRAb were affinity enriched on recombinant TSHR antigen before IgG subclass analysis (19). Of three sera samples processed, one contained IgG1 only, one IgG1 + IgG4, and one only IgG4. Since they studied sera selected for very high TRAb levels, this finding suggests that long-term antigen stimulation may increase TRAb concentrations and eventually lead to subclass switching from IgG1 to IgG4. M22, a stimulating monoclonal anti-TSH-R antibody (TRAb), is found to be IgG1 (20). Although IgG subclasses in TRAb were not investigated in our study, a positive correlation between IgG4 or the ratio of IgG4/IgG and TSAb was observed in overall patients with GD. Patient 2 showed strong TSAb activity in spite of negative TgAb or TPOAb, suggesting that TSAb is at least partly present in the IgG4 fraction. Patient 3, who rapidly changed to hypothyroidism and possessed persistent thyroid enlargement (Fig. 1), is quite similar to recent cases reported independently by Nishihara *et al.* and Kawashima *et al.* (21,22). Thus, both in stimulating and blocking TRAbs, IgG4-positive plasma cells may be involved in the pathogenesis of GD.

The exchange of IgG half-molecules among IgG4 results in bispecific characteristics (23). Notably, McLachlan *et al.* showed that in patients with GO and elevated TRAb, an IgG4 shift toward TgAb was observed (24). We speculate that IgG4 could have a bispecific nature and consist of TgAb and TRAb. Considering that the levels of TgAb and TPOAb, as well as TRAb, are elevated in GD (13), these autoantibodies may be related to bispecific IgG4 molecules. Another biological relevance of this exchange of half-molecules in IgG4 is that it generates antibodies that inhibit formation of large immune complexes and immune inflammation by IgGs of other subclasses (23). For example, Guo *et al.* reported that the IgG1 but not the IgG4 subfraction of TPOAb is associated with antibody-dependent cytotoxicity (25).

In the present study, ultrasound examination revealed that the elevated IgG4 group had significantly more hypoechoic areas than the nonelevated IgG4 group (Table 1), as similarly observed in IgG4 thyroiditis (3). Moreover, low echo scoring was positively correlated with serum IgG4 levels. Since hypoechoic areas reflect lymphocyte infiltration and fibrosis in the thyroid gland (10), this area may be related to lymphoplasmacytes that produce IgG4 in the thyroid. In the elevated IgG4 group, disease was controlled with low doses of ATD or treatment with ATD and/or L-T4 one year following the first visit (Table 2). In cases of HT, the degree of hypoechoic areas correlated with the prevalence of hypothyroidism, resulting from fibrosis (26). Thus, the clinical course of patients with IgG4 elevation may be related to fibrosis on the basis of ultrasonographic findings, which resemble the alterations found in HT. In this context, the relationship between IgG4 and Hashitoxicosis (27,28), which has concurrent features of GD and HT, should be studied in the future.

There are several limitations of our study. First, there was a lack of pathologic studies performed in the patients. Second, the patients showed an absence of manifestations of other organs in IgG4-RD. Third, the number of patients with elevated IgG4 is rather small. For this reason, there is some concern for either a type I error (false positive) or a type II error (false negative). Finally, information on alterations in serum IgG4 levels during treatment and clinical course was not collected. As a future study, longitudinal follow-up studies to examine pathophysiological relations and therapeutic outcomes are warranted.

In conclusion, the current study proposes a novel subgroup of patients with GD who show elevated serum IgG4 levels. In comparison to the majority of patients with GD, these patients were older, had more hypoechoic areas, and may be responsive to ATD or prone to be hypothyroid after ATD treatment. IgG4-RD generally shows positive response to steroid therapy. In patients with GD and elevated serum IgG4 levels, steroid therapy should be considered to avoid adverse effects of ATD, radioiodine treatment, or surgical intervention. Thus, measurement of serum IgG4 levels may help to distinguish this new entity and may offer diagnostic and potential therapeutic options for GD. Further investigations on the relationship between GD and IgG4-RD using a larger patient population and longer observation times are warranted.

Acknowledgments

We are thankful to Takamasa Minaga, Yuko Matsumoto, Kana Hayakawa, Tomomi Funahashi, Shinsuke Uraki, Syogo Ueda, Seigo Kurisu, Takahiro Hayakawa, Kaori Miyata, Takeshi Shimada, Tatsuya Ishibashi, Tomoyuki Takagi, Takayuki Nakagawa, and the member of The First Department of Medicine for collecting and analyzing data. This

work was supported by the Research Program of Intractable Diseases provided by the Ministry of Health, Labor, and Welfare of Japan.

Author Disclosure Statement

No competing financial interests exist.

References

1. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaido T, Nakayama K, Usuda N, Kiyosawa K 2001 High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med **344:**732–738.
2. Stone JH, Zen Y, Deshpande V 2012 IgG4-related disease. N Engl J Med **366:**539–551.
3. Li Y, Nishihara E, Hirokawa M, Taniguchi E, Miyauchi A, Kakudo K 2010 Distinct clinical, serological, and sonographic characteristics of Hashimoto's thyroiditis based with and without IgG4-positive plasma cells. J Clin Endocrinol Metab **95:**1309–1317.
4. Dahlgren M, Khosroshahi A, Nielsen GP, Deshpande V, Stone JH 2010 Riedel's thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum. Arthritis Care Res (Hoboken) **62:**1312–1318.
5. Hennessey JV 2011 Clinical review: Riedel's thyroiditis: a clinical review. J Clin Endocrinol Metab **96:**3031–3041.
6. Watanabe T, Maruyama M, Ito T, Fujinaga Y, Ozaki Y, Maruyama M, Kodama R, Muraki T, Hamano H, Arakura N, Kadoya M, Suzuki S, Komatsu M, Shimojo H, Notohara K, Uchida M, Kawa S 2013 Clinical features of a new disease concept, IgG4-related thyroiditis. Scand J Rheumatol **42:**325–330.
7. Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Yoshino T, Nakamura S, Kawa S, Hamano H, Kamisawa T, Shimosegawa T, Shimatsu A, Nakamura S, Ito T, Notohara K, Sumida T, Tanaka Y, Mimori T, Chiba T, Mishima M, Hibi T, Tsubouchi H, Inui K, Ohara H 2012 Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. Mod Rheumatol **22:**21–30.
8. Masaki Y, Kurose N, Yamamoto M, Takahashi H, Saeki T, Azumi A, Nakada S, Matsui S, Origuchi T, Nishiyama S, Yamada K, Kawano M, Hirabayashi A, Fujikawa K, Sugiura T, Horikoshi M, Umeda N, Minato H, Nakamura T, Iwao H, Nakajima A, Miki M, Sakai T, Sawaki T, Kawanami T, Fujita Y, Tanaka M, Fukushima T, Eguchi K, Sugai S, Umehara H 2012 Cutoff values of serum IgG4 and histopathological IgG4+ plasma cells for diagnosis of patients with IgG4-related disease. Int J Rheumatol **2012:** 580814.
9. Mourits MP, Prummel MF, Wiersinga WM, Koornneef L 1997 Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf) **47:**9–14.
10. Werner SC 1977 Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. J Clin Endocrinol Metab **44:**203–204.
11. Yoshida A, Adachi T, Noguchi T, Urabe K, Onoyama S, Okamura Y, Shigemasa C, Abe K, Mashiba H 1985 Echographic findings and histological feature of the thyroid: a reverse relationship between the level of echo-amplitude and lymphocytic infiltration. Endocrinol Jpn **32:**681–690.
12. Yamamoto M, Nishimoto N, Tabeya T, Naishiro Y, Ishigami K, Shimizu Y, Yajima H, Matsui M, Suzuki C, Takahashi H, Imai K, Shinomura Y 2012 [Usefulness of measuring serum IgG4 level as diagnostic and treatment marker in IgG4-related disease]. Nihon Rinsho Meneki Gakkai Kaishi **35:**30–37.
13. DeGroot LJ, Quintans J 1989 The causes of autoimmune thyroid disease. Endocr Rev **10:**537–562.
14. Schauer U, Stemberg F, Rieger CH, Borte M, Schubert S, Riedel F, Herz U, Renz H, Wick M, Carr-Smith HD, Bradwell AR, Herzog W 2003 IgG subclass concentrations in certified reference material 470 and reference values for children and adults determined with the binding site reagents. Clin Chem **49:**1924–1929.
15. Parkes AB, McLachlan SM, Bird P, Rees Smith B 1984 The distribution of microsomal and thyroglobulin antibody activity among the IgG subclasses. Clin Exp Immunol **57:**239–243.
16. Davies TF, Weber CM, Wallack P, Platzer M 1986 Restricted heterogeneity and T cell dependence of human thyroid autoantibody immunoglobulin G subclasses. J Clin Endocrinol Metab **62:**945–949.
17. Caturegli P, Kuppers RC, Mariotti S, Burek CL, Pinchera A, Ladenson PW, Rose NR 1994 IgG subclass distribution of thyroglobulin antibodies in patients with thyroid disease. Clin Exp Immunol **98:**464–469.
18. Weetman AP, Fung HY, Richards CJ, McGregor AM 1990 IgG subclass distribution and relative functional affinity of thyroid microsomal antibodies in postpartum thyroiditis. Eur J Clin Invest **20:**133–136.
19. Latrofa F, Chazenbalk GD, Pichurin P, Chen CR, McLachlan SM, Rapoport B 2004 Affinity-enrichment of thyrotropin receptor autoantibodies from Graves' patients and normal individuals provides insight into their properties and possible origin from natural antibodies. J Clin Endocrinol Metab **89:**4734–4745.
20. Sanders J, Evans M, Premawardhana LD, Depraetere H, Jeffreys J, Richards T, Furmaniak J, Rees Smith B 2003 Human monoclonal thyroid stimulating autoantibody. Lancet **362:**126–128.
21. Nishihara E, Hirokawa M, Takamura Y, Ito M, Nakamura H, Amino N, Miyauchi A 2013 IgG4 thyroiditis in a Graves' disease patient with large goiter developing hypothyroidism. Thyroid **23:**1496–1497.
22. Kawashima ST, Tagami T, Nakao K, Nanba K, Tamanaha T, Usui T, Naruse M, Minamiguchi S, Mori Y, Tsuji J, Tanaka I, Shimatsu A 2013 Serum levels of IgG and IgG4 in Hashimoto thyroiditis. Endocrine [Epub ahead of print].
23. Aalberse RC, Schuurman J 2002 IgG4 breaking the rules. Immunology **105:**9–19.
24. McLachlan SM, Bahn R, Rapoport B 1992 Endocrine ophthalmopathy: a re-evaluation of the association with thyroid autoantibodies. Autoimmunity **14:**143–148.
25. Guo J, Jaume JC, Rapoport B, McLachlan SM 1997 Recombinant thyroid peroxidase-specific Fab converted to immunoglobulin G (IgG) molecules: evidence for thyroid cell damage by IgG1, but not IgG4, autoantibodies. J Clin Endocrinol Metab **82:**925–931.
26. Marcocci C, Vitti P, Cetani F, Catalano F, Concetti R, Pinchera A 1991 Thyroid ultrasonography helps to identify

patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. J Clin Endocrinol Metab **72:**209–213.
27. Fatourechi V, McConahey WM, Woolner LB 1971 Hyperthyroidism associated with histologic Hashimoto's thyroiditis. Mayo Clin Proc **46:**682–689.
28. Fonseca V, Thomas M, Havard CW 1988 Hashitoxicosis and autoantibody interference with thyroid function tests. J R Soc Med **81:**546–547.

Address correspondence to:
Takashi Akamizu, MD, PhD
The First Department of Medicine
Wakayama Medical University
811-1 Kimi-idera
Wakayama 641-8509
Japan

E-mail: akamizu@wakayama-med.ac.jp

Human Pathology (2012) 43, 536–549

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Characteristic tubulointerstitial nephritis in IgG4-related disease☆,☆☆

Yutaka Yamaguchi MD[a], Yukiko Kanetsuna MD[b], Kazuho Honda MD[c], Nobuaki Yamanaka MD[d], Mitsuhiro Kawano MD[e], Michio Nagata MD[f,*] on behalf of the Japanese study group on IgG4-related nephropathy

[a]*Yamaguchi's Pathology Laboratory, Matsudo, Chiba, 270-2231 Japan*
[b]*Department of Pathology, The Jikei University Kashiwa Hospital, Kashiwa, Chiba, 277-8567 Japan*
[c]*Department of Pathology, Tokyo Women's Medical University, Tokyo, 162-8666 Japan*
[d]*Tokyo Kidney Research Institute, Tokyo, 113-8602 Japan*
[e]*Division of Rheumatology, Department of Internal Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, 920-8641 Japan*
[f]*Department of Kidney and Vascular Pathology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, 305-8575 Japan*

Received 31 March 2011; revised 28 May 2011; accepted 1 June 2011

Keywords:
IgG4-related disease;
Tubulointerstitial nephritis;
Tubulointerstitial deposition;
Interstitial fibrosis/sclerosis

Summary Nephropathy associated with IgG4-related disease is characterized by tubulointerstitial nephritis. To better identify its pathology, the present study analyzed clinicopathologic features of IgG4-related tubulointerstitial nephritis cases from across Japan. Sixteen cases were identified as IgG4-related nephropathy using the criterion of high serum IgG4 levels (>135 mg/dL) with abnormal kidney computed tomography or elevated serum creatinine levels. Male predominance (75%) and advanced age (average, 62.0 years) were noted. Eight cases displayed no autoimmune pancreatitis. Renal computed tomography abnormalities were found in 12 of 13 cases examined. Renal dysfunction was found in 15 of 16 cases at biopsy. Distinctive features of tubulointerstitial lesions included (1) well-demarcated borders between involved and uninvolved areas; (2) involvement of the cortex and medulla, often extending beyond the renal capsule and with occasional extension to retroperitoneal fibrosis; (3) interstitial inflammatory cells comprising predominantly plasma cells and lymphocytes, with a high prevalence of IgG4-positive cells often admixed with fibrosis; (4) peculiar features of interstitial fibrosis resembling a "bird's-eye" pattern comprising fibrosis among inter–plasma cell spaces; and (5) deposits visible by light and immunofluorescent microscopy in the tubular basement membrane, Bowman capsule, and interstitium that are restricted to the involved portion, sparing normal parts. Ultrastructural analysis revealed the presence of myofibroblasts with intracellular/pericellular collagen accompanied by plasma cell accumulation from an early stage. Histology could not discriminate between IgG4-related tubulointerstitial nephritis with and without autoimmune pancreatitis. In conclusion, the distinctive histologic features of IgG4-related tubulointerstitial nephritis can facilitate the differential diagnosis of tubulointerstitial nephritis, even without autoimmune pancreatitis or an abnormal computed tomography suggesting a renal tumor.

☆ Conflict of interest: none.
☆☆ Disclosures: none.
* Corresponding author.
E-mail address: nagatam@md.tsukuba.ac.jp (M. Nagata).

0046-8177/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2011.06.002

1. Introduction

IgG4-related disease is a systemic disease associated with high serum IgG4 levels that was first identified in Japan and is now emerging worldwide [1-4]. IgG4-related disease encompasses various uncommon clinical manifestations, including autoimmune pancreatitis (AIP), Mikulicz disease, and Küttner disease, despite a relatively common background pathology: interstitial fibrosis/sclerosis and lymphoplasmacytic infiltration [4-9]. IgG4-related disease is associated with high IgG4 serum levels in approximately 70% to 80% of cases. The kidney is an organ particularly affected by IgG4-related disease, and chronic renal failure occasionally develops [10-14]. In practice, IgG4-related nephropathy is occasionally found by abnormal computed tomography (CT), revealing a tumor that is sometimes treated by performing an unnecessary nephrectomy [15].

Among several forms of IgG-related disease in the kidney (IgG4-related nephropathy), previous reports have documented that tubulointerstitial nephritis (TIN) is common and may determine a prognosis [10-12,16-22]. IgG4-related TIN is often accompanied by AIP; however, several cases without AIP have also been reported [6,14,16,18].

Generally, the histology of TIN is fairly nonspecific; thus, the diagnosis of IgG4-related TIN, particularly in cases without AIP, is problematic. Because steroid therapy has been found to be effective in AIP [1,4,23,24], a proper diagnosis of IgG4-related TIN is very important so that steps can be taken to halt disease progression.

Several case reports have described the histologic features of IgG4-related TIN. Most were single or a few cases associated with AIP and were subjected to renal biopsy, revealing various features ranging from mild interstitial inflammation to diffuse tubulointerstitial fibrosis [10-14,17-22]. Some reports have demonstrated IgG4 immunostaining, but most did not refer to the relationship between the presence of IgG4-positive cells and disease progression because of the small number of cases and the inconsistent severity of the interstitial injury. In particular, whether the different pathologic profiles noted in previous reports are based on different stages of the same disease or are histologic variants has not been determined. Recently, Saeki et al [25] documented clinicopathologic characteristics of IgG4-related TIN. Although its pathology was briefly summarized in their publication, detailed morphological considerations, including histologic staging and features of progression, were not well discussed.

The present study analyzed 16 cases of IgG4-related TIN from across Japan (including 4 cases [cases 5, 8, 11,and 13] that appeared in the report of Saeki et al) to better define the clinical and pathologic characteristics of this condition, facilitate its diagnosis, and discuss its pathogenesis.

2. Subjects and methods

2.1. Subjects

The study group collected 16 cases from across Japan. The diagnostic criterion for IgG4-related nephropathy was the presence of either AIP with renal dysfunction or elevated serum IgG4 levels (>135 mg/dL) without AIP. The diagnosis of AIP was made according to Asian criteria, as previously reported [26]. Patients with clinically suspected Castleman disease or with high titers of anti–double-stranded DNA, anti-Sm, or anti-Sjogren syndrome A were not included. Renal dysfunction was defined by more than 1.1 mg/dL of serum creatinine. Among the 16 cases, 4 (cases 5, 8, 11, 13) had appeared in the recent report by Saeki et al [25]; the remaining 12 cases were newly enrolled and had not been published. The study was approved by the ethical committees of Kanazawa University.

2.2. Histopathology

Renal biopsy samples were processed by standard techniques for light microscopy (LM), immunostaining, and electron microscopy (EM). Immunostaining was performed either with immunofluorescence (IF, 5 cases with frozen sections) or with peroxidase-antiperoxidase methods (11 cases with paraffin sections) using polyclonal antibodies against IgG, IgA, IgM, C3, C1q, and κ and λ light chains (Dako, Kyoto, Japan). EM was performed in 10 cases, using standard techniques.

2.3. Immunohistochemistry for IgG subclass staining

IgG subclass staining was performed in all cases by a standard method using an avidin-biotin immunoperoxidase technique with antigen retrieval by microwave. The primary antibodies were mouse monoclonals against each IgG subclass (IgG1, IgG2, IgG3, and IgG4) (Zymed Laboratories Inc, San Francisco, CA), applied at a 1:100 dilution.

2.4. Statistics

All data are presented as absolute numbers or means ± SE. Statistical differences between groups were evaluated using Student unpaired t test. $P < .05$ was deemed to indicate statistical significance.

3. Results

3.1. Clinical features

All cases consisted of Japanese patients, most of whom were men (75%) and older than 50 years (average, 62.0 years; Table 1). Renal dysfunction was found in 15 cases (94%), and

Table 1 Clinical data

Case	Age	Sex	Kidney CT	IgG (mg/dL), normal value (800-1600 mg/dL)	IgG4 (mg/dL), normal value (48-105 mg/dL)	Low C3	SCr (mg/dL)	Proteinuria (g/d)	Salivary gland swelling	Others
With AIP										
1	45	M	Attenuation	2947	2120	+	1.9	0.15	+	DM
2	59	M	Attenuation	3570	602	+	1.2	0.4	+	
3	60	F	Swelling	1706	142	NE	1.05	14 (NS)	−	Lung tumor
4	67	M	NE	6328	781	NE	3.56	1.1	−	M-protein
5	69	M	NE	4110	1340	+	2.2	0.55	−	
6	72	M	Nodular	3019	936	NE	0.84	−	−	
7	75	M	Swelling	1569	300	+	1.1	−	−	
8	78	M	Attenuation	3935	185	−	6.17	1.38	−	HD
				3398 ± 1510	801 ± 671		2.3 ± 1.8	0.72 ± 0.5		
Without AIP										
9	49	F	Swelling	3143	1520	−	2.7	0.93	+	
10	54	F	NE	2864	905	−	1.38	0.21	−	
11	54	M	Attenuation	5040	1780	−	1.8	0.49	+	
12	56	M	Swelling	3639	2110	−	2.2	−	−	
13	58	F	Nodular	2850	1470	−	1.15	−	−	Liver tumor
14	64	M	Normal	4292	260	+	1.4	0.67	−	
15	64	M	Nodular	5510	980	NE	1.3	−	−	
16	68	M	Nodular	4027	1510	+	2.29	0.65	+	Hydronephrosis
				3920 ± 991	1311 ± 579		1.8 ± 0.6	0.65 ± 0.6		

Abbreviations: NE, not examined; SCr, serum creatinine; DM, diabetes mellitus; HD, hemodyalisis; NS, nephrotic syndrome.
NOTE. IgG4 levels in 3 cases among AIP group were not examined at biopsy because of poststeroid therapy for AIP.

proteinuria (>400 mg/d) was found in 10, including 1 case with nephrotic syndrome, as identified by the presence of membranous glomerulonephritis. Abnormal kidney CT, including swelling and a nodular or an irregular pattern, was found in 12 (92%) of 13 cases examined (Fig. 1).

Extrarenal comorbidities were composed of AIP (8 cases), diabetes mellitus (2 cases), and hydronephrosis caused by peritoneal fibrosis (1 case). Salivary gland abnormalities were found in 7 cases, with swelling (4 cases) or accumulation on gallium scintigraphy without noticeable swelling (1 case). CT-detected pseudotumors were found in 2 cases, in the lung and the liver, respectively. The liver biopsy showed accumulation of IgG4-positive plasma cells in the tumor.

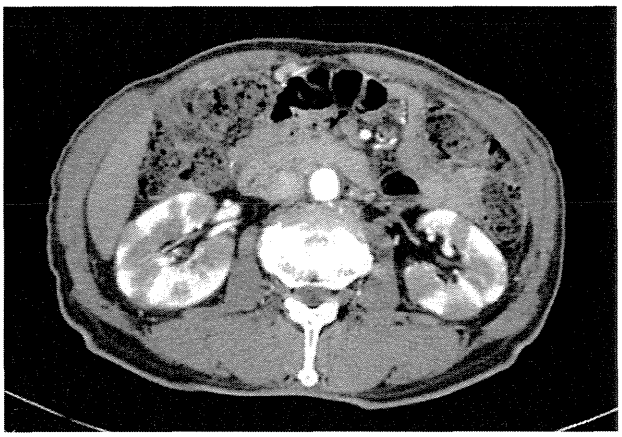

Fig. 1 Representative enhanced CT scan in case 6 reveals multiple nodular lesions with frequent effacement in the bilateral kidneys. Note that the enhanced area includes both the cortex and medulla. Retroperitoneal fibrosis is also present.

Serum IgG and IgG4 levels were measured in all cases, revealing high serum IgG levels (mean, 3659.3 mg/dL) and high serum IgG4 levels (mean, 1162.9 mg/dL). A serum hypo-C3 level was found in 6 of 12 cases examined. When we stratified the cases into 2 groups, with and without AIP, no statistical difference in IgG, IgG4, serum creatinine level, or proteinuria between the 2 groups was noted, as shown in Table 1.

3.2. Pathologic findings

Morphological findings are summarized in Table 2 (LM, 16 cases), Table 3 (EM, 10 cases), and Table 4 (immunohistochemistry, 16 cases).

3.2.1. Tubulointerstitium

The most distinctive and common histology observed in all samples by LM was interstitial inflammatory cell infiltration and fibrosis (Fig. 2A), with the 2 often admixed as various features. The margins of such interstitial lesions were relatively well defined (Fig. 2B). Although generally unusual for TIN, interstitial fibrosis in this disease involved the deep renal medulla (Fig. 2C). In addition, fibrosis extended to the capsule and perirenal areas in 6 cases, occasionally spreading into and effacing the retroperitoneal fibrosis (Fig. 2D). In the areas of interstitial fibrosis,

Table 2 Light microscopic findings

Case	Lymphoplasmacytic infiltration	Eosinophilia	Interstitial fibrosis	Stages	Bird's eye	Deposition		Other infiltration	
						Tubular wall	Bowman capsule	Perirenal	Medulla
With AIP									
1	++	++	+++	B + C	+++	−	−	−	−
2	+++	++	++	A + B + C	++	+	−	−	−
3	+++	++	+++	A + B + C	+++	+	−	−	−
4	+++	++	+++	B + C	+++	−	−	−	+
5	++	+	+++	C + D	++	+	−	−	−
6	++	−	++	A + B + C	++	−	−	+	−
7	+++	++	+	A + B	+	−	−	−	−
8	++	−	+++	C + D	++	+	+	−	+
Without AIP									
9	+++	++	+++	C	+++	++	+	−	−
10	+++	++	+++	A + C	+++	++	++	+	−
11	++	+	+++	C + D	++	++	+	−	−
12	++	−	+++	C + D	+++	−	−	−	+
13	+++	+	++	B + C	++	+	−	+	−
14	+++	++	+++	B + C	+++	+	+	−	−
15	+	+	+	A + B + C	+	−	−	+	−
16	+++	++	+++	B + C	++	+	−	++	−

Table 3 Electron microscopic findings

Case	Pericellular fibrosis			Pericellular fibrosis				
	Epi	Im	Mes	Glomerulus	Interstitium	TBM	Bowman's capsule	Vascular
With AIP								
1	+	−	−	−	+	++	−	−
2	++	−	−	−	+	++	−	−
3	+	++	−	−	−	++	++	+
4	++	−	−	−	++	+++	−	−
5	++	−	−	−	++	++	−	+
8	++	+	−	−	−	+	++	−
Without AIP								
9	++	−	−	−	+	+	−	−
10	−	−	−	−	−	++	++	+
12	+	−	−	−	+	+	−	−
14	++	++	++	++	+	++	+	+

Abbreviations: Epi, subepithelium; Im, intramembrane; Mes, mesangium; TBM, tubular basement membrane.

Table 4 Immunofluorescence findings

Case	IgG	IgG1	IgG2	IgG3	IgG4	IgA	IgM	κ/λ	C3	C4	C1q
1	G, B, T, I	G, B, T, I	None	None	G, B, T, I	None	None	G, B, T, I	None	None	None
9	T, I	T, I	None	T, I	B, T, I	None	None	T, I	B, T, I	None	None
10	G, B, T, I	G, B, T, I	G, B, T, I	G, B, T, I	G, B, T, I	None	None	G, B, T, I	B, T	B, T	B, T
14	B, T, I	B, T, I	None	B, T, I	B, T, I	T, I	None	G, B, T, I	T, I	None	T, I
15	T, I	T, I	None	None	T, I	None	None	T, I	None	None	None

Abbreviations: G, glomerular basement membrane; B, Bowman capsule; T, TBM; I, interstitium.

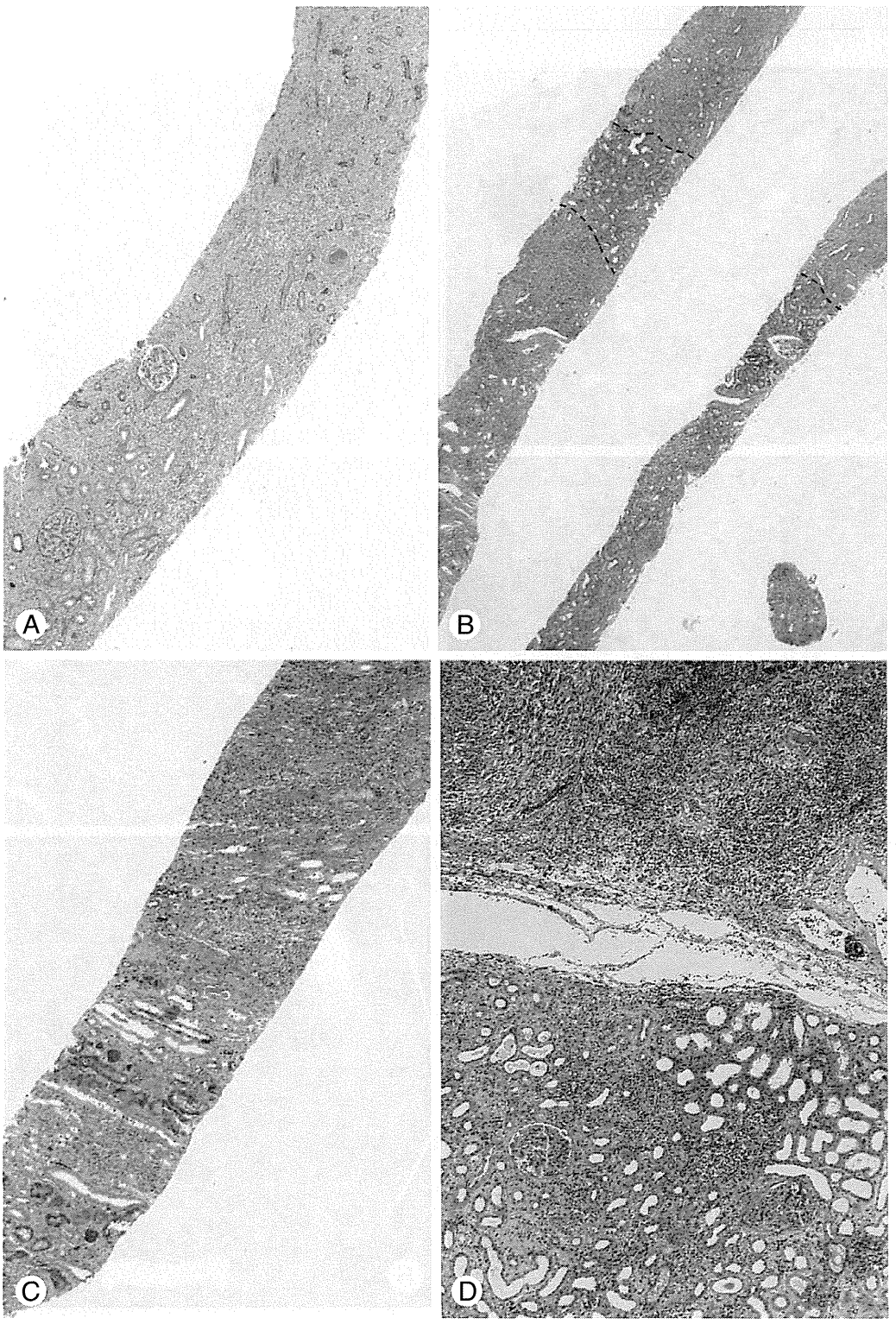

Fig. 2 Representative light microscopic findings of renal biopsy. (A), Marked tubular attenuation with diffuse interstitial infiltration and fibrosis (PAS staining). This is the most common histologic feature noted in our series. (B), Focal tubulointerstitial involvement in the cortex and medulla showing clear demarcation (dashed line) between involved and uninvolved areas (PAS). (C), Extension of fibrosis into the deep medulla. Note tubular attenuation and focal inflammatory cell accumulation on a background of connective tissue deposition (Massons trichrome stain). (D), Subcapsular and perirenal involvement (upper) in a case with retroperitoneal fibrosis. Occasional lymph follicular features in the extrarenal fibrosis are associated (PAS). Original magnification: A, ×20; B, ×10; C, ×20; D, ×20.