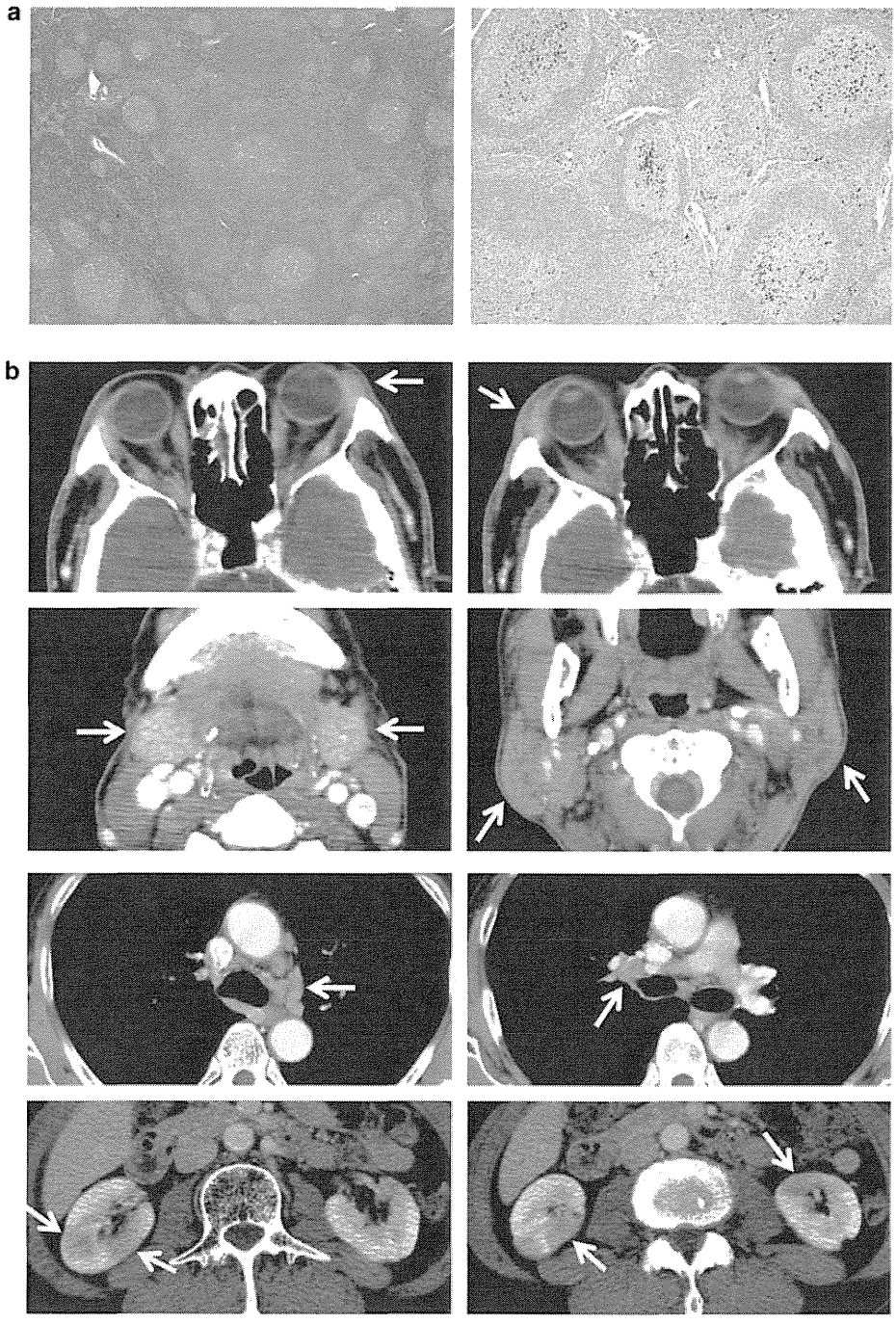

Figure 3 Histological features and radiological images of a patient with IgG4$^+$ progressively transformed germinal centers with progression to systemic disease. (**a**) The affected lymph node from patient no. 11 (initial lymphadenopathy of the submandibular node had been detected 10 years before) 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)

MODERN PATHOLOGY (2012), 1–10

Clinicopathologic analysis of IgG4-related skin disease

Yasuharu Sato[1,9], Mai Takeuchi[1,9], Katsuyoshi Takata[1], Kyotaro Ohno[1], Noriko Iwaki[1], Yorihisa Orita[2], Naoe Goto[3], Akira I Hida[4], Toshiyuki Iwamoto[5], Naoko Asano[6], Toshihiro Ito[7], Hiroyuki Hanakawa[2], Hiroyuki Yanai[8] and Tadashi Yoshino[1]

[1]*Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan;* [2]*Department of Otolaryngology, Head and Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan;* [3]*Department of Immunopathology, Gifu University Graduate School of Medicine, Gifu, Japan;* [4]*Department of Pathology, Matsuyama Red Cross Hospital, Matsuyama, Japan;* [5]*Department of Pathology, Chuden Hospital, Hiroshima, Japan;* [6]*Department of Clinical Laboratory, Nagoya University Hospital, Nagoya, Japan;* [7]*Department of Pathology and Experimental Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan and* [8]*Department of Diagnostic Pathology, Okayama University Hospital, Okayama, Japan*

IgG4-related disease is a recently recognized systemic syndrome characterized by mass-forming lesions with lymphoplasmacytic infiltration, increase in the number of IgG4$^+$ cells in affected tissues and elevation of serum IgG4 levels. In 2009, we were the first to report skin lesions in patients with IgG4-related disease, but no large case series has been reported and clinicopathological findings remain unclear. To clarify these features, we herein report 10 patients (9 men and 1 woman; median age, 64 years; age range, 46–81 years) with IgG4-related skin disease. All patients had erythematous and itchy plaques or subcutaneous nodules on the skin of the head and neck, particularly in the periauricular, cheek, and mandible regions, except for one patient, whose forearm and waist skin were affected. In addition, eight patients had extracutaneous lesions: these were found on the lymph nodes in six patients, the lacrimal glands in three patients, the parotid glands in three patients, and the kidney in one patient. Histologically examined extracutaneous lesions were consistent with IgG4-related disease; five of six lymph node lesions showed progressively transformed germinal centers-type IgG4-related lymphadenopathy. Cases of IgG4-related skin disease were classified into two histological patterns: those exhibiting a nodular dermatitis pattern and those with a subcutaneous nodule pattern. The infiltrate was rich in plasma cells, small lymphocytes, and eosinophils; the majority of the plasma cells were IgG4$^+$. The IgG4$^+$ cell count was 49–396 per high-power field (mean ± s.d., 172 ± 129), with an IgG4$^+$/IgG$^+$ cell ratio ranging from 62 to 92%. Serum IgG4 levels were elevated in all examined patients. In conclusion, patients with IgG4-related skin disease had uniform clinicopathology. Lesions were frequently present on the skin of the periauricular, cheek, and mandible regions, and were frequently accompanied by IgG4-related lymphadenopathy.
Modern Pathology advance online publication, 23 November 2012; doi:10.1038/modpathol.2012.196

Keywords: IgG4-related disease; lymphadenopathy; periauricular region; progressively transformed germinal centers; skin

IgG4-related disease is a recently recognized syndrome characterized by mass-forming lesions with lymphoplasmacytic infiltration, an increased number of IgG4$^+$ cells in the affected tissues, and elevated serum IgG4 levels. It is usually found in middle-aged and older patients, with men predominantly affected, and normally has a favorable clinical response to steroid therapy.[1–4]

IgG4-related disease can affect multiple organs, including the pancreas, hepatobiliary tract, lacrimal glands, salivary glands, lungs, kidneys, retroperitoneum, prostate, aorta, and lymph nodes. In most patients with this disease, two or more sites in various combinations are involved.[2–4]

Correspondence: Dr Y Sato, 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
[9]These two authors contributed equally to this work.
Received 9 August 2012; revised 1 October 2012; accepted 1 October 2012; published online 23 November 2012

Table 1 Clinical features of patients with IgG4-related skin disease

Patient	*Age/sex*	*Presentation*	*Laboratory findings*	*Treatment*
1	77/M	Erythematous, itchy, and ill-defined plaque on the skin of the preauricular, cheek, temporal, and buttock regions for 4 years	IgG4 261 mg/dl, IgG 1099 mg/dl, serum IgG4/IgG ratio 18%, IgA 408 mg/dl, IgM 60 mg/dl, IgE 22907 IU/ml, CRP 0.31 mg/dl, Eo 18%	Lesion persisted despite topical steroid therapy. Patient responded well to oral steroid therapy; however, the skin lesions relapsed when steroid administration was tapered
2	70/M	Erythematous, itchy, and ill-defined plaque on the skin of the left periauricular and mandible regions, accompanied by left submandibular lymphadenopathy. History of left parotid gland swelling and left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) 3 years ago	IgG4 431 mg/dl, IgG 1253 mg/dl, serum IgG4/IgG ratio 34%, IgA 215 mg/dl, IgM 26 mg/dl, IgE 1182 IU/ml, CRP 0.19 mg/dl, Eo 4.1%	Patient responded well to oral steroid therapy, but left submandibular lymphadenopathy increased in size when steroid administration was tapered
3	65/M	Eythematous, itchy, and ill-defined plaque on the skin of the postauricular region. A history of right submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) 6 years ago, and progression to systemic IgG4-related lymphadenopathy 3 years later	IgG4 1120 mg/dl, IgG 2077 mg/dl, serum IgG4/IgG ratio 54%, IgA 80 mg/dl, IgM 56 mg/dl, IgE 910 IU/ml, CRP 0.47 mg/dl, Eo 8%	Skin lesions persisted despite topical steroid therapy, but the patient responded well to oral steroid therapy
4	58/M	Erythematous, itchy, and ill-defined plaque on the skin of the forearm and waist, accompanied by systemic IgG4-related lymphadenopathy. History of left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) 6 years ago, and bilateral lacrimal gland swelling (IgG4-related dacryadenitis) 1 year ago	IgG4 1700 mg/dl, IgG 4539 mg/dl, serum IgG4/IgG ratio 38%, IgA 72 mg/dl, IgM 29 mg/dl, IgE 977 IU/ml, CRP 0.08 mg/dl, Eo 14%	Patient responded well to oral steroid therapy; however, the skin lesions relapsed when steroid administration was tapered
5	63/F	Erythematous, itchy, and ill-defined plaque on the skin of the preauricular, cheek, and mandible region for 3 years, accompanied by left parotid gland swelling (IgG4-related parotitis)	IgG4 415 mg/dl, IgG 929 mg/dl, serum IgG4/IgG ratio 45%, IgA 181 mg/dl, IgM 49 mg/dl, IgE 1579 IU/ml, CRP 0.11 mg/dl	Patient responded well to oral steroid therapy; however, the skin lesions relapsed when steroid administration was tapered
6	77/M	Erythematous, itchy, and ill-defined plaque on the skin of the left preauricular and cheek region for 3 years. One year later, right submandibular lymphadenopathy occurred (PTGC-type IgG4-related lymphadenopathy). Three years from initial presentation, bilateral lacrimal gland swelling developed	IgE 875 IU/ml, Eo 11.6%	Follow-up
7	47/M	Erythematous, itchy and ill-defined plaque on the skin of the left mandible and left forearm. History of left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) 5 years ago, and bilateral lacrimal gland swelling 2 years ago	IgG4 216 mg/dl, IgG 1574 mg/dl, serum IgG4/IgG ratio 14%, IgA 302 mg/dl, IgM 78 mg/dl, IgE 1550 IU/ml, CRP 0.19 mg/dl, Eo 9%	Patient responded well to oral steroid therapy; however, the skin lesions relapsed when steroid administration was tapered
8	81/M	Subcutaneous nodule in the left postauricular region for 2 months, accompanied by IgG4-related kidney disease. History of IgG4-related mediastinal lymphadenopathy 9 months ago	IgG4 907 mg/dl, IgG 4654 mg/dl, serum IgG4/IgG ratio 20%, IgA 110 mg/dl, IgM 21 mg/dl, CRP 0.52 mg/dl, Eo 3%	NA
9	46/M	Subcutaneous nodules in the bilateral postauricular and posterior neck region for 2 years	IgG4 166 mg/dl, IgG 1187 mg/dl, serum IgG4/IgG ratio 14%, IgA 156 mg/dl, IgM 57 mg/dl, CRP 0.14 mg/dl, Eo 14%	Follow-up
10	63/M	Subcutaneous nodule in the right postauricular region for 3 months	NA	Follow-up: a mild decrease in skin lesion size

Abbreviations: NA, not available; PTGC, progressively transformed germinal centers.

In 2009, we first reported skin lesions in patients with IgG4-related disease.[5] However, no large case series has been reported so far, and the clinicopathological findings remain unclear.[6,7] Herein, we report the clinicopathological findings in 10 Japanese patients with IgG4-related skin disease.

Materials and methods

Case Selection

All patients with IgG4-related skin disease were diagnosed on the basis of their clinical and pathological features recorded by the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

The clinical records and pathology materials for all cases were reviewed, and cases of multicentric Castleman's disease, malignant lymphoma, and other lymphoproliferative disorders (including rheumatoid arthritis and other immune-mediate conditions) were histologically and clinically excluded from this study.

Histological Examination and Immunohistochemistry

Surgical biopsy 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 (H&E) and Elastica-van Gieson. Immunohistochemistry was performed on paraffin sections using an automated

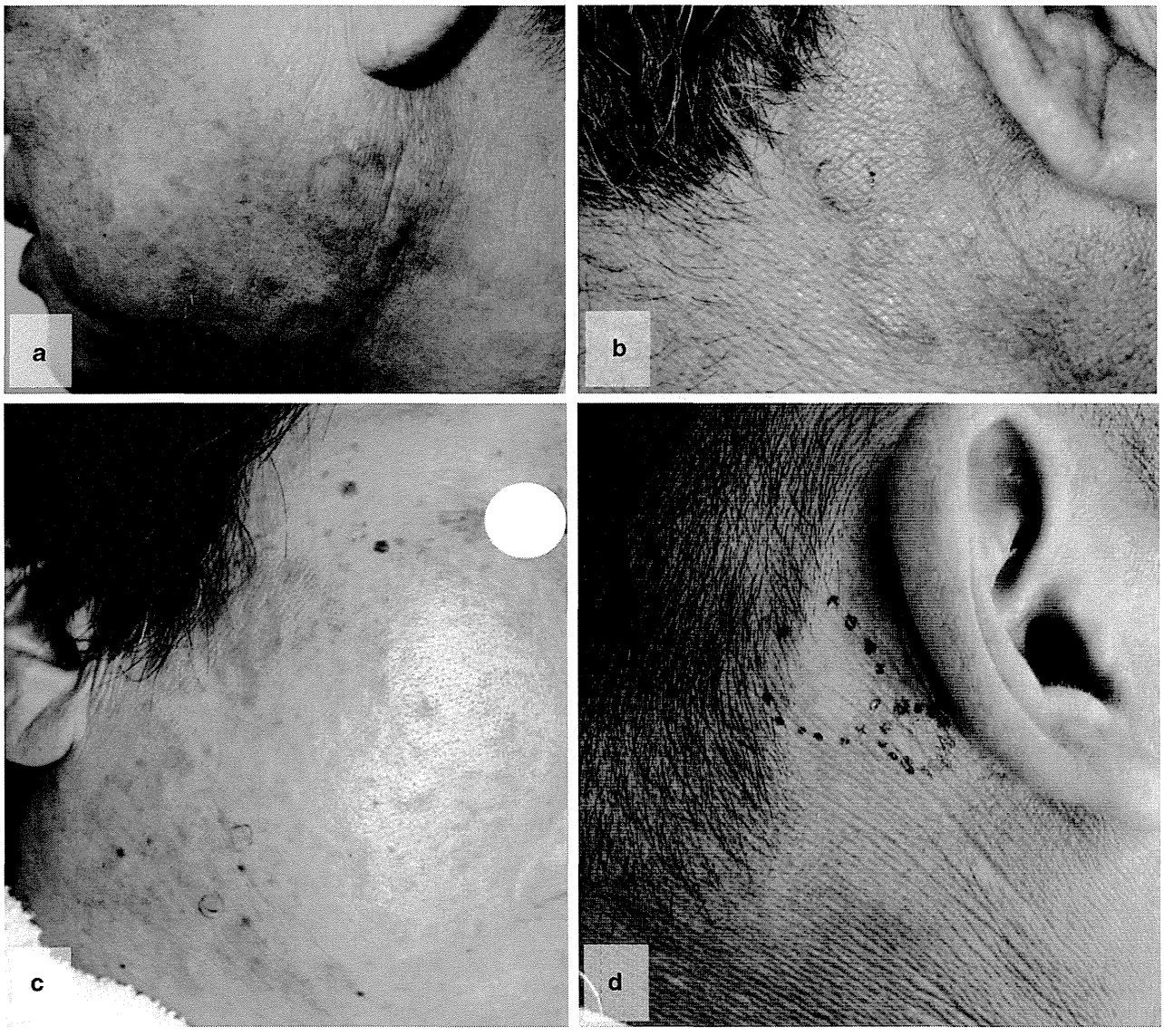

Figure 1 Macroscopic findings of IgG4-related skin disease. (**a**) Patient 2, (**b**) Patient 3, (**c**) Patient 5, and (**d**) Patient 9.

Table 2 Clinical and pathologic summary of IgG4-related skin disease

Patient	*Distribution of skin lesions*	*Extracutaneous lesions*	*Histological features*	*IgG4$^+$ cell count per HPF (IgG4$^+$/IgG$^+$ cell ratio)*	*Eosinophil count per HPF*
1	Preauricule, cheek, temporal, and buttock	None	Perivasucular and periadnexal lymphoid nodular infiltrates in the dermis	340 (85.1%)	40.3
2	Periauricule and mandible	Left parotid gland swelling and left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy)	Perivasucular and periadnexal lymphoid nodular infiltrates in the dermis	49 (79%)	1.3
3	Postauricule	Right submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) and progression to systemic IgG4-related lymphadenopathy	Perivasucular and periadnexal lymphoid nodular infiltrates in the dermis. Obliterative phlebitis (+)	174 (92.3%)	19
4	Forearm and waist	Left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy), progression to systemic IgG4-related lymphadenopathy, and IgG4-related dacryoadenitis (bilateral)	Perivasucular and periadnexal nodular lymphoid infiltrates in the dermis	78 (89%)	8.3
5	Preauricule, cheek, and mandible	IgG4-related parotitis	Perivasucular and periadnexal nodular lymphoid infiltrates with lymphoid follicle in the dermis and subcutis	118 (79.3%)	5
6	Preauricule and cheek	Right submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy) and bilateral lacrimal gland swelling	Perivasucular and periadnexal nodular lymphoid infiltrates with lymphoid follicle in the dermis and subcutis Fibrosis (+)	396 (84%)	49.3
7	Mandible and forearm	Left submandibular lymphadenopathy (PTGC-type IgG4-related lymphadenopathy), progression to systemic IgG4-related lymphadenopathy, and bilateral lacrimal gland swelling	Perivasucular and periadnexal nodular lymphoid infiltrates in the dermis and subcutis Fibrosis (+)	304 (82.4%)	289
8	Postauricule	Mediastinal IgG4-related lymphadenopathy and IgG4-related kidney disease	Subcutaneous lymphoid nodule with lymphoid follicle	96 (80.1%)	1.5
9	Postauricule and posterior neck	Left parotid gland nodule	Subcutaneous lymphoid nodule with lymphoid follicle. Fibrosis (+)	175 (80%)	20.6
10	Postauricule	NA	Subcutaneous lymphoid nodule with lymphoid follicle Fibrosis (+)	100 (61.9%)	175

Abbreviations: HPF, high-power field; NA, not available; PGTC, progressively transformed germinal centers.

Bond Max stainer (Leica Biosystems, Melbourne, Australia). The primary antibodies used were as follows: CD20 (L26 (1:400); Dako), CD3 (LN10 (1:200); Novocastra), CD10 (56C6 (1:100); Novocastra), bcl-2 (3.1 (1:400); Novocastra), 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^+ cells was estimated for areas with the highest density of positive cells. Three different high-power fields (HPFs; $\times 10$ eyepiece and $\times 40$ objective lenses) in each section were counted, and the average number of positive cells per HPF was calculated;[3] eosinophil numbers were also counted.

Unless specified otherwise, all data are represented as the mean ± s.d.

In Situ Hybridization

In situ hybridization of κ- and λ-chains was performed by an automated Bond Max stainer (Leica Biosystems).

Results

Clinical Features

The subjects included in this study were nine men and one woman, with a median age of 64 years (age range, 46–81 years) (Table 1). Patients had erythematous and itchy plaques or subcutaneous nodules on the skin of the face, head, and neck, particularly in the periauricular, cheek, and mandible regions, with the exception of one patient in whom the skin of the forearm and waist region were affected (Figure 1). In addition, 8 of 10 patients had extracutaneous lesions; these were found on the lymph nodes in 6 patients (60%), the lacrimal glands in 3 patients (30%), the parotid glands in 3 patients (30%), and the kidney in 1 patient (10%). Histologically examined extracutaneous lesions were consistent with IgG4-related disease. Interestingly, the histological results for five of six lymph node lesions demonstrated progressively transformed germinal centers-type IgG4-related lymphadenopathy, and three patients progressed to systemic IgG4-related lymphadenopathy (Tables 1 and 2).

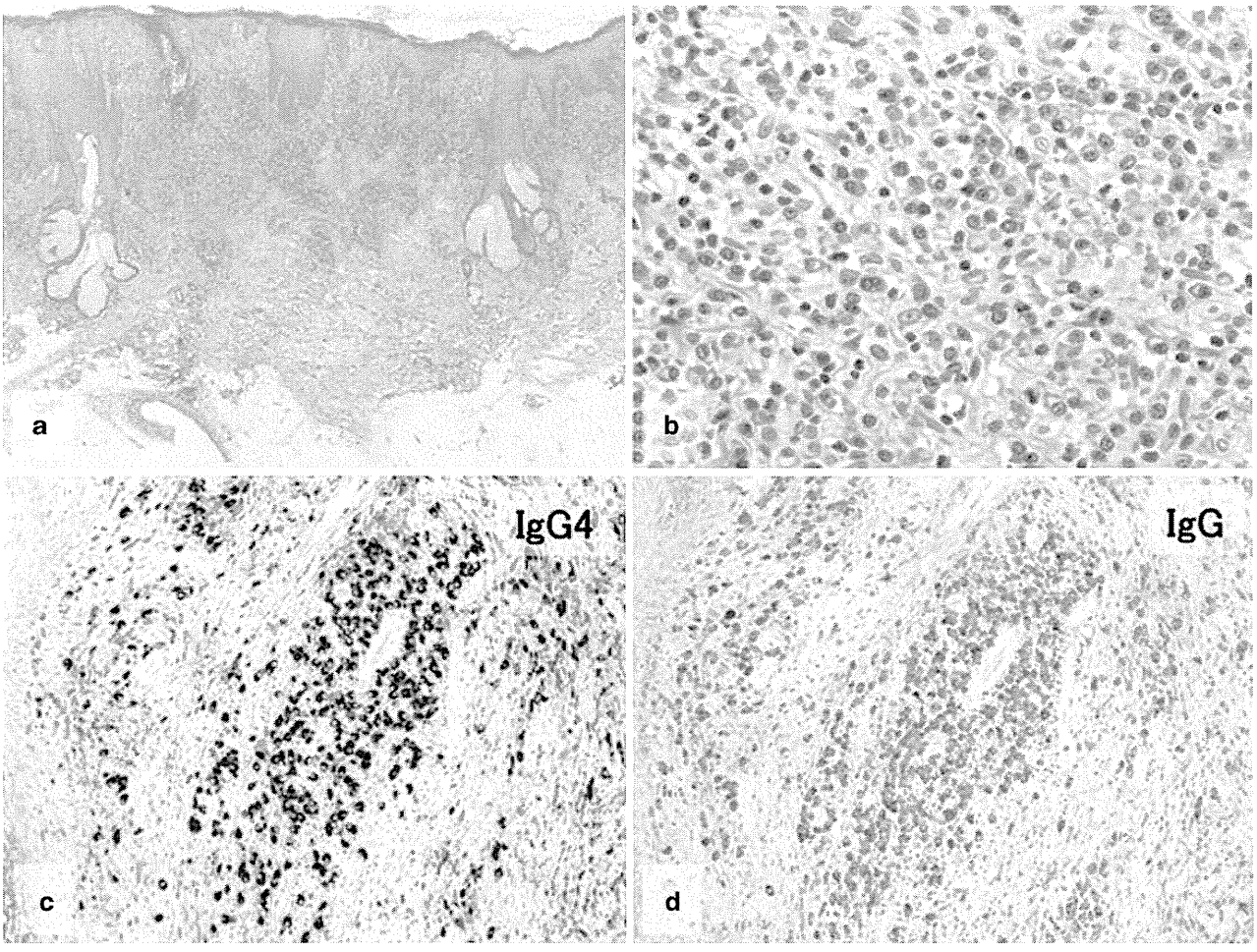

Figure 2 Patient 1: (**a**) This case showed a nodular dermatitis pattern (H&E). (**b**) Dermal involvement by perivascular and periadnexal lymphoplasmacytic nodular infiltration with eosinophils is observed (H&E). (**c**) IgG4 immunostaining, and (**d**) IgG immunostaining.

All eight patients in whom serum IgG4 levels were measured had elevated IgG4 levels (652 ± 543 mg/dl); the serum IgG4/IgG ratios ranged from 14 to 54% (29.5 ± 15.2). Serum IgE levels were elevated in seven of the seven patients in whom serum IgE levels were measured, and peripheral blood eosinophil counts were increased in six of eight patients in whom the eosinophil counts were measured.

Six patients were treated with systemic steroid therapy, which decreased the size of various lesions. However, the lesions increased in size when steroid administration was tapered in five patients.

Pathological Features

Cases of IgG4-related skin disease were classified into two histological patterns: those exhibiting a nodular dermatitis pattern and those with a subcutaneous nodule pattern (Table 2).

Patients 1–7 exhibited a nodular dermatitis pattern. Their skin lesions showed dermal involvement by perivascular and periadnexal lymphoplasmacytic nodular infiltration, with or without lymphoid follicles. Patients 5–7 demonstrated subcutaneous involvement by perivascular lymphoplasmacytic nodular infiltration. Patients 6 and 7 had fibrosis without a storiform pattern (Figures 2–4).

Patients 8–10 demonstrated a subcutaneous nodule pattern. Their skin lesions exhibited predominantly subcutaneous lymphoplasmacytic nodular infiltration with lymphoid follicles, and patients 9 and 10 had fibrosis without a storiform pattern (Figure 5).

Obliterative phlebitis was detected in only one patient (Patient 3, Figure 4).

The inflammatory cells were rich in mature and immature plasma cells and small lymphocytes, with varied numbers of eosinophils; the eosinophil counts were 1.3–289/HPF.

On immunohistological examination, the lymphoid infiltrates were a mixed population of $CD20^+$ B cells in nodular aggregates and $CD3^+$ T cells. The germinal centers were $CD10^+$ and Bcl-2^-. Light-chain restriction by *in situ* hybridization was not detected in any of the cases. Although the majority of the plasma cells were $IgG4^+$, the number of $IgG4^+$ cells varied. The $IgG4^+$ cell count was 49–396 cells/HPF (183 ± 121), with an $IgG4^+/IgG^+$ cell ratio of 62–92%.

Seven of eight patients with extracutaneous lesions were histologically examined, and the find-

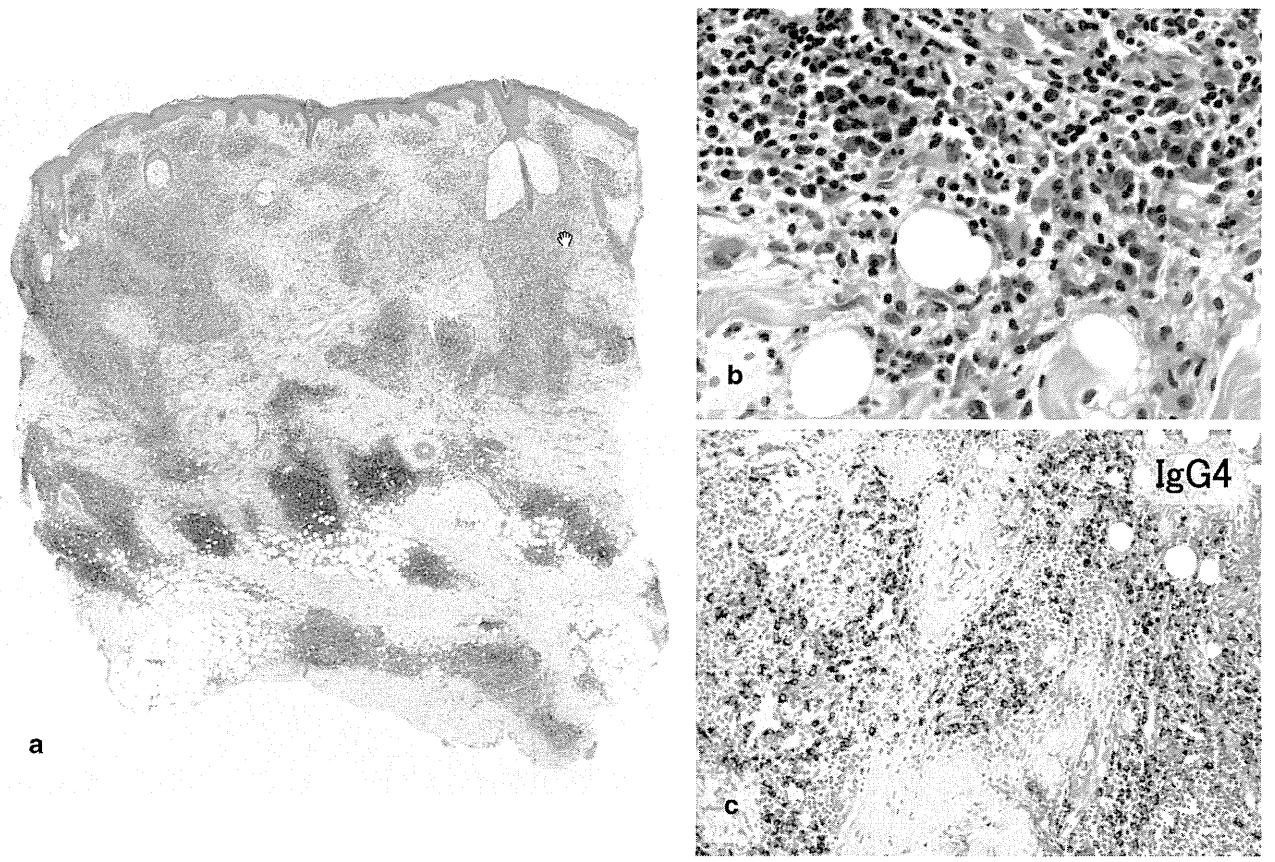

Figure 3 Patient 6: (**a**, **b**) Perivascular and periadnexal nodular lymphoplasmacytic infiltrates with eosinophils in the dermis and subcutis is observed. Fibrosis is seen in the subcutis (H&E). (**c**) Many plasma cells are $IgG4^+$.

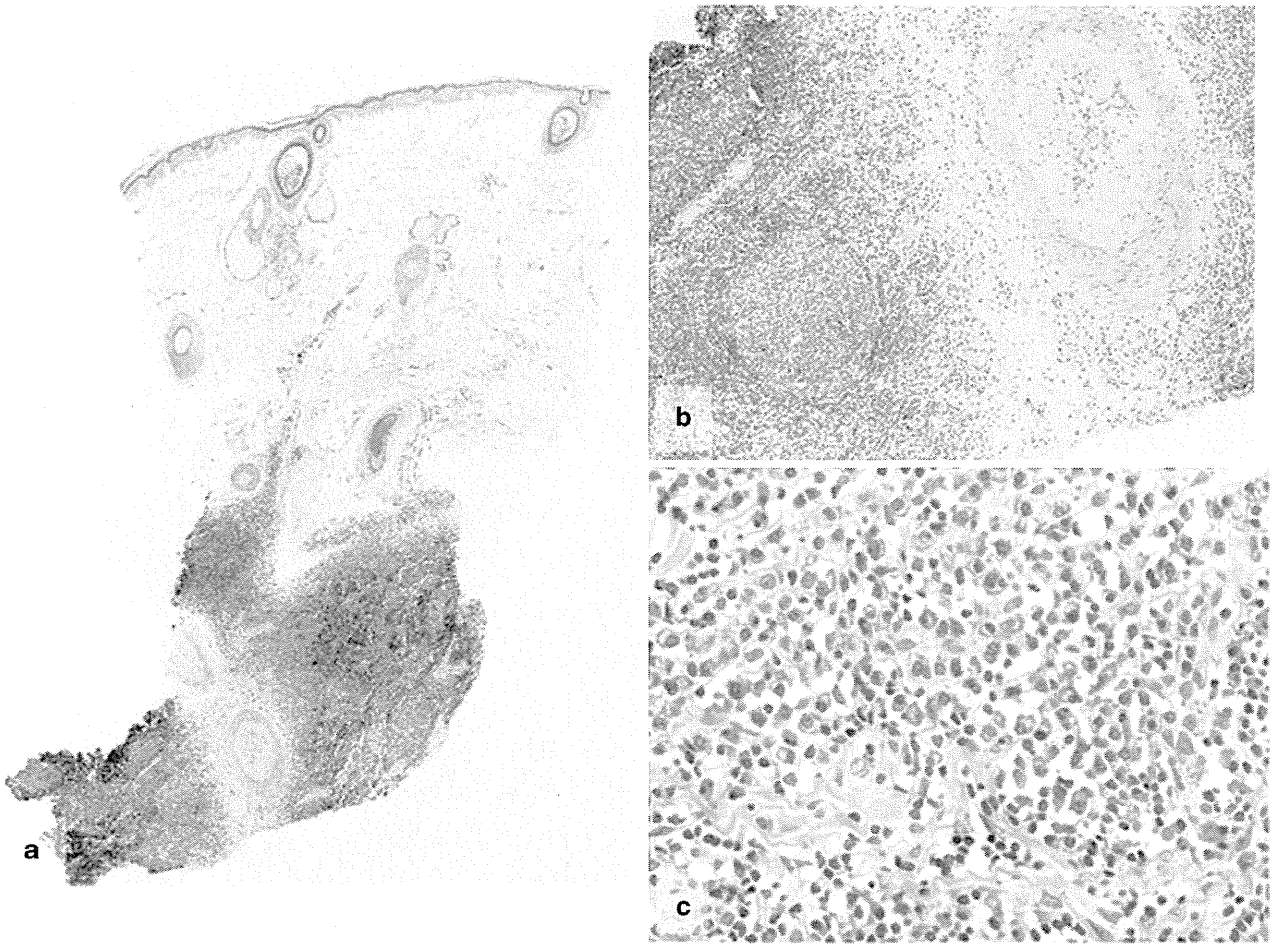

Figure 4 Patient 10: (**a**) This case showed a subcutaneous lymphoid nodule pattern (H&E). (**b**, **c**) Subcutis involvement by lymphoplasmacytic infiltration with eosinophils and lymphoid follicles is observed. Perivascular mild fibrosis is seen (H&E).

ings were consistent with IgG4-related disease (Table 2). Five of six lymph node lesions demonstrated progressively transformed germinal centers-type IgG4-related lymphadenopathy (Figure 6).

Discussion

IgG4-related disease frequently involves various organs, such as the lacrimal glands, salivary glands, lymph nodes, lungs, mediastinum, pancreas, hepatobiliary tract, retroperitoneum, aorta, kidneys, and prostate. 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, although lactate dehydrogenase levels remain unchanged. Patients often have an excellent response to steroid therapy.[1–4]

In this report, we have described the clinicopathological details of patients with IgG4-related skin disease. The patients presented with uniform clinicopathology: the lesions frequently involved the skin of the periauricular, cheek, and mandible regions. Interestingly, half of the patients had submandibular progressively transformed germinal centers-type IgG4-related lymphadenopathy, which was recently recognized as a new type of IgG4-related disease.[8] This type of IgG4-related lymphadenopathy frequently involves the submandibular lymph nodes, and is accompanied by extranodal lesions, particularly in the lacrimal and salivary glands.[8] Interestingly, 6 of 10 patients in this study with IgG4-related skin disease had lacrimal or parotid gland lesions.

The histological features of IgG4-related skin disease are rather nonspecific, being indistinguishable from primary cutaneous lymphoid hyperplasia and other reactive processes. Nonetheless, this diagnosis may have to be considered, particularly for lesions rich in plasma cells and eosinophils, and especially if accompanying mass lesions are observed in sites commonly involved in IgG4-related disease, such as the lacrimal and salivary glands, and lymph nodes. In addition, the presence of large numbers of $IgG4^+$ cells together with a high proportion of $IgG4^+/IgG^+$ cells provides a conclusive diagnosis.[3]

The 2012 Consensus Statement on the pathology of IgG4-related disease emphasizes that the characteristic histopathological appearance of dense

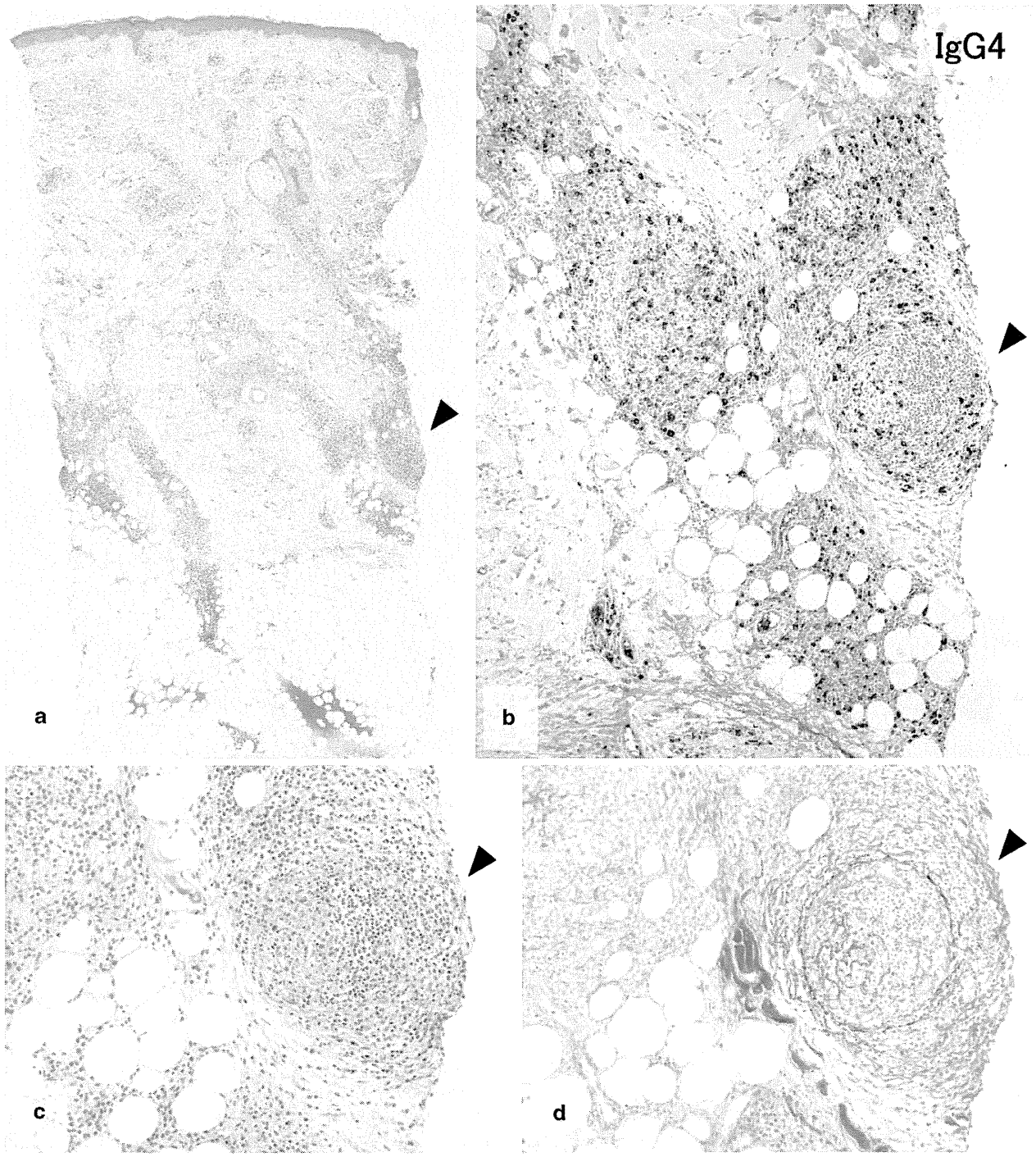

Figure 5 Patient 3: Dermal to subcutis involvement by perivascular and periadnexal lymphoplasmacytic nodular infiltration is observed. Obliterative phlebitis is seen (arrowhead). (**a**) H&E, (**b**) IgG4 immunostaining, (**c**) H&E, and (**d**) Elastica-van Gieson.

lymphoplasmacytic infiltrates, a storiform pattern of fibrosis, and obliterative phlebitis are the most critical factors in diagnosis.[3] However, although 4 of our 10 cases exhibited fibrosis, it was not storiform pattern fibrosis. In addition, obliterative phlebitis was detected in only one patient. The number of $IgG4^+$ cells and the $IgG4^+/IgG^+$ cell ratio (>40%) were considered to be of secondary importance. The $IgG4^+$ cell counts required for diagnosis differ among the affected organs, ranging from 10 to 200 cells/HPF.[3] According to the Consensus Statement, 200 $IgG4^+$ cells/HPF are required for the diagnosis of IgG4-related skin disease.[3] In our case series, the $IgG4^+$ cell count ranged from 49 to 396 cells/HPF (183 ± 121). Thus, 7 of 10 cases with IgG4-related skin disease did not meet this diagnostic criteria. However, these criteria were on the basis of a limited number of published

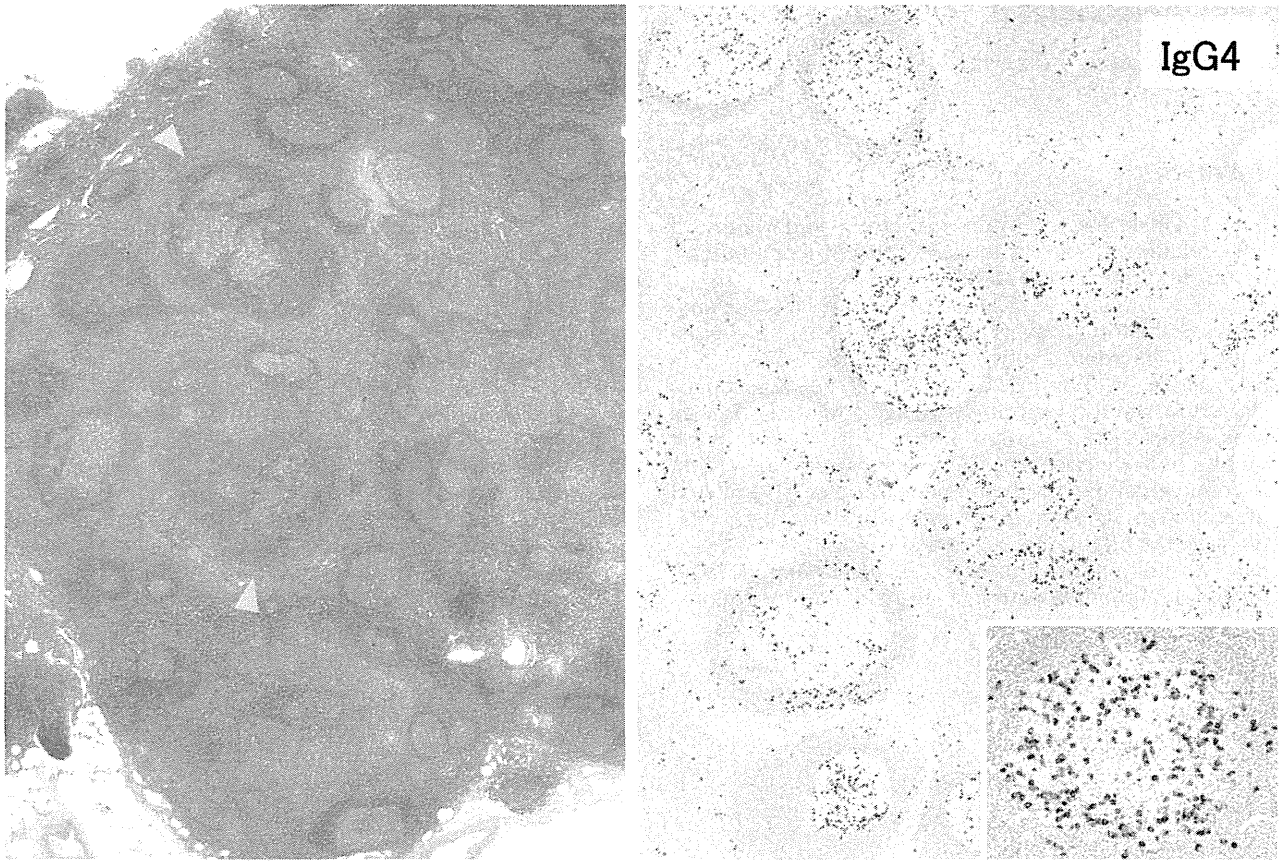

Figure 6 Patient 7: Submandibular lymph node lesion demonstrating progressively transformed germinal centers-type IgG4-related lymphadenopathy. The progressively transformed germinal centers is characterized by scattered large expansile follicles with unusual appearance against a background of normal reactive follicles (arrowhead). (Left) H&E, (right) IgG4 immunostaining.

studies on IgG4-related skin disease.[3] Therefore, the cutoff point for the $IgG4^+$ cell count used to diagnose IgG4-related skin disease needs to be reconsidered. In fact, in a recently published case report of IgG4-related skin disease, the $IgG4^+$ cell count was 47 cells/HPF.[7]

In our cases of IgG4-related skin disease and previously published studies,[6,7] $IgG4^+$ cell counts were 47 to 425/HPF, and the $IgG4^+/IgG^+$ cell ratio was 62 to 100%. On the basis of these results, we propose the following histological diagnostic criteria for IgG4-related skin disease: $IgG4^+$ cell count of >50/HPF and an $IgG4^+/IgG^+$ cell ratio of $>60\%$. Needless to say, attaining the IgG4 immunostain threshold does not necessarily imply a diagnosis of IgG4-related disease. For the diagnosis of IgG4-related disease, careful correlation with the histopathological features of the sample and clinical findings is required.[3]

Hyper-IL-6 syndromes, such as multicentric Castleman's disease, rheumatoid arthritis, and immune-mediated conditions, sometimes fulfill the diagnostic criteria for IgG4-related disease.[9,10] Recently, we encountered a case of cutaneous involvement of multicentric Castleman's disease that fulfilled the diagnostic criteria for IgG4-related disease.[11] Therefore, it is important to differentiate between IgG4-related skin disease and cutaneous involvement of multicentric Castleman's disease. In our cases, hyper-IL-6 syndromes were histologically and clinically excluded. These were differentiated by the fact that hyper-IL-6 syndromes are characterized by elevated serum levels of IgG, IgA, IgM, and C-reactive protein; thrombocytosis; anemia, hypoalbuminemia; and hypocholesterolemia. In contrast, IgG4-related disease does not have any of these characteristics.[3,5,9,10]

In conclusion, patients with IgG4-related skin disease had uniform clinicopathology, with lesions frequently found on the skin of the face, head, and neck, particularly the periauricular, cheek, and mandible regions; these were frequently accompanied by progressively transformed germinal centers-type IgG4-related lymphadenopathy.

Acknowledgements

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.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Sato Y, Ohshima K, Ichimura K, *et al.* Ocular adnexal IgG4-related disease has uniform clinicopathology. Pathol Int 2008;58:465–470.
2 Sato Y, Notohara K, Kojima M, *et al.* IgG4-related diseases: historical overview and pathology of hematological disorders. Pathol Int 2010;60:247–258.
3 Deshpande V, Zen Y, Chan JK, *et al.* Consensus statement on the pathology of IgG4-related disease. Mod Pathol 2012;25:1181–1192.
4 Stone JH, Khosroshahi A, Deshpande V, *et al.* Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. Arthritis Rheum 2012;64:3061–3067.
5 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.
6 Cheuk W, Lee KC, Chong LY, *et al.* IgG4-related sclerosing disease. A potential new etiology of cutaneous pseudolymphoma. Am J Surg Pathol 2009; 33:1713–1719.
7 Kakuchi Y, Yamada K, Suzuki Y, *et al.* IgG4-related skin lesions in a patient with IgG4-related chronic sclerosing dacryoadenitis and sialoadenitis. Intern Med 2011;50:1465–1469.
8 Sato Y, Inoue D, Asano N, *et al.* Association between IgG4-related disease and progressively transformed germinal centers of lymph node. Mod Pathol 2012;25:956–967.
9 Sato Y, Yoshino T. IgG4-related lymphadenopathy. Int J Rheumatol 2012;2012:572–539.
10 Asano N, Sato Y. Rheumatoid lymphadenopathy with abundant IgG4$^+$ plasma cells: a case of mimicking IgG4-related disease. J Clin Exp Hematopathol 2012;52:57–61.
11 Takeuchi M, Sato Y, Takata K, *et al.* Cutaneous multicentric castleman's disease mimicking IgG4-related disease. Pathol Res Pract 2012; doi:10.1016/j.prp.2012.09.006 (in press).

Mod Rheumatol (2012) 22:859–870
DOI 10.1007/s10165-011-0589-2

ORIGINAL ARTICLE

Clinical and histological changes associated with corticosteroid therapy in IgG4-related tubulointerstitial nephritis

Ichiro Mizushima · Kazunori Yamada · Hiroshi Fujii · Dai Inoue · Hisanori Umehara · Masakazu Yamagishi · Yutaka Yamaguchi · Michio Nagata · Masami Matsumura · Mitsuhiro Kawano

Received: 11 November 2011 / Accepted: 26 December 2011 / Published online: 20 January 2012

Abstract
Objectives This study aimed to investigate the clinicopathological changes induced by corticosteroid therapy in immunoglobulin (Ig)G4-related tubulointerstitial nephritis (TIN).
Methods We studied six IgG4-related TIN patients receiving renal biopsies before and after corticosteroid therapy. Their clinical data and histological findings were evaluated before and after therapy.
Results Elevated serum creatinine levels rapidly improved after corticosteroid therapy except for two patients, in whom it persisted. Abnormal radiological findings improved in all patients, although focal cortical atrophy persisted in three. Histologically, TIN-like dense lymphoplasmacytic infiltration, interstitial fibrosis, IgG4-positive plasma cell, CD4+CD25+ T cell, and Foxp3+ cell infiltration were characteristic before therapy. After therapy, the area with cell infiltration decreased and regional fibrosis became evident in the renal interstitium. The number of IgG4-positive plasma cells and Foxp3+ cells significantly diminished even in the early stage of therapy, whereas low to moderate numbers of CD4+ and CD8+ T cells still infiltrated where inflammation persisted in the later stage.
Conclusions Our study shows that persistent renal insufficiency associated with macroscopic atrophy and microscopic fibrosis is not so rare in IgG4-related TIN. Pathologically, the behavior of regulatory T cells during the clinical course is quite similar to that of IgG4-positive plasma cells, and the behavior pattern of those cells is distinctive.

Keywords IgG4-related disease · Tubulointerstitial nephritis · IgG4-positive plasma cell · Regulatory T cell · Corticosteroid therapy

I. Mizushima · K. Yamada · H. Fujii · M. Kawano (✉)
Division of Rheumatology, Department of Internal Medicine, Kanazawa University Graduate School of Medicine, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8640, Japan
e-mail: mk0920@mac.com

D. Inoue
Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

H. Umehara
Department of Hematology and Immunology, Kanazawa Medical University, Kanazawa, Ishikawa, Japan

M. Yamagishi
Division of Cardiology, Department of Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

Y. Yamaguchi
Yamaguchi Pathology Laboratory, Matsudo, Chiba, Japan

M. Nagata
Department of Kidney and Vascular Pathology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan

M. Matsumura
Research Center for Medical Education, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

Introduction

Immunoglobulin (Ig)G4-related disease (IgG4-RD) is a recently recognized systemic inflammatory disease with multiorgan involvement [1–5], including the kidney. Since 2004, accumulated case reports and case series have defined the radiographic and histopathological characteristic findings of IgG4-related kidney disease [6–20]. Two large

studies [21, 22] demonstrated clinicopathological features of IgG4-related tubulointerstitial nephritis (TIN) that included high levels of serum IgG4, IgG, and IgE; hypocomplementemia; and TIN with copious IgG4-positive plasma cell infiltration with fibrosis. However, the clinical course and histological changes occurring after corticosteroid therapy have not yet been well characterized. In addition, although IgG4-RD is thought to be steroid responsive, not all cases achieve recovery of normal renal function [23], with, for example, one case report describing a patient who required maintenance hemodialysis despite corticosteroid therapy [24]. Therefore, in order to establish the optimal treatment plan to prevent progressive kidney damage, renal re-biopsy seems to be necessary. We undertook this study to evaluate the influence of corticosteroid therapy on the clinical and histopathological findings in IgG4-related TIN.

Materials and methods

Patients and materials

Between 1 September 2005 and 31 August 2010, we identified 11 IgG4-RD patients with kidney involvement, including renal parenchymal and pelvic lesions. We diagnosed IgG4-RD according to the provisional diagnostic criteria for IgG4-RD of Masaki et al. [25]. Renal biopsies were performed in six patients in whom IgG4-related TIN was highly suspected because of renal dysfunction, elevation of renal tubular markers, or abnormal imaging finding, and we diagnosed them with IgG4-related TIN based on diagnostic criteria proposed by Kawano et al. [26] and Raissian et al. [22]. Two patients (patients 1 and 5 in Table 1) had been included in our earlier studies [27, 28], two (patient 1 and 6) in a report by Saeki et al. [21], and one (patient 1) in a report by Yamaguchi et al. [29].

All six patients underwent re-biopsy while receiving corticosteroid therapy, as re-evaluation of the extent of cell infiltration and fibrosis was necessary to assess the future dosage regimen of corticosteroid. One patient (patient 1 in Table 1) underwent re-biopsy 14 months after the start of therapy, one (patient 2) 7 months later, three (patients 3, 4, and 5) 4 months later, and one (patient 6) only 1 month later. These biopsy specimens were obtained randomly, not specifically from the mass lesions. We examined these 12 specimens histologically and immunohistochemically. With regard to the extent of the renal interstitial lesion, diffuse TIN was defined as being present when $\geq$80% of the renal interstitium in renal biopsy specimens was affected and focal TIN when <80% was affected. We also retrospectively evaluated the clinical and radiographic findings of these six patients. Serial laboratory data during the clinical course were analyzed, and the computed tomography (CT) findings of renal lesions were investigated before and after corticosteroid therapy in all patients. This study received institutional ethics board approval, and informed consent for all data and samples was obtained from each patient. The research was in compliance with the Declaration of Helsinki.

Single immunostaining

Bouin's fluid- or formalin-fixed and paraffin-embedded renal specimens of six patients with IgG4-related TIN were used for the immunostaining of IgG4, CD138, CD4, CD8, and Foxp3. The immunostaining was performed using a monoclonal antibody against human IgG4 (Zymed Laboratory, San Francisco, CA, USA), CD138 (AbD Serotec, Oxford, UK), CD4 (Nichirei, Tokyo, Japan), CD8 (Nichirei), and Foxp3 (AbD Serotec). The deparaffinized sections were microwaved in citrate buffer (pH 6.0) for 15 min. Cells positive for IgG4, CD138, CD4, CD8, or Foxp3 were counted in five different high-power fields (HPF: 10× eyepiece and 40× lens) with intense cell infiltration.

Dual fluorescent immunostaining of CD4 and CD25

All Bouin's fluid-fixed and paraffin-embedded renal specimens were used for dual fluorescent immunostaining of CD4 and CD25. The deparaffinized sections were microwaved in citrate buffer (pH 6.0) for 20 min and incubated with normal donkey serum for protein blocking for 30 min. The specimens were incubated with a mouse monoclonal antibody to CD25 (Leica Microsystems, Wetzlar, Germany) and a rabbit monoclonal antibody to CD4 (Spring Bioscience, CA, USA) overnight at 4°C. Then, the specimens were incubated for 1 h at room temperature with Alexa Fluor 488-labeled donkey anti-mouse IgG antibodies and Alexa Fluor 594-labeled donkey anti-rabbit IgG antibodies (Molecular Probes, Carlsbad, CA, USA) and observed under a laser microscope and digitally merged. No positive staining was observed when the primary antibodies were replaced with normal donkey serum in the negative control of the staining procedures.

Statistical analysis

Statistical analysis was performed using the Wilcoxon signed rank test for continuous non-normally distributed data. Significant differences were defined as $P < 0.05$.

Results

Patient profiles

We analyzed six patients [four men and two women; average age 71.0 (range 59–79) years] with IgG4-related

Table 1 Clinicopathological characteristics of six patients with immunoglobulin (Ig)G4-related tubulointerstitial nephritis

No.	Age/sex	Cr at pre-Tx (*N* 0.6–1)	IgG (*N* 870–1,600)	IgG4 (*N* < 105)	IgE (*N* < 250)	CH50 (*N* 32–47)	C3 (*N* 65–135)	C4 (*N* 13–35)	ANA	RF	U-β2MG (*N* < 250)
1	59/M	1.15	2,850	1,470	456	34	81	16	(−)	(−)	335
2	79/M	0.54	4,756	409	457	8	41	3	(+)	(−)	496
3	77/W	0.59	2,256	984	292	60	110	27	(+)	(−)	78
4	68/M	1.90	3,830	736	242	3	33	1	(+)	(+)	7,375
5	75/W	2.55	3,695	486	1,226	2	18	2	(+)	(−)	35,490
6	68/M	7.26	4,661	1,120	335	5	10	7	(+)	(+)	3,240

No.	Age/sex	U-NAG (*N* < 10)	Eosinophilia	Allergy	U-Pr	CT findings	Initial renal biopsy findings	Initial Tx of post-biopsy	Cr at re-biopsy (*N* 0.6–1)	Period between initial biopsy and re-biopsy (months)
1	59/M	1.6	(+)	(−)	(−)	LDLs	fTIN	PSL 30	1.06	14
2	79/M	19	(−)	(−)	(−)	(−)	fTIN + EndPGN	PSL 20	0.51	7
3	77/W	0.4	(−)	Rhinitis	(−)	LDLs + P	fTIN	PSL 20	0.63	4
4	68/M	8.8	(−)	BA	(−)	LDLs	fTIN + IgAGN	PSL 30	1.20	4
5	75/W	5.9	(−)	(−)	(+)	S	dTIN + HSPN	PSL 30	1.05	4
6	68/M	16.9	(−)	(−)	(+)	S	dTIN + EndPGN	PSL 30	1.45	1

Conversion factor for Cr: mg/dl to μmol/l, ×88.4

ANA antinuclear antibody, *BA* bronchial asthma, *CH50* serum CH50 at initial renal biopsy (U/ml), *Cr* serum creatinine (mg/dl), *CT* computed tomography, *C3* serum C3 at initial renal biopsy (mg/dl), *C4* serum C4 at initial renal biopsy (mg/dl), *dTIN* diffuse tubulointerstitial nephritis, *EndPGN* endocapillary proliferative glomerulonephritis *fTIN* focal tubulointerstitial nephritis, *HSPN* Henoch–Schönlein purpura nephritis, *IgAGN* IgA nephropathy, *IgG* serum immunoglobulin G at initial renal biopsy (mg/dl), *IgG4* serum immunoglobulin G4 at initial renal biopsy (mg/dl), *IgE* serum immunoglobulin E at initial renal biopsy (IU/ml), *LDLs* multiple low-density lesions of the renal parenchyma, *P* diffuse thickening of the renal pelvic wall, *post-biopsy* post-renal biopsy, *PSL* prednisolone mg/day, *re-biopsy* renal re-biopsy, *RF* rheumatoid factor, *S* diffuse bilateral renal swelling, *Tx* treatment, *U-β2MG* urine β2-microglobulin at initial renal biopsy (μg/l), *U-NAG* urine *N*-acetyl-β-D-glucosaminidase at initial renal biopsy (U/l), *U-Pr* proteinuria

TIN. None had been treated with corticosteroid or any other immunosuppressants before the diagnosis. All patients had other organ involvement associated with IgG4-RD: four (66.7%) dacryoadenitis and/or sialoadenitis, six (100%) multiple lymphadenopathy, and three (50.0%) lung lesions. Pancreatic lesion was detected in only one patient (16.7%). Prostate, liver, and joint lesion was detected in one patient each. All patients were treated with prednisolone at an initial dose of 20–30 mg/day after the initial renal biopsy (Table 1).

Laboratory findings and changes associated with corticosteroid therapy

At presentation, all patients showed elevated levels of serum IgG4 (average 867.5 mg/dl, range 409–1,470 mg/dl, normal range <105 mg/dl) and IgG (average 3,675 mg/dl, range 2,256–4,756 mg/dl, normal range 870–1,700 mg/dl). Five patients (83.3%) showed elevated serum IgE levels (average 501.3 IU/ml, range 242–1,226 IU/ml, normal range <250 IU/ml), and only one patient had eosinophilia (eosinophils >5%). Four patients (66.7%) had hypocomplementemia. Antinuclear antibodies were positive in five patients (83.3%) and rheumatoid factor in two (33.3%). Serum creatinine concentration was elevated in four patients (66.7%). Urine β2-microglobulin (β2-MG) excretion was elevated in five patients (83.3%) and urine *N*-acetyl-β-D-glucosaminidase (NAG) level in two (33.3%), whereas two patients (33.3%) had proteinuria (Table 1). In three patients who showed severe renal dysfunction (serum creatinine >1.5 mg/dl) before corticosteroid therapy (patients 4, 5, and 6 in Table 1), serum creatinine rapidly declined within 1 month after the start of therapy. However, some degree of renal dysfunction persisted in two of them (Fig. 1a). Four patients had hypocomplementemia before corticosteroid therapy (patients 2, 4, 5, and 6 in Table 1), and corticosteroid therapy promptly improved serum complement levels within 2 months in three of them; recovery of serum complement level was very slow in one other (Fig. 1b). On the other hand, urine β2-MG and NAG concentrations fluctuated despite the corticosteroid therapy (data not shown).

Computed tomography findings and changes associated with corticosteroid therapy

Computed tomography revealed some radiologic findings of the renal parenchymal lesions in five patients. Among them, multiple low-density lesions on enhanced CT (Fig. 2a, c, e) were the most frequent findings and were observed in three patients (patients 1, 3, and 4 in Table 1), one of whom also had diffuse thickening of the renal pelvic wall (Fig. 2c). On the other hand, the renal parenchymal

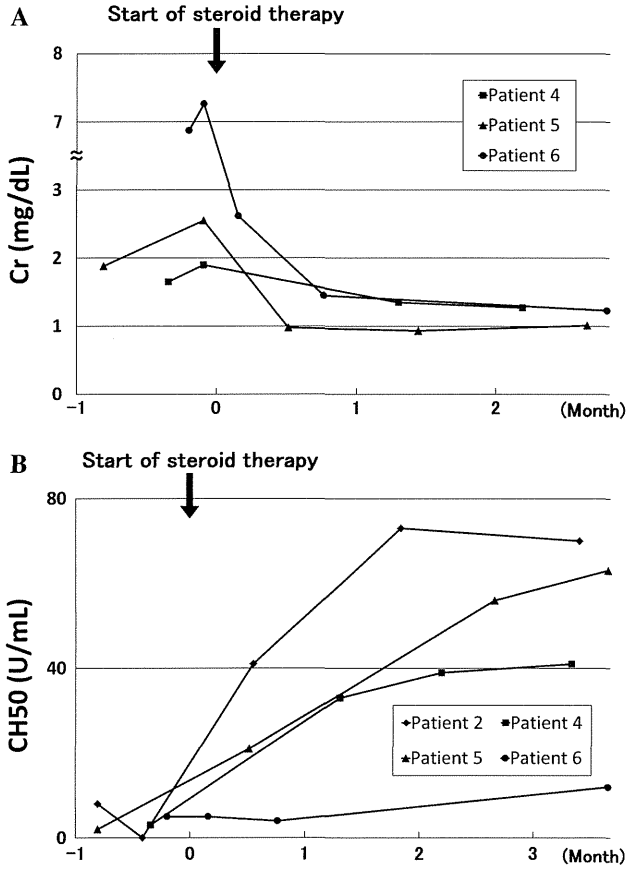

Fig. 1 Patient's clinical course. Serum creatinine concentrations rapidly declined within 1 month after corticosteroid therapy in patients 4, 5, and 6, who showed severe renal dysfunction before therapy (**a**). Hypocomplementemia was improved by corticosteroid therapy within 2 months in patients 2, 4, and 5, but response to therapy was insufficient in patient 6 (**b**)

lesion observed in two patients (patients 5 and 6 in Table 1) who underwent only plain CT because of severe renal dysfunction before corticosteroid therapy was diffuse bilateral renal swelling (Fig. 2g, i). No radiological abnormal findings were apparent in only one patient (patient 2 in Table 1).

On the whole, corticosteroid therapy quickly ameliorated renal lesions. Three patients with multiple low-density lesions on enhanced CT showed recovery of contrast enhancement of the renal cortex after therapy. However, scar-like focal cortical atrophy persisted in two of them (Fig. 2b, f). Two patients with diffuse bilateral renal swelling showed apparent improvement of the renal swelling after therapy (Fig. 2h, j). A few small scar-like focal cortical atrophy persisted in one of them (Fig. 2h), whereas relatively uniform contrast enhancement was observed in the other (Fig. 2j). Diffuse thickening of the renal pelvic wall became thinner after therapy in patient 3 (Fig. 2d).

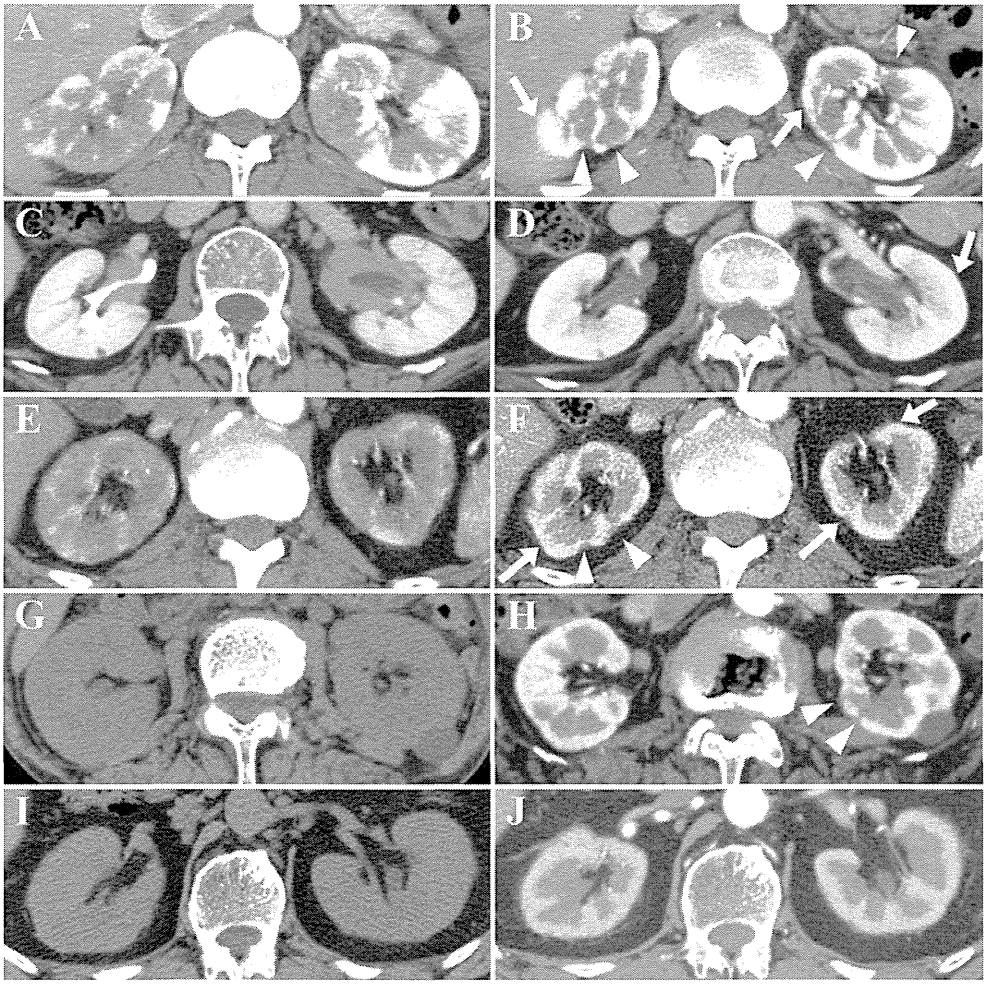

Fig. 2 Contrast-enhanced (**a**–**f**, **h**, **j**) or plain (**g**, **i**) computed tomography (CT) findings of immunoglobulin (Ig)G4-related renal lesions and changes induced by corticosteroid therapy. Multiple low-density lesions on enhanced CT (**a** patient 1, **c** patient 3, **e** patient 4), diffuse bilateral renal swelling on plain CT (**g** patient 5, **i** patient 6), and diffuse thickening of the renal pelvic wall (**c**) were observed before therapy. Contrast enhancement of the renal cortex recovered after therapy in all patients with multiple low-density lesions (**b**, **d**, **f** *arrows* show recovering lesions), though some atrophic areas of decreased enhancement remained (**b**, **f** *arrowheads* show atrophic lesions). Diffuse bilateral renal swelling was improved with small areas of decreased enhancement 4 months after therapy in patient 5 (**h** *arrowheads* show lesions with decreased enhancement) and with relatively uniform contrast enhancement 1 month after therapy in patient 6 (**j**). Diffuse thickening of the renal pelvic wall became less marked 4 months after therapy in patient 3 (**d**)

Histological and immunohistochemical findings and changes associated with corticosteroid therapy

Histological findings of six renal specimens before corticosteroid therapy were as follows: Dense lymphoplasmacytic infiltration and sparse distribution of atrophic renal tubules in the renal interstitium were present (Fig. 3a–c). The findings of renal tubulitis were mild (Fig. 3a, e, f). The interstitial lesions were often localized, and the border of the lesion was fairly clear (Fig. 3b). On the other hand, the interstitial lesion was diffuse in two patients (patients 5 and 6 in Table 1) with severe renal dysfunction whose plain CT images before therapy showed diffuse bilateral renal swelling (Fig. 3c). In one case (patient 6), inflammation extended beyond the renal capsule (Fig. 3d). Fibrosis with occasional infiltrating cells was observed (Fig. 3e, f). Lymph follicles were not evident in any patient. Glomerular lesions were observed in four: one showed Henoch–Schönlein purpura nephritis [28], another IgA nephropathy, and the other two focal endocapillary proliferation. Immunohistochemically, in addition to IgG4-positive plasma cells (Fig. 5a), Foxp3+ cells (Fig. 5b) and CD4+CD25+ T cells (Fig. 5c) were detected in the interstitial lesions of IgG4-related TIN. CD4+ T cells (Fig. 6a) and CD8+ T cells (Fig. 6b) were also found there.

The re-biopsy specimen obtained 1 month after corticosteroid therapy from patient 6 showed that areas with intense cell infiltration remained (Fig. 4a), but infiltration of IgG4-positive plasma cells (Fig. 5d), Foxp3+ cells (Fig. 5e), and CD4+CD25+ T cells (Fig. 5f) considerably decreased, and there was little obvious fibrosis of the renal

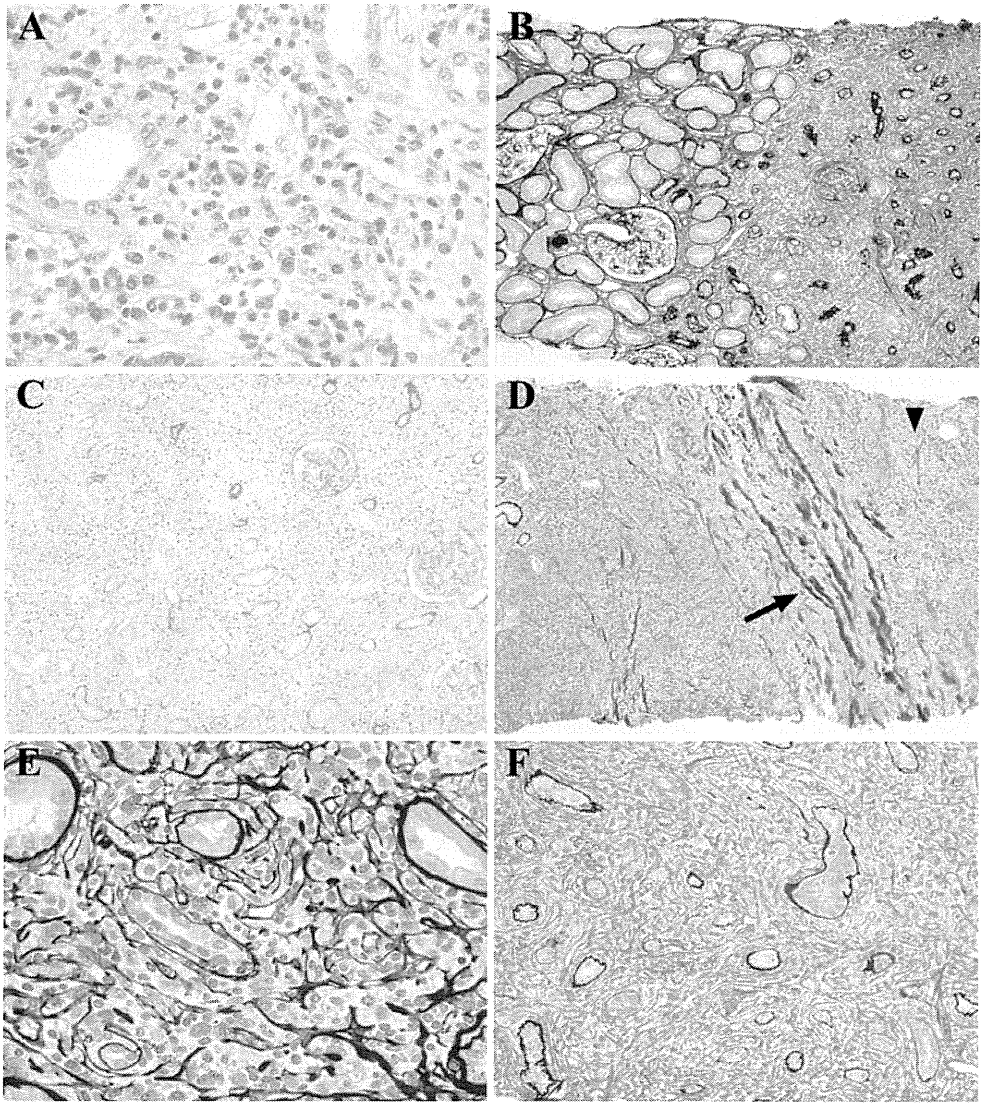

Fig. 3 Light microscopy findings of the renal interstitium before corticosteroid therapy. Severe lymphoplasmacytic infiltration with tubular atrophy was observed (**a** patient 6, **b** patient 4, **c** patient 6). Interstitial lesions were often focal, and the borderline between lesion and nonlesion was fairly clear (**b**). In two patients with severe renal dysfunction, interstitial lesion was diffuse (**c**). Inflammatory lesion beyond the renal capsule was detected (**d** patient 6, *arrow* shows the renal capsule, and *arrowhead* shows inflammation beyond the renal capsule). A characteristic fibrosis that appeared to surround infiltrating cells was observed (**e** patient 4, **f** patient 5) [**a** Hematoxylin and eosin (H&E) staining ×400; **b**, **d** periodic acid-methenamine-silver (PAM)-H&E staining ×100; **c** periodic acid-Schiff (PAS) staining ×100; **e**, **f** PAM-H&E staining ×400]

interstitium (Fig. 4a). The re-biopsy specimen 14 months after therapy from patient 1 demonstrated an obvious decrease of cell infiltration except for small localized infiltrations, whereas patchy marked fibrosis remained (Fig. 4b). In the re-biopsy specimens 4 months after therapy from patient 5 who showed severe renal dysfunction before therapy, we observed mixed lesions where cell infiltration was dense or fibrosis was predominant or neither of these features was noted (Fig. 4c, d, e, respectively). In the re-biopsy specimens 4 months after therapy from patient 3 who showed normal renal function before therapy, we observed the same three components as in patient 5, but fibrosis was not marked. In one patient with diffuse renal interstitial cell infiltration and sparse distribution of atrophic tubules (patient 6), tubular atrophy and sparseness of tubular distribution seemed to partially improve after therapy (Figs. 3c, 4f). In the immunostaining specimens, cell count of various infiltrating cells in the lesions with intense cell infiltration before and after corticosteroid therapy revealed that the characteristic infiltrating cells, such as IgG4-positive plasma cells and Foxp3+ cells, showed significant decreases after corticosteroid therapy (Figs. 5d, e, 7a–c). Other CD4+ T cells and CD8+ T cells were relatively persistent, even in the later stage of therapy

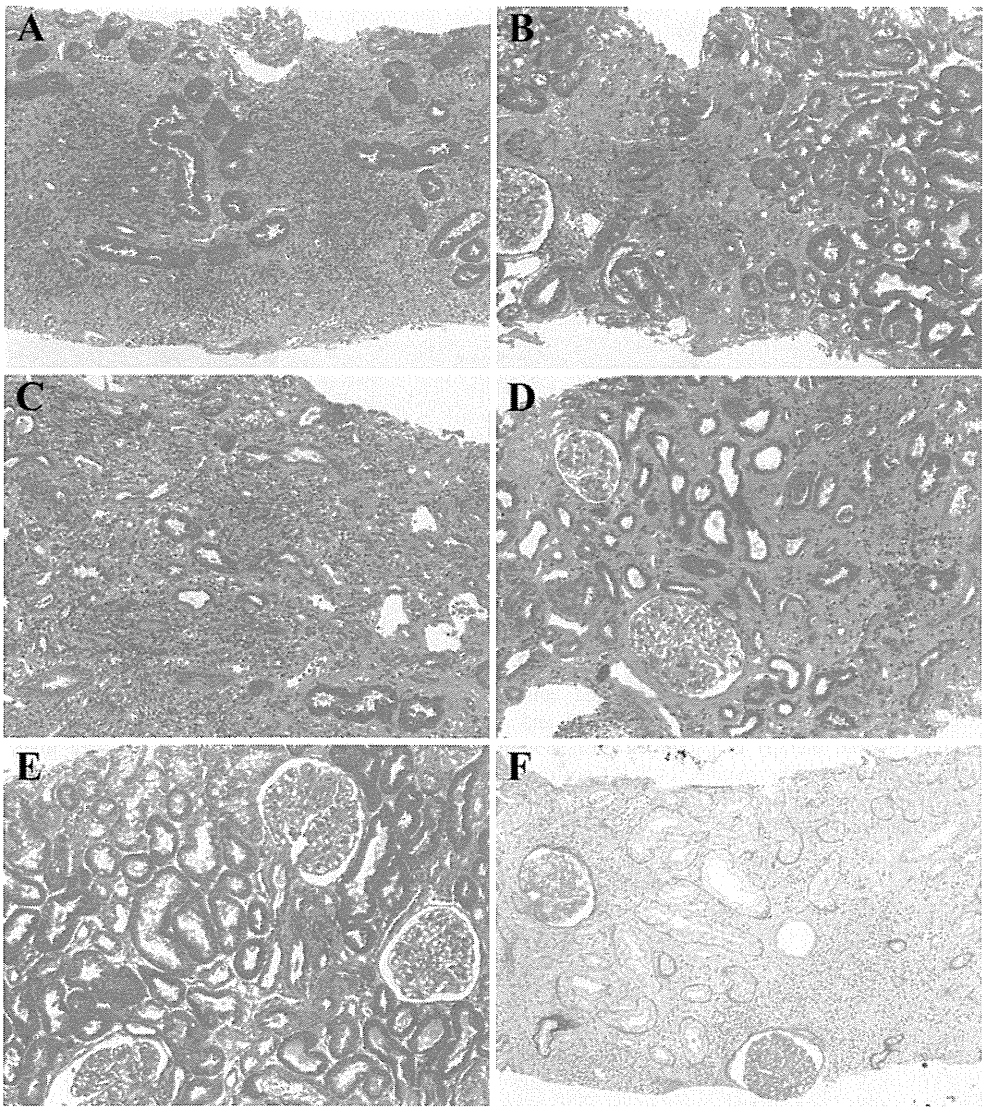

Fig. 4 Changes in light microscopy findings of the renal interstitium after corticosteroid therapy. Azan staining showed that interstitial fibrosis was not evident 1 month after corticosteroid therapy in patient 6 (**a**), whereas localized severe fibrosis was observed 14 months after therapy in patient 1 (**b**). There were mixed lesions where cell infiltration was dense (**c**) or fibrosis was predominant (**d**) or neither of them was noted (**e**) 4 months after therapy in patient 5. Interstitium in the posttreatment specimen of patient 6 seemed to show recovery of some renal tubules 1 month after corticosteroid therapy (**f**) [**a–e** Azan staining ×100, **f** periodic acid-Schiff (PAS) staining ×100]

(Figs. 6c, d, 7d, e). The average number of IgG4-positive plasma cells in five different HPF decreased from 156.4/HPF (range 102–210) to 2.0/HPF (range 0–3) in patient 1; 41.8/HPF (range 36–60) to 7.2/HPF (range 1–15) in patient 2; 50.2/HPF (range 25–77) to 12.2/HPF (range 5–36) in patient 3; 27.6/HPF (range 9–46) to 5.0/HPF (range 3–11) in patient 4; 62.2/HPF (range 45–80) to 4.8/HPF (range 3–7) in patient 5; and 73.2/HPF (range 50–108) to 20.8/HPF (range 19–22) in patient 6 (Fig. 7a). In the same way, the average number of Foxp3+ cells decreased from 35.4/HPF (range 26–52) to 8.4/HPF (range 1–16) in patient 1; 25.4/HPF (range 18–45) to 3.8/HPF (range 2–6) in patient 2; 18.0/HPF (range 10–23) to 1.2/HPF (range 0–3) in patient 3; 41.8/HPF (range 36–46) to 4.8/HPF (range 4–6) in patient 4; 33.2/HPF (range 30–35) to 3.0/HPF (range 0–5) in patient 5; and 23.0/HPF (range 19–30) to 3.2/HPF (range 1–5) in patient 6 (Fig. 7c).

Discussion

This study examined the clinicopathological features in patients with IgG4-related TIN before and after corticosteroid therapy. The characteristic laboratory findings before therapy were elevated serum IgE levels or eosinophilia, high incidence of hypocomplementemia, and hyper-β2