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Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis

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IgG4-related disease is a recently recognized multi-organ disorder characterized by high levels of serum IgG4 and dense infiltration of IgG4-positive cells into several organs. Although the pancreas was the first organ recognized to be affected by IgG4-related disorder in the syndrome of autoimmune pancreatitis, we present here clinicopathological features of 23 patients diagnosed as having renal parenchymal lesions. These injuries were associated with a high level of serum IgG4 and abundant IgG4-positive plasma cell infiltration into the renal interstitium with fibrosis. In all patients, tubulointerstitial nephritis was the major finding. Although 14 of the 23 patients did not have any pancreatic lesions, their clinicopathological features were quite uniform and similar to those shown in autoimmune pancreatitis. These included predominance in middle-aged to elderly men, frequent association with IgG4-related conditions in other organs, high levels of serum IgG and IgG4, a high frequency of hypocomplementemia, a high serum IgE level, a patchy and diffuse lesion distribution, a swirling fibrosis in the renal pathology, and a good response to corticosteroids. Thus, we suggest that renal parenchymal lesions actually develop in association with IgG4-related disease, for which we propose the term 'IgG4-related tubulointerstitial nephritis.'

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IgG4-related disease represents a recently recognized group of multi-organ diseases characterized by a high level of serum IgG4 and dense infiltration of IgG4-positive cells into multiple organs.[1–3] The condition was first described in relation to the pancreas (that is, autoimmune pancreatitis (AIP)),[4] and has since been expanded to various organ systems. At present, many other inflammatory conditions affecting multiple organs are considered to fall within the category of IgG4-related disease,[1–3] including sclerosing cholangitis,[5] sialadenitis,[2,6] retroperitoneal fibrosis,[7] interstitial pneumonitis,[8] inflammatory pseudotumor,[1–3] and periaortitis.[9] Although much attention is now being focused on these conditions, and the number of case reports of IgG4-related disease has been increasing, there are still few clinicopathological data on the involvement of organs other than the pancreas. The aim of this study was to elucidate the clinicopathological characteristics of renal parenchymal lesions associated with IgG4-related disease.

RESULTS

Patient profiles

Patients were all Japanese (20 men and 3 women) with an average age of 65.2 ± 10.1 (40–83) years at the time of diagnosis of renal disease. Although seven patients fulfilled the criteria for Sjögren's syndrome, as revised by the Japanese Ministry of Welfare in 1999, none of them met the criteria for systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis, rheumatoid arthritis, or sarcoidosis. None of the organ specimens showed any evidence of malignant lymphoma by the DNA PCR method or immunohistochemistry. At the time of diagnosis of renal involvement, one patient had been treated with low-dose corticosteroid (prednisolone 2.5 mg daily) because of AIP (patient 5 in Table 1). Three patients had been treated with the corticosteroid because of AIP (patients 19 and 22 in Table 1) or idiopathic thrombocytic purpura (patient 8 in Table 1), but the treatment was discontinued when they developed renal involvement. The other 19 patients had not received corticosteroids or immunosuppressants before the appearance of renal lesions.

Clinical features

Of the 23 patients, 22 (95.7%) had some accompanying extrarenal lesions: sialadenitis in 19 (82.6%), lymphadenopathy in 10 (43.5%), AIP in 9 (39.1%), dacryoadenitis in 7 (30.4%), lung lesions (interstitial pneumonitis and nodular lesions) in 6 (26.0%), and others in 3 (pseudotumor of the liver, prostatitis, and idiopathic thrombotic purpura) (Table 1). Among the extrarenal lesions, 15 (represented in bold in Table 1) had been recognized before diagnosis of the renal lesions, and 10 of these 15 lesions in patients 3, 5, 8, 19, and 22 had improved with steroid therapy, or spontaneously, at the time of diagnosis of the renal lesions. The other 39 lesions were diagnosed at the same time as the renal lesions. Clinical symptoms were mostly associated with extrarenal lesions, such as gland swelling and lymphadenopathy. Fever, arthralgia, skin eruption, and edema were observed in 3, 5, 1, and 2 patients, respectively. In 21 of the 23 patients, the renal lesions were observed by physicians because of urinary abnormalities, renal dysfunction, and/or abnormalities revealed by radiological examinations, including computed tomography (CT) and gallium citrate scintigraphy, during follow-up or further examinations of the IgG4-related extrarenal lesions. In patients 13 and 14, renal lesions were observed because of renal dysfunction in the absence of extrarenal lesions. The results of renal histological and laboratory findings alerted the attending physicians to IgG4-related disease, who then carried out measurements of serum IgG4 levels and IgG4 immunostaining. (Additional gallium citrate scintigraphy showed gallium-67 accumulation of both the salivary glands in patient 13.)

Laboratory findings at the time of diagnosis of the renal lesions

Urinary protein excretion was <0.3 g/day in 19 of the 23 patients (Table 1). Urinary protein excretion >1.0 g/day was shown in two patients with membranous nephropathy (patients 14 and 22). Hematuria (urinary red blood cells >3 per h.p.f. (high-power field)) was shown in eight patients. Although mostly mild, hematuria with urinary red blood cells >20 per h.p.f. was evident in three patients with glomerular lesions (patients 14, 22, and 23). Hematological examinations revealed anemia (hemoglobin <10.0 g/dl) in only one patient (hemoglobin 9.0 g/dl in patient 20). Although white blood cell counts were within the normal range in most patients (mild leukocytosis was observed in 2 patients), eosinophila (eosinophils >5%) was observed in 11 patients (47.8%). Platelet counts were within the normal range in all patients. Renal function varied from normal to renal failure (serum creatinine 0.67–6.87 mg/dl). Elevated creatinine levels (serum creatinine >1.2 mg/dl) were shown in 13 of the 23 patients. Abnormal liver function at the time of diagnosis of the renal lesions was observed in three patients; AST (aspartate transaminase) 53 IU/l (range: 13–33), ALT (alanine transaminase) 101 IU/l (range: 10–47), ALP (alkaline phosphatase) 914 IU/l (range: 115–359) in patient 4, AST 16 IU/l, ALT 20 IU/l, ALP 573 IU/l in patient 5, and AST 55 IU/l, ALT 115 IU/l, ALP 1690 IU/l in patient 22. Pancreatic swelling due to AIP had improved by this time in patients 5 and 22, and no patient showed CT abnormalities in the liver or pancreatobiliary system.

All patients showed elevated levels of serum IgG (2721–8841 mg/dl, mean 4836 ± 1499 mg/dl, normal range 870–1700). Although serum IgA and IgM levels were within the normal ranges in all patients, elevation of the serum IgE level (272–4442 IU/ml, normal range <250 IU/ml) was observed in 10 of 14 evaluated patients (71.4%). Serum IgG4 levels before steroid therapy ranged from 587 to 4630 mg/dl (mean 1520 ± 909, normal range <105 mg/dl). In 16 of the 23 patients (69.6%), the serum CH50 level was found to be decreased, together with a reduction in the serum level of C3, C4, or both. In two patients (nos 20 and 21), only the C3 level was decreased. The rheumatoid factor was positive in 7 of 18 evaluated patients (38.9%). Although anti-nuclear antibodies were positive in 69.6%, anti-SS-A, anti-SS-B, anti-Sm, and anti-RNP antibodies were all negative, and the levels of anti-DNA antibodies were not increased in any of the patients. The level of CRP was <1.5 mg/dl in 22 of the 23 patients, and 8.7 mg/dl in 1 patient (patient 7). Cryoglobulin, M-protein, myeloperoxidase-ANCA, and proteinase-3-ANCA were not observed in any of the patients.

Imaging studies

CT revealed abnormal renal parenchymal lesions in 16 of the 23 patients (69.6%) (Table 1). Among them, diffuse swelling of the bilateral kidneys was evident in seven patients. Patchily distributed hypoattenuated lesions in the renal cortex, being single or multiple, round or wedge shaped, were found in 10 patients (Figure 1). Renal pelvic tumor and calicectasis were observed in addition to the renal parenchymal lesions in patients 2 and 4, respectively. In patient 1, thickening of the renal pelvic wall was observed, although no renal

Table 1 | Clinicopathological features of 23 patients with IgG4-related tubulointerstitial nephritis

No	Age/sex	Renal biopsy findings	U-Pr/U-B	Cr at biopsy	IgG (*N*:870–1700)	IgG4 (*N*<105)	IgE (*N*<250)	Low CH50	Low C3	Low C4	ANA	RF	Extrarenal lesions	Renal CT findings	Tx of pre-/post-biopsy	Cr at 1 M after Tx	References
1	58/F	TIN	0.23 g/day/(±)	0.67	7319	4630	4442	(–)	(–)	(–)	(–)	(+)	La, Sa, AIP, Ly	Thickening of renal pelvis	(–)/PSL40	0.59	
2	76/F	TIN	(–)/(–)	0.69	2721	769	267	(–)	(–)	(–)	(+)	(–)	La, Sa, Ly, Lu	Pa, pelvic tumor	(–)/PSL20	0.64	
3	76/M	TIN	(–)/(–)	0.71	3486	1030	NA	(+)	(–)	( )	(–)	(–)	*Sa*, AIP	Pa	(–)/(–)	0.69	Saeki *et al.*[33,34]
4	40/F	TIN	(–)/(–)	0.74	3450	2400	272	(+)	(–)	(–)	(–)	(+)	La, Sa, Lu	Pa, S, calicectasis	(–)/Pulse+PSL60	0.58	Shimoyama *et al.*[35]
5	52/M	TIN	(–)/(–)	0.80	3180[a]	1430[a]	NA	(–)	(–)	(–)	(–)	(+)	*Sa*, *AIP*, *Lu*	Pa	PSL2.5/PSL10	0.7	Nakamura *et al.*[36]
6	56/M	TIN mesPGN	(+)/(–)	0.9	5680	1920	248	(+)	( )	(+)	(+)	(–)	La, Sa, Ly	Pa	(–)/PSL50	0.9	Yamamoto *et al.*[37]
7	70/M	TIN	(±)/( )	0.9	3496	623	NA	(+)	(+)	(+)	(+)	( )	AIP	S	( )/PSL30	0.8	
8	61/M	TIN	( )/( )	1.09	6569	730[a]	1049[a]	(+)	(+)	(+)	(+)	NA	*Sa*, *AIP*, *ITP*, Ly	Pa	( )/PSL60[b]	0.97	Saeki *et al.*[14,33,34]
9	74/M	TIN	(+)/( )	1.1	4387	1320	560	(+)	(+)	(+)	(+)	(+)	*Sa*	S	( )/PSL30	0.9	Nakada *et al.*[38]
10	58/M	TIN	( )/( )	1.15	2850	1470	456	( )	( )	( )	(+)	NA	Pseudo-tumor (liver)	Pa	( )/PSL30	1.06	
11	62/M	TIN+IgAGN	(–)/(±)	1.3	8194	NA	704	(+)	(+)	(+)	(+)	(+)	La, Sa, AIP, Ly	S	(–)/Pulse+PSL30	1.1	
12	75/M	TIN	(+)/(–)	1.34	5380	587	NA	(+)	(+)	(+)	(+)	(+)	Sa, Ly, Lu	Pa	(–)/PSL30	1.14	
13	68/M	TIN	(–)/(–)	1.37	2995	670	2323	(+)	(+)	(+)	(+)	(–)	Sa	Normal	(–)/PSL40	1.19	Saeki *et al.*[14,33]
14	83/M	TIN+MN	2.3 g/day/(3+)	1.48	3144	924	32	(+)	(+)	(+)	(+)	NA	(–)	Normal	(–)/PSL40	1.39	Saeki *et al.*[39]
15	60/M	TIN	(–)/(–)	1.7	8841	1028	NA	(+)	(+)	(+)	(+)	(–)	*Ly*, *Lu*, Sa	Normal	(–)/PSL30	NA	Takamura *et al.*[40]
16	60/M	TIN+mesPGN	(+)/(±)	1.75	5188	305[a]	NA	(+)	(+)	(+)	(+)	(+)	Sa, Ly	S	(–)/PSL50	1.55	Saeki *et al.*[14,33,34]
17	61/M	TIN	NA	2.0	8005	2390	858	(+)	( )	(+)	(+)	(–)	Sa, Ly	S	(–)/unknown	NA	
18	55/M	TIN	(+)/(–)	2.1	5040	1780	NA	(–)	(–)	(–)	(+)	(+)	*AIP*, Sa	Pa	(–)/PSL40	1.3	Saeki *et al.*[33]
19	69/M	TIN	0.25 g/day/(±)	2.36	4001	1340	NA	(+)	(+)	(+)	(–)	NA	*AIP*	Normal	(–)/PSL30[b]	2.1	
20	64/M	TIN	NA	2.9	5100	1360	92	(–)	(+)	(–)	(+)	(–)	La, Sa	Normal	(–)/PSL50	NA	
21	76/M	TIN	0.3 g/day/(+)	5.4	2963	1800	125	(–)	(+)	(–)	(–)	(–)	*Sa*	Normal (nonenhanced)	(–)/PSL40	2.9	
22	78/M	TIN+MN	1.4 g/day/(2–)	6.17	3731	1860	NA	(+)	(+)	(–)	(–)	(–)	*Sa*, *AIP*	Pa	(–)/PSL20[b]	HD	Saida *et al.*[10]
23	68/M	TIN+endocap	(2+)/(2+)	6.87	4661	1120	335	(+)	( )	(+)	(+)	(–)	La, Sa, Ly, Lu, P	S (nonenhanced)	(–)/PSL30	1.45	

Abbreviations: ANA, antinuclear antibody; Cr, serum creatinine (mg/dl); CT, computed tomography; endocap, endocapillary hypercellularity; IgAGN, IgA nephropathy; IgG, serum IgG (mg/dl); IgE, serum IgE (IU/ml); IgG4, serum IgG4 (mg/dl); Low CH50,C3,C4, low titer of serum CH50,C3,C4; M, month; mesPGN, mesangioproliferative glomerulonephritis; MN, membranous nephropathy; NA, not available; pre-/post-biopsy, pre-renal biopsy/post-renal biopsy; RF, rheumatoid factor; TIN, tubulointerstitial nephritis; Tx, treatment; U-B, hematuria; U-Pr, proteinuria.

[a]Value under steroid therapy. AIP, autoimmune pancreatitis; ITP, idiopathic thrombocytic purpura; La, dacryoadenitis; Lu, lung lesion; Ly, lymphadenitis; P, prostatitis; Pa, patchy lesion; PSL, prednisolone mg/day; S, diffuse swelling; Sa, sialadenitis (bold and italic extrarenal lesions were diagnosed before diagnosis of renal lesion).

[b]History of steroid treatment. HD, hemodialysis.

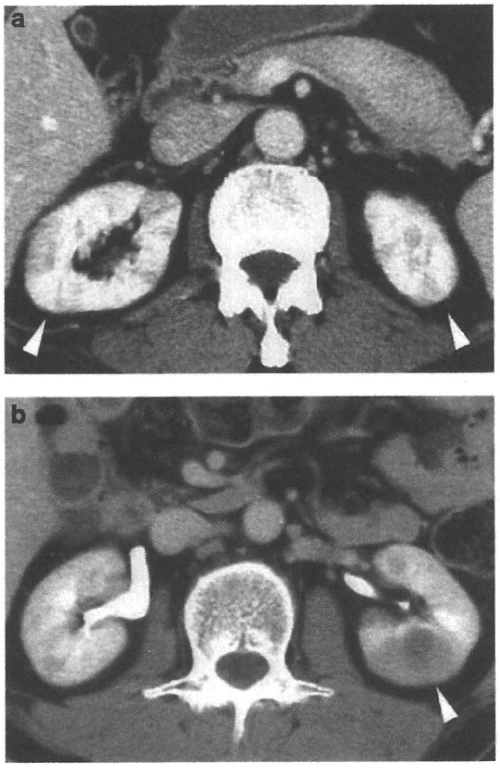

Figure 1 | Contrast-enhanced renal CT findings in patients with IgG4-related nephropathy. (**a**) Multiple wedge-shaped low-attenuation lesions in both renal cortices are evident (arrows, patient 3); (**b**) A tumor-like less-enhanced mass (arrow) is evident in the left kidney (patient 8). CT, computed tomography.

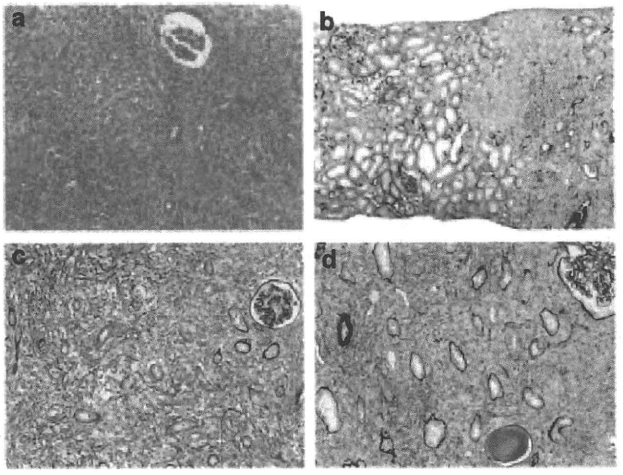

Figure 2 | Light microscopy findings in the renal tissues. (**a**) Diffuse marked renal interstitial inflammation consisting of lymphocytes and plasma cells with fibrosis is demonstrated, and most tubules are diminished (patient 8, HE; original magnification ×200); (**b**) Patchy distribution of dense lymphoplasmacytic infiltrates with irregular fibrosis, showing a clear margin (patient 16, PAM-Masson; original magnification ×80); (**c, d**) Collagen fibers exhibit a swirling pattern or an arabesque outline in a PAM-stained preparation, and the inflammatory cells show infiltration into the collagen fibers (panel **c**; patient 13, PAM-Masson; original magnification ×200, panel **d**; patient 21, PAM-Masson; original magnification ×250). HE, hematoxylin and eosin; PAM, periodic acid-methenamine.

parenchymal lesion was evident. Gallium citrate scintigraphy was performed before therapy in 17 patients (nos 2–9, 11–18, and 21), and all but 2 (nos 2 and 14) showed gallium-67 accumulation in both the kidneys. Gallium citrate scintigraphy performed during therapy showed no gallium-67 accumulation in the kidneys (patients 10 and 21).

Renal pathology

Tubulointerstitial nephritis was a dominant feature in all patients (Table 1). Light microscopy demonstrated dense cell infiltration with fibrosis and tubular atrophy. The infiltrate was predominantly composed of plasma cells and lymphocytes, and also eosinophils in some patients. Distribution of the lymphoplasmacytic infiltration was not only diffuse but also patchy, sometimes with a clear margin (Figure 2a and b). Tubule atrophy or diminishment developed according to the severity of cell infiltration and fibrosis. In the fibrotic interstitium, collagen fibers exhibited a swirling pattern or an arabesque outline in periodic acid-methenamine silver-stained preparations, and inflammatory cells infiltrated the collagen fibers, producing a characteristic pattern (Figure 2c and d). On light microscopy, the glomeruli were unremarkable in 18 patients, except for evidence of focal global sclerosis. One patient (no. 14) showed membranous nephropathy. Mild mesangioproliferative glomerulonephritis was evident in three patients (nos 6, 11, and 16), and focal segmental endocapillary hypercellularity was observed in one (no. 23). Direct immunofluorescence was evaluated in 14 patients (nos 1, 2, 5, 6, 9, 10, 11, 13, 14, 16, 18, 19, 21, and 22). In three patients (nos 1, 18, and 21), deposition of immunoglobulins and complement components was not confirmed in the glomeruli or the interstitium. In the patient with membranous nephropathy (no. 14), diffuse and global IgG and C3 deposits along the glomerular capillary walls, and focal deposits of IgG and C3 along the tubular basement membranes were observed. In patient 22, no deposition was observed in the glomeruli, and parts of the tubular basement membranes were positive for C3 deposition. Although the glomeruli on light microscopy were also unremarkable in the patient, electron microscopy revealed segmental subepithelial deposits on the glomerular basement membranes, and therefore, we diagnosed the patient as having tubulointerstitial nephritis with membranous nephropathy.[10] In three patients with mild mesangioproliferative glomerulonephritis (nos 6, 11, and 16), no apparent deposit was evident on the tubular basement membranes. In two of these patients (nos 6 and 16), mild IgG and IgA deposits (IgA was not dominant) were observed in the mesangial areas. In the other patient (no. 11), dominant IgA deposition was observed in the mesangial area, and we diagnosed the patient as having tubulointerstitial nephritis with IgA nephropathy. In four of

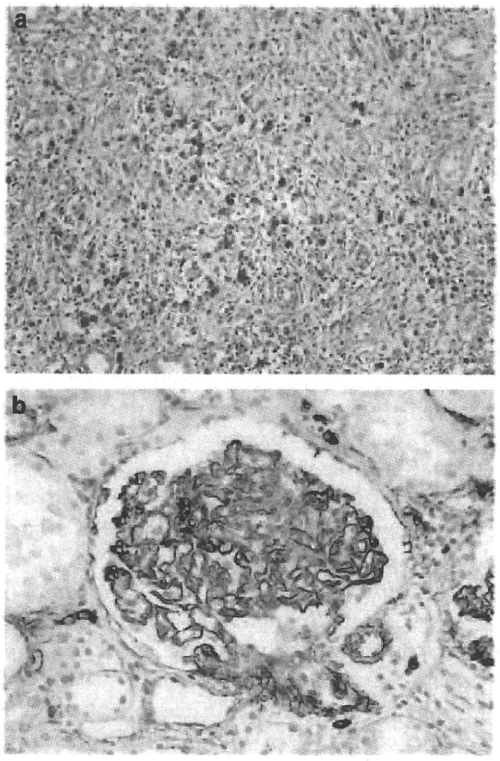

Figure 3 | Immunostaining of IgG4 in renal tissues. (**a**) Numerous IgG4-positive plasma cells are evident in the renal interstitium (patient 13, original magnification ×250); (**b**) In the patient with membranous nephropathy (patient 14), diffuse staining of glomerular capillary walls is also shown in addition to the IgG4-positive infiltrating plasma cells in the renal interstitium (original magnification ×400).

the other six patients (nos 5, 9, 13, and 19), segmental staining for IgG or C3 was shown in the glomeruli, with no deposits on the tubular basement membrane; faint or mild segmental mesangial staining for IgG in patients 5, 9, and 19, and mild segmental staining for C3 along the glomerular capillary wall in patient 13. In the other two patients (nos 2 and 10), nonspecific faint segmental staining for C3 was evident on the tubule basement membranes, without any deposits in the glomeruli.

Immunostaining for IgG4 revealed infiltration of numerous IgG4-positive plasma cells (IgG4-positive plasma cells/IgG-positive plasma cells >40%; IgG4-positive plasma cells >10 per h.p.f.) into the renal interstitium (Figure 3a). In the patient with membranous nephropathy (no. 14), IgG4 immunostaining also showed diffuse reactivity in the glomerular capillary walls, and focal staining of the tubule basement membrane, in addition to the infiltrating plasma cells (Figure 3b).

Treatment and course

The treatment regimen was decided according to the opinion of each attending physician (Table 1). Of the 23 patients, 21 were treated with prednisolone (initial dose 10–60 mg/day) for renal lesions. Intravenous methylprednisone pulse therapy (500–1000 mg for 3 days) was also conducted in three patients. One patient (no. 3) was followed up without therapy because both AIP and renal lesions improved spontaneously. Among patients treated with corticosteroid, the clinical data after 4 weeks were evaluable in 19. In 18 of those patients, abnormalities of renal function, complement level, and imaging features were all improved by therapy. Extrarenal lesions were also improved. In one patient with renal failure (no. 22), renal function did not recover, and maintenance hemodialysis became necessary.

DISCUSSION

Lesions of the kidney associated with IgG4-related disease include those affecting the renal parenchyma, and lesions of other areas.[7,11] Renal parenchymal lesions associated with IgG4-related disease were first described in 2004 as case reports of tubulointerstitial nephritis associated with AIP.[12,13] Thereafter, the number of case reports of tubulointerstitial nephritis associated with IgG4-related disease has been increasing,[14–18] although most of such cases have been accompanied by AIP and their number is still limited.

The clinicopathological features of IgG4-related disease have been described most extensively for cases with pancreatic involvement (that is, AIP).[19–22] It predominantly affects middle-aged to elderly men. The clinical symptoms are mild and the condition usually comes to clinical attention because of imaging abnormalities of the pancreas or obstructive jaundice. In most patients, extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, lymphadenopathy, and retroperitoneal fibrosis occur during the clinical course, sometimes simultaneously and often metachronously.[23] Laboratory examinations reveal characteristically increased levels of serum IgG and IgG4 (increased serum IgG4 is observed in 68–90% of Japanese patients with AIP),[22] and a recent study has shown that the serum IgE level is also frequently elevated.[24] Antinuclear antibodies and rheumatoid factor are often positive, but anti-SSA and anti-SSB antibodies are negative,[21] and hypocomplementemia is observed in 17–36% of the patients.[25] CT scan or magnetic resonance imaging shows diffuse pancreatic enlargement or focal masses. Corticosteroid therapy is usually quite effective for normalizing pancreatic lesions, and also the clinical and laboratory parameters. Histopathologically, dense lymphoplasmacytic infiltration, swirling or storiform fibrosis, and abundant IgG4-positive plasma cell infiltration revealed by IgG4 immunostaining are characteristic, and several studies have shown that IgG4-related disease shows similar pathological features, regardless of the specific type of organ involved.[8,26] In this study, we diagnosed 23 patients as having renal parenchymal lesions associated with IgG4-related disease on the basis of the renal pathological features described above, in addition to high levels of serum IgG4. As an elevated serum IgG4 level is characteristic of IgG4-related disease, but not diagnostic (sensitivity 75%, specificity 93% in AIP),[20] histopathological examination including IgG4

immunostaining is useful for diagnosis of this disease in addition to an elevated serum IgG4 level. Although 14 of the 23 patients did not have any pancreatic lesions, the clinical features were quite uniform and similar to those shown in AIP. Our results suggested that renal parenchymal lesions actually developed in association with IgG4-related disease, but not in association with AIP.

Tubulointerstitial nephritis is caused by various factors, including infections, drug reactions, urinary tract obstruction, autoimmune conditions, plasma cell dyscrasias, and metabolic disorders. Many patients in the present series showed hypergammaglobulinemia, hypocomplementemia, and positivity for anti-nuclear antibodies, being reminiscent of systemic lupus erythematosus, but none of them met the criteria for it. Although seven of the patients fulfilled the ordinary criteria for Sjögren's syndrome, typical Sjögren's syndrome does not show elevation of the serum IgG4 level and abundant IgG4-positive plasma cell infiltration.[3] Recent studies have shown that there are considerable differences between IgG4-related disease and Sjögren's syndrome, although distributions of the involved organs are similar,[2,3] and therefore it is important to recognize IgG4-related disease and to distinguish it from Sjögren's syndrome.[27] The clinical symptoms, laboratory findings such as hypocomplementemia, a low CRP level and negativity for ANCA, and also radiological findings, differed from those of drug-induced tubulointerstitial nephritis, infection, and ANCA-related vasculitis. The clinicopathological features of tubulointerstitial nephritis associated with IgG4-related disease are thus unique and distinct from those of other renal diseases.

Despite the characteristic clinicopathological features, the pathogenesis of IgG4-related disease remains poorly understood, although autoimmune or allergic mechanisms have been discussed.[5,19,20] Cornell *et al.*[28] demonstrated IgG4 immune-complex deposition in the renal tubule basement membranes of patients with tubulointerstitial nephritis associated with AIP, suggesting an immune-complex mechanism. In the present series, an apparent deposition of immune complex on the tubular basement membranes was evident in only one of the patients with membranous nephropathy by direct immunofluorescence. On the other hand, the relationship between glomerular lesions and IgG4-related disease is also poorly understood. Although the major pathological feature of this disease is tubulointerstitial nephritis, the glomeruli were also affected in a small number of cases in this study, and this has also been described previously,[12,15,29,30] membranous nephropathy being the most frequent feature among them. The significance of IgG4 has been documented in idiopathic membranous nephropathy, in which the predominance of Th2 cytokines is a common feature,[31,32] and also in IgG4-related disease,[5] suggesting a possible relationship between them. In view of the possible association of renal lesions with IgG4-related disease, glomerular lesions should be examined closely in addition to interstitial lesions. Further large-scale and detailed clinicopathological studies including electron microscopy examinations will be necessary to elucidate the pathogenesis of IgG4-related renal lesions.

As the concept of IgG4-related disease was proposed relatively recently, it remains largely unrecognized by most clinicians. However, accurate diagnosis of IgG4-related disease is very important because steroid therapy is usually quite effective.[19–22] In this study, the renal lesions had improved with corticosteroid therapy in most patients at the time of the 4-week follow-up. However, renal function did not recover in one patient (no. 22) with renal failure.[10] Although in this study we were unable to characterize the clinicopathological differences between patients with better or worse renal function, because it was a retrospective analysis and only one patient showed worsened renal function after treatment, it is important to be aware that renal failure may also occur in IgG4-related disease if the diagnosis is delayed. Nephrologists should be aware of the condition in patients with tubulointerstitial nephritis, and measure the serum IgG4 level, especially when there is associated sialadenitis, lymphadenopathy, hypergammaglobulinemia, eosinophilia, hypocomplementemia,[41] and a patchy lesion distribution. In addition, clinicians should be vigilant for the development of renal lesions at any time when following the course of involvement of other organs in patients with any IgG4-related disease, such as AIP. In five of the present patients, renal lesions developed during remission of the condition in other organs.

In conclusion, renal parenchymal lesions associated with IgG4-related disease appear to have characteristic clinicopathological features in comparison with those of other renal diseases, and therefore we propose the term 'IgG4-related tubulointerstitial nephritis' for this condition.

MATERIALS AND METHODS

Patients and methods

A total of 153 patients with suspected IgG4-related disease were collected retrospectively from 22 collaborating institutions in Japan between September 2004 and August 2009, among whom 30 were diagnosed as having renal parenchymal abnormalities by their physicians. Among these 30 patients, we diagnosed 23 as having renal parenchymal lesions associated with IgG4-related disease. (In Table 1, patients nos 3, 4, 5, 6, 8, 9, 13–16, 18 and 22 have been previously reported in references 10, 14, and 33–40.) The diagnosis was based on a high serum IgG4 level (>135 mg/dl) and numerous infiltration of IgG4-positive plasma cells into the renal interstitium (IgG4-positive plasma cells/IgG-positive plasma cells >40%; IgG4-positive plasma cells >10 per h.p.f.) with fibrosis. Although the serum IgG4 level was not measured in one patient, we also diagnosed this patient as having renal parenchymal involvement associated with IgG4-related disease because of the presence of tubulointerstitial nephritis with infiltration of numerous IgG4-positive plasma cells into the renal interstitium with typical AIP and Mikulicz's disease. In the other 7 of 30 patients, renal parenchymal abnormalities were diagnosed on the basis of radiographic abnormalities. Five of these patients were diagnosed as having IgG4-related sialadenitis and dacryoadenitis and showed multiple hypoattenuating lesions in the renal cortex by contrast-enhanced

CT, with normal urinalysis values and normal renal function. One other patient (under hemodialysis) was diagnosed as having IgG4-related sclerosing cholangitis and end-stage renal disease, with evidence of a mass lesion in the right kidney by nonenhanced CT. Renal biopsy was not performed in these six patients. Another patient showed a right renal mass lesion and a high serum IgG4 level, with normal urinalysis values and mild renal dysfunction. As the tissue obtained by CT-guided renal biopsy of the mass lesion did not include any renal parenchyma, even though marked lymphoplasmacytic infiltration was observed in the adipose tissues, we excluded this patient.

In the 23 patients diagnosed as having renal parenchymal lesions associated with IgG4-related disease, we retrospectively examined the clinical features, data from laboratory and imaging studies, and the clinical response to treatment. Whole-body CT imaging was evaluated in all patients (contrast-enhanced CT in 21 patients). Gallium citrate scintigraphy was performed in 19 of the 23 patients, before therapy in 17 and during therapy in 2. The diagnosis of AIP was made in accordance with the 2006 Japan Pancreas Society revised criteria.[42] Sialadenitis and dacryoadenitis were diagnosed on the basis of physical findings, and the results of imaging studies (CT and gallium citrate scintigraphy) and/or biopsy.

The study was approved by the review board of the Nagaoka Red Cross Hospital and the boards of the various collaborating institutions. All data and samples from patients were collected with their informed consent, and the research was conducted in compliance with the principles of the Declaration of Helsinki.

Renal pathology

Renal pathological examination was conducted at the request of the attending physicians because of urinary abnormalities, renal dysfunction, and/or radiological abnormalities. Renal tissues were obtained by nondirected medical needle biopsy in 21 patients, by open biopsy of the mass lesion in 1 patient (no. 8), and by autopsy in 1 patient (no. 3), who died of lung cancer. All renal tissue specimens were examined by light microscopy. Direct immunofluorescence studies were conducted in 17 patients, and we evaluated 14 specimens because 3 were considered inadequate. For routine light microscopy studies, renal biopsy specimens were fixed in formalin or alcohol-Bouin, embedded in paraffin, and stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver, and Masson's trichrome. For immunostaining, formalin-fixed, paraffin-embedded biopsy specimens were cut into 3-μm thick sections, and the sections were immunostained using anti-IgG antibody (Dako, Glostrup, Denmark) and mouse monoclonal antibody against human IgG4 (Zymed Laboratory, San Francisco, CA, USA, or The Binding Site, Birmingham, UK). For direct immunofluorescence studies, tissues were snap frozen, and sections were treated with fluorescein isothiocyanate-conjugated rabbit anti-human IgG, IgA, IgM, C1q, C3c, or fibrinogen (Dako, Carpinteria, CA, USA).

DISCLOSURE

All the authors declared no competing interests.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Analysis of Serum IgG Subclasses in Churg-Strauss Syndrome—The Meaning of Elevated Serum Levels of IgG4

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Abstract

Objective Mikulicz's disease (MD) is characterized by symmetrical and persistent enlargement of the lacrimal and salivary glands. Recently it has been categorized as an 'Ig (immunoglobulin) G4-related disease.' It presents with elevated serum levels of IgG4 and abundant infiltration of IgG4-bearing plasmacytes in involved organs. Allergic symptoms are often observed in patients with IgG4-related disease. On the other hand, allergic diseases are often complicated with Churg-Strauss syndrome (CSS). Here we focused on CSS and analyzed the relation of IgG4 in its pathogenesis.

Materials and Methods We analyzed five patients (2 men and 3 women) with CSS and 51 patients (20 men and 31 women) with MD who presented at Sapporo Medical University Hospital since 2001. We measured the serum concentrations of IgG subclasses in the patients with MD and CSS, and evaluated renal specimens from CSS patients, staining them for anti-IgG4 antibody.

Results We surprisingly found elevated serum levels of IgG4 not only in MD but also in CSS patients. The renal specimens in CSS patients revealed the infiltration of IgG4-positive plasmacytes.

Conclusion IgG4-bearing plasmacytes may be involved in the pathogenesis of CSS, and it is possible that an allergic reaction plays an important role in the pathogenesis of IgG4-related disease.

Key words: allergy, autoimmune pancreatitis, Churg-Strauss syndrome, IgG4, Mikulicz's disease, IgG4-related disease

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Introduction

Mikulicz's disease (MD) is characterized by symmetrical and persistent enlargement of the lacrimal and salivary glands (1). MD has been categorized as primary Sjögren's syndrome since 1953 (2), but more recently it is considered to be an 'Ig (immunoglobulin) G4-related disease (3)' because MD presents with elevated serum levels of IgG4 (4) and abundant infiltration of IgG4-bearing plasmacytes in lacrimal and submandibular glands (5). The origin of MD is unknown, but it has been associated with allergic symptoms, such as bronchial asthma and allergic rhinitis in approximately half of MD patients (6). In contrast, Churg-Strauss syndrome (CSS) is a rare systemic necrotizing vasculitis involving small vessels (arterioles, capillaries and venules). CSS invariably involves the lungs and may, additionally, affect a wide variety of other tissues and organs. In most cases of CSS, patients suffer from bronchial asthma or allergic rhinitis. Both MD and CSS are clinically quite different diseases except for the allergic symptoms. Here, we analyzed the serum levels of IgG subclasses in CSS patients, and considered the meaning of elevated levels of IgG4 in MD.

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Table 1. The Clinical Diagnostic Criteria of IgG4-related Mikulicz's Disease (The Japanese Medical Society for Sjögren's Syndrome, 2008)

1.	Persistent (>3 months), symmetrical swelling of the lacrimal, parotid and submandibular glands, involving at least two pairs.
2.	Serologically high levels of immunoglobulin (Ig) G4 (≥1.35 g/L).
3.	Marked IgG4-positive plasmacyte infiltration (≥50% IgG4-positive/IgG-positive cells in five high power fields) into lacrimal and salivary gland tissues.

In terms of diagnosis, IgG4-related Mikulicz's disease is defined as satisfying Item 1 and either Item 2 and/or 3. This form of systemic IgG4-related disease often accompanies multiple organ lesions. Sarcoidosis, Castleman's disease, Wegener's granulomatosis and malignant lymphoma need to be considered as differential diagnoses.

Patients and Methods

Study patients and materials

We analyzed five patients (2 men and 3 women) with CSS and 51 patients (20 men and 31 women) with MD who presented at Sapporo Medical University Hospital since 2001. The CSS patients met the criteria of the American College of Rheumatology (ACR) for diagnosing CSS (7), and the MD patients were categorized according to the criteria of the Japanese Medical Society for Sjögren's syndrome (2008) (Table 1) (8). Sapporo Medical University Ethics Committee approved this clinical study. Written informed consent was obtained from all patients. Serum samples were obtained pre-therapy, and stored at -80℃. Formalin-fixed paraffin-embedded blocks of renal tissue from the patients with CSS were analyzed.

Cases 1 and 2 of CSS were relapsed cases and prescribed with a small quantity of prednisolone. The rest of the CSS cases and MD cases were incipient. The mean age of subjects in the study was 35.6±14.1 years in the CSS patients, and 58.7±13.4 years in MD patients. In the CSS group, there was mononeuritis multiplex in all patients, pulmonary involvement and glomerulonephritis in 3 patients. In the MD group, there was autoimmune pancreatitis in 9 patients, tubulointerstitial nephritis in 8 patients, and pulmonary involvement in 5 patients, and retroperitoneal fibrosis in 10 patients (Table 2).

Clinical data

We analyzed the counts of leukocytes and eosinophils, and the values of IgE, total complement activity (CH50), rheumatoid factor (RF), myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA), and C-reactive protein (CRP), which were examined in the course of medical treatment.

Nephelometry

Pre-therapy serum levels of IgG subclasses from patients were measured with a Behring nephelometer (Dade Behring, Deerfield, IL, USA) using IgG subclasses (BS-NIA IgG1-4; The Binding Site, Birmingham, UK) as antibodies for 0.4 mL serum samples. Diluted samples (N-dilution liquid; Oriental Yeast, Tokyo, Japan, in 1: 20-100) of standard and control sera were introduced into the reaction tubes of the nephelometer. Appropriate anti-IgG subclass reagents and reaction buffer (N-responsive buffer liquid; Oriental Yeast) were added. Dispersion strength according to irradiation from a light-emitting diode was measured at a wavelength of 840 nm, and contrasted to dispersion by available light after 10 seconds and again after 6 minutes. IgG subclass concentrations in test samples were calculated relative to calibration curves, obtained using nephelometric IgG subclass standard sera. A control serum was assayed to confirm the validity of calibration curves and the accuracy of IgG subclass determinations.

Immunohistochemistry

For anti-IgG4 antibody immunostaining, the monoclonal antibodies were reacted for 24 hours at 4℃ with steam after endogenous peroxidase activity was stopped in each section. Primary antibodies comprised anti-IgG4 antibodies (Mouse anti-human IgG4; The Binding Site, Birmingham, UK) diluted 1 : 500. Secondary antibodies (Biotinylated anti-mouse IgG (H+L); Vector, Burlingame, CA, USA) were diluted 1 : 500. Nuclear-staining was performed using Hematoxylin after indirect peroxidase staining in renal specimens from the patients with CSS.

Results

Leukocytosis was observed in all patients with CSS, but in only two patients (3.9%) with MD. Eosinophilia was also detected in all CSS cases and in 14 cases (27.5%) with MD. The eosinophil count in MD was not as high as that in CSS.

Table 2. The Clinical and Serological Data of Five Patients with Churg-Strauss Syndrome and the Mean Data of IgG4-related Mikulicz's Disease

	CSS					Average of MD 51 cases
	Case 1	Case 2	Case 3	Case 4	Case 5	
Age (y.o.), Sex (M:F)	19 F	25 M	38 F	37 M	51 F	58.7±13.4, 20:31
Organ failure	PH	GIB	MM	P	PH	AIP 9 cases
	MM	HAA		MM	GN	TIN 8 cases
	GN	MM		GN	MM	P 5 cases
Leukocytes (/μL)	47,700	13,700	20,500	16,300	18,800	5,868±1,630
Eosinophils (/μL)	36,872	8,220	10,455	6,846	10,077	320± 281
IgG (g/L)	20.80	9.74	15.10	20.60	21.90	26.78±16.07
IgG1 (g/L, %)	11.40, 38.11	3.81, 47.69	4.93, 43.82	11.60, 49.33	9.36, 42.01	11.94±5.59, 41.13±6.29
IgG2 (g/L, %)	8.56, 28.61	3.24, 40.56	4.55, 40.44	7.33, 31.17	6.49, 29.13	8.28±2.47, 30.75±8.06
IgG3 (g/L, %)	0.20, 0.66	0.19, 2.40	0.20, 1.78	0.42, 1.80	0.39, 1.75	0.67±0.57, 2.35±1.54
IgG4 (g/L, %)	9.76, 32.62	0.75, 9.35	1.57, 13.96	4.16, 17.69	6.04, 27.11	8.91±7.33, 25.76±9.89
IgE (IU/mL)	75	253	650	1120	3300	304.1±321.7
CH50 (U/mL)	66.0	64.7	54.8	48.7	52.3	36.7±12.3
RF (IU/mL)	73	40	172	10	370	29.1±56.0
MPO-ANCA (EU)	222	720	262	279	640	All normal
CRP (mg/L)	138.5	51.2	6.0	85.9	78.9	2.5±3.3
Prognosis	Alive	Alive	Alive	Dead	Dead	All alive

CSS: Churg-Strauss syndrome, MD: IgG4-related Mikulicz's disease, M: male, F: female, PH: pulmonary hemorrhage, MM: mononeuropathy multiplex, GN: glomerulonephritis, GIB: gastrointestinal bleeding, HAA: hepatic artery aneurysm, P: pneumonia, AIP: autoimmune pancreatitis, TIN: tubulointerstitial nephritis, CH50: total complement activity, RF: rheumatoid factor, MPO-ANCA: myeloperoxidase anti-neutrophil cytoplasmic antibodies, CRP: C-reactive protein

Serologically, the mean total IgG level was 17.62±5.14 g/L in CSS and 26.78±16.07 g/L in MD. Both diseases presented with hypergammaglobulinemia. The three cases in CSS and 11 cases (21.6%) with MD showed elevated serum levels of IgE (normal range, <450 IU/mL). The serum IgE levels in CSS patients showed a tendency to be significantly higher than those in MD patients. As for the total complement activity, all cases with CSS presented with elevated levels of CH50 (normal range, 30.0-50.0 U/mL). On the other hand, 13 cases (25.5%) with MD disclosed hypocomplementemia. There were four RF-positive cases with CSS, and 13 RF-positive cases (25.5%) with MD. All patients with CSS had MPO-ANCA, but it was not detected in the MD patient group. CSS patients showed positive CRP (normal range, <3.0 mg/L), but there were only seven CRP-positive patients (13.7%) with MD.

With regard to IgG subclass, the amount of IgG1 and IgG4 was 8.22±3.64 g/L and 4.46±3.63 g/L in CSS patients, and 11.94±5.59 g/L and 8.91±7.33 g/L in MD patients. It tended to be elevated serum levels of IgG4 in the CSS group. The mean ratios of each IgG subclass to total IgG are shown in Fig. 1. Surprisingly, there were no significant differences in IgG subclasses between CSS and MD groups. The ratio of IgG1 and IgG4 to total IgG was 44.19±4.49% and 20.15±9.55% in CSS, and 41.13±6.29% and 25.76± 9.89% in MD (Table 2).

Tissue specimens from the kidneys of 3 patients with CSS revealed infiltration of numerous IgG4-producing cells by anti-IgG4 antibody staining (Fig. 2).

Discussion

Recently, worldwide attention has been drawn to the new concept of 'systemic IgG4-related plasmacytic syndrome (SIPS) (3),' which originated from Japan. Untilrecently this disease was referred to by various names, 'IgG4-positive multi-organ lymphoproliferative syndrome (IgG 4 + MOLPS) (6),' 'IgG4-related sclerosing disease (10),' but we found and recognized that they were the same. Thus recently, the name was finally unified to 'IgG4-related disease' at the IgG4+MOLPS Study Group Meeting granted from Ministry of Health, Labour and Welfare, Japan. However recently, there is a tendency to label all pathogenesis, which presents with elevated levels of serum IgG4 and infiltration of IgG4-bearing plasmacyte in the involved organ, 'IgG4-related disease (11, 12).' The confusion occurs in the diagnosis and interpretation of this disease (13). We consider that there is 'IgG4-related disease' in a narrow and wide sense. Original 'IgG4-related disease,' called in a narrow sense, includes MD, autoimmune pancreatitis (AIP), and the diseases, which complicate them. The basic characteristics of these diseases, except IgG4, are the swelling of the in-

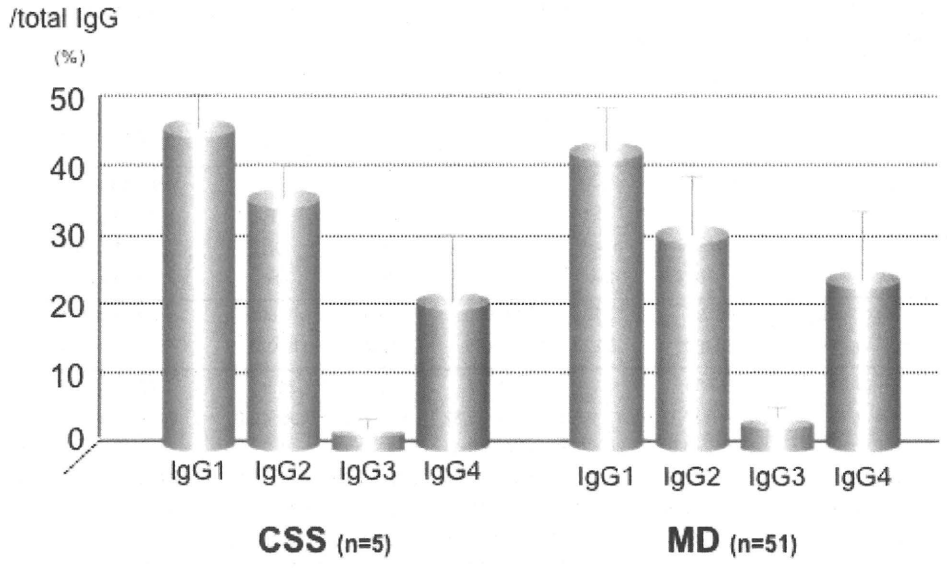

Figure 1. The ratio of each IgG subclass to total IgG in patients with Churg-Strauss syndrome and Mikulicz's disease. The pattern of the ratio is similar in patients with Churg-Strauss syndrome and Mikulicz's disease.

Figure 2. The renal specimen with anti-IgG4 antibody stain in a patient with Churg-Strauss syndrome. Renal biopsy revealed the infiltration of IgG4-bearing plasma cells in Churg-Strauss syndrome.

volved organ and lack of systemic inflammation. The other characteristics are shown in Table 3. The 'IgG4-related disease' without the above features means 'IgG4-related disease' in a broad sense. We do not know whether this view is correct, and whether both are actually the same disease, however we want to describe here 'IgG4-related disease' in a narrow sense.

Before the present study, there has been no report on serum IgG subclasses in patients with CSS. This study revealed elevated serum levels of IgG4 in CSS patients as well as in MD patients. The inflammation with IgG4-bearing plasmacytes was also detected in the renal specimen of CSS. Is CSS an 'IgG4-related disease' in a narrow sense? CSS is basically vasculitis, which may involve systemic organs. It shows systemic inflammation, and does not present with swelling of the involved organ. CSS is clinically quite different from MD and AIP. It could be considered that CSS is not included in 'IgG4-related disease' in the narrow sense.

Why is the level of serum IgG4 elevated in CSS? There are few little reports on IgG subclasses in other eosinophilic disorders or allergic diseases other than CSS. It was reported that elevated serum concentrations of IgG4 is sometimes observed in severe asthma (14), but it was not observed in mild or typical asthma (15). Anti-aspergillus fumigatus IgG4-antibodies were detected in the patients with allergic bronchopulmonary aspergillosis complicated with cystic fibrosis (16). It will be necessary to comprehensively analyze IgG subclasses in other disorders, but it is traditionally suggested that the IgG4 is concerned with allergy (17, 18). Kamisawa et al analyzed the allergic manifestations in AIP, and reported that they were detected in about half of the AIP patients (19). CSS is based on allergic disorders, such as bronchial asthma or chronic sinusitis. It is an allergy to be common in CSS and 'IgG4-related disease' in a narrow sense. Therefore, it is considered that an allergic reaction may play an important role in the mechanism of elevated serum levels of IgG4.

The detailed mechanism of the elevated levels of serum IgG4 in CSS and 'IgG4-related disease' in a narrow sense is still unknown. It has been considered that Th2 cytokines, such as interleukin (IL)-4, IL-5 and IL-10 are very important in allergy. It is known that they act on the proliferation and the induction of eosinophils, and the class switching to IgE (20, 21). IL-5 is the representative cytokine, which activates eosinophils (22), and the signals of IL-4/IL-13 and ligation of the CD40 induce IgE class switching (23, 24). Zen et al reported that the cytokines, mainly interleukin

Table 3. The Clinical and Histological Characteristics of IgG4-related Disease

1. Elevated serum levels of IgG4
2. Pathological characteristics;
   1) Abundant infiltration of lymphocytes and plasma cells
   2) Infiltration of IgG4-positive plasmacytes
   3) Infiltration of eosinophils
   4) Fibrosis around glands (Sclerosing lesions)
   5) Obstructive phlebitis
3. Efficacy of glucocorticoid for a short term
4. Spatio-temporal complications

(IL)-10, promote the production of IgG4 in AIP (9). As for IL-10, it decreases IL-4-induced IgE switching. IgE versus IgG4 production can be differentially regulated by IL-10 (25). On the other hand, Saito et al analyzed the cytokine profile in CSS and reported that IL-10 is reversely decreased in the active stage of CSS, and increased in the inactive stage (26, 27). CD4+ T cells from patients with active CSS rather tend toward Th17 (27). We do not know whether this difference leads to the difference between CSS and 'IgG4-related disease' in a narrow sense. The allergy, which is based on Th2 cytokines, is important, but it is suggested that the mechanism by which regulatory T cells are influential differes in the two diseases. To resolve this issue, we have to further analyze the relationship between IgG4 and cytokine profiles.

The novel findings were a shock to us, as we considered that only elevated levels of serum IgG4 and infiltration of IgG4-positive plasma cells in the involved organs were very important in diagnosing 'IgG4-related disease.' However we must remember that they simultaneously gave us a new start. In other words, 'IgG4-related disease' is carefully diagnosed based not only on IgG4 but also on other factors, such as physical findings and images. This study also suggested that IgG4 itself is not the fundamental cause of 'IgG 4-related disease.' It may be true that the elevated levels of serum IgG4 and the infiltration of plasmacytes with IgG4 are only part of the process of the some immunological reactions.

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Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis

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IgG4-related disease is a recently recognized multi-organ disorder characterized by high levels of serum IgG4 and dense infiltration of IgG4-positive cells into several organs. Although the pancreas was the first organ recognized to be affected by IgG4-related disorder in the syndrome of autoimmune pancreatitis, we present here clinicopathological features of 23 patients diagnosed as having renal parenchymal lesions. These injuries were associated with a high level of serum IgG4 and abundant IgG4-positive plasma cell infiltration into the renal interstitium with fibrosis. In all patients, tubulointerstitial nephritis was the major finding. Although 14 of the 23 patients did not have any pancreatic lesions, their clinicopathological features were quite uniform and similar to those shown in autoimmune pancreatitis. These included predominance in middle-aged to elderly men, frequent association with IgG4-related conditions in other organs, high levels of serum IgG and IgG4, a high frequency of hypocomplementemia, a high serum IgE level, a patchy and diffuse lesion distribution, a swirling fibrosis in the renal pathology, and a good response to corticosteroids. Thus, we suggest that renal parenchymal lesions actually develop in association with IgG4-related disease, for which we propose the term 'IgG4-related tubulointerstitial nephritis.'

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KEYWORDS: autoimmune pancreatitis; corticosteroid; fibrosis; IgG4; tubulointerstitial nephritis

IgG4-related disease represents a recently recognized group of multi-organ diseases characterized by a high level of serum IgG4 and dense infiltration of IgG4-positive cells into multiple organs.[1–3] The condition was first described in relation to the pancreas (that is, autoimmune pancreatitis (AIP)),[4] and has since been expanded to various organ systems. At present, many other inflammatory conditions affecting multiple organs are considered to fall within the category of IgG4-related disease,[1–3] including sclerosing cholangitis,[5] sialadenitis,[2,6] retroperitoneal fibrosis,[7] interstitial pneumonitis,[8] inflammatory pseudotumor,[1–3] and periaortitis.[9] Although much attention is now being focused on these conditions, and the number of case reports of IgG4-related disease has been increasing, there are still few clinicopathological data on the involvement of organs other than the pancreas. The aim of this study was to elucidate the clinicopathological characteristics of renal parenchymal lesions associated with IgG4-related disease.

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RESULTS

Patient profiles

Patients were all Japanese (20 men and 3 women) with an average age of 65.2 ± 10.1 (40–83) years at the time of diagnosis of renal disease. Although seven patients fulfilled the criteria for Sjögren's syndrome, as revised by the Japanese Ministry of Welfare in 1999, none of them met the criteria for systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis, rheumatoid arthritis, or sarcoidosis. None of the organ specimens showed any evidence of malignant lymphoma by the DNA PCR method or immunohistochemistry. At the time of diagnosis of renal involvement, one patient had been treated with low-dose corticosteroid (prednisolone 2.5 mg daily) because of AIP (patient 5 in Table 1). Three patients had been treated with the corticosteroid because of AIP (patients 19 and 22 in Table 1) or idiopathic thrombocytic purpura (patient 8 in Table 1), but the treatment was discontinued when they developed renal involvement. The other 19 patients had not received corticosteroids or immunosuppressants before the appearance of renal lesions.

Clinical features

Of the 23 patients, 22 (95.7%) had some accompanying extrarenal lesions: sialadenitis in 19 (82.6%), lymphadenopathy in 10 (43.5%), AIP in 9 (39.1%), dacryoadenitis in 7 (30.4%), lung lesions (interstitial pneumonitis and nodular lesions) in 6 (26.0%), and others in 3 (pseudotumor of the liver, prostatitis, and idiopathic thrombotic purpura) (Table 1). Among the extrarenal lesions, 15 (represented in bold in Table 1) had been recognized before diagnosis of the renal lesions, and 10 of these 15 lesions in patients 3, 5, 8, 19, and 22 had improved with steroid therapy, or spontaneously, at the time of diagnosis of the renal lesions. The other 39 lesions were diagnosed at the same time as the renal lesions. Clinical symptoms were mostly associated with extrarenal lesions, such as gland swelling and lymphadenopathy. Fever, arthralgia, skin eruption, and edema were observed in 3, 5, 1, and 2 patients, respectively. In 21 of the 23 patients, the renal lesions were observed by physicians because of urinary abnormalities, renal dysfunction, and/or abnormalities revealed by radiological examinations, including computed tomography (CT) and gallium citrate scintigraphy, during follow-up or further examinations of the IgG4-related extrarenal lesions. In patients 13 and 14, renal lesions were observed because of renal dysfunction in the absence of extrarenal lesions. The results of renal histological and laboratory findings alerted the attending physicians to IgG4-related disease, who then carried out measurements of serum IgG4 levels and IgG4 immunostaining. (Additional gallium citrate scintigraphy showed gallium-67 accumulation of both the salivary glands in patient 13.)

Laboratory findings at the time of diagnosis of the renal lesions

Urinary protein excretion was <0.3 g/day in 19 of the 23 patients (Table 1). Urinary protein excretion >1.0 g/day was shown in two patients with membranous nephropathy (patients 14 and 22). Hematuria (urinary red blood cells >3 per h.p.f. (high-power field)) was shown in eight patients. Although mostly mild, hematuria with urinary red blood cells >20 per h.p.f. was evident in three patients with glomerular lesions (patients 14, 22, and 23). Hematological examinations revealed anemia (hemoglobin <10.0 g/dl) in only one patient (hemoglobin 9.0 g/dl in patient 20). Although white blood cell counts were within the normal range in most patients (mild leukocytosis was observed in 2 patients), eosinophila (eosinophils >5%) was observed in 11 patients (47.8%). Platelet counts were within the normal range in all patients. Renal function varied from normal to renal failure (serum creatinine 0.67–6.87 mg/dl). Elevated creatinine levels (serum creatinine >1.2 mg/dl) were shown in 13 of the 23 patients. Abnormal liver function at the time of diagnosis of the renal lesions was observed in three patients; AST (aspartate transaminase) 53 IU/l (range: 13–33), ALT (alanine transaminase) 101 IU/l (range: 10–47), ALP (alkaline phosphatase) 914 IU/l (range: 115–359) in patient 4, AST 16 IU/l, ALT 20 IU/l, ALP 573 IU/l in patient 5, and AST 55 IU/l, ALT 115 IU/l, ALP 1690 IU/l in patient 22. Pancreatic swelling due to AIP had improved by this time in patients 5 and 22, and no patient showed CT abnormalities in the liver or pancreatobiliary system.

All patients showed elevated levels of serum IgG (2721–8841 mg/dl, mean 4836 ± 1499 mg/dl, normal range 870–1700). Although serum IgA and IgM levels were within the normal ranges in all patients, elevation of the serum IgE level (272–4442 IU/ml, normal range <250 IU/ml) was observed in 10 of 14 evaluated patients (71.4%). Serum IgG4 levels before steroid therapy ranged from 587 to 4630 mg/dl (mean 1520 ± 909, normal range <105 mg/dl). In 16 of the 23 patients (69.6%), the serum CH50 level was found to be decreased, together with a reduction in the serum level of C3, C4, or both. In two patients (nos 20 and 21), only the C3 level was decreased. The rheumatoid factor was positive in 7 of 18 evaluated patients (38.9%). Although anti-nuclear antibodies were positive in 69.6%, anti-SS-A, anti-SS-B, anti-Sm, and anti-RNP antibodies were all negative, and the levels of anti-DNA antibodies were not increased in any of the patients. The level of CRP was <1.5 mg/dl in 22 of the 23 patients, and 8.7 mg/dl in 1 patient (patient 7). Cryoglobulin, M-protein, myeloperoxidase-ANCA, and proteinase-3-ANCA were not observed in any of the patients.

Imaging studies

CT revealed abnormal renal parenchymal lesions in 16 of the 23 patients (69.6%) (Table 1). Among them, diffuse swelling of the bilateral kidneys was evident in seven patients. Patchily distributed hypoattenuated lesions in the renal cortex, being single or multiple, round or wedge shaped, were found in 10 patients (Figure 1). Renal pelvic tumor and calicectasis were observed in addition to the renal parenchymal lesions in patients 2 and 4, respectively. In patient 1, thickening of the renal pelvic wall was observed, although no renal

Table 1 | Clinicopathological features of 23 patients with IgG4-related tubulointerstitial nephritis

No	Age/sex	Renal biopsy findings	U-Pr/U-B	Cr at biopsy	IgG (*N*:870–1700)	IgG4 (*N*<105)	IgE (*N*<250)	Low CH50	Low C3	Low C4	ANA	RF	Extrarenal lesions	Renal CT findings	Tx of pre-/post-biopsy	Cr at 1 M after Tx	References
1	58/F	TIN	0.23 g/day/(±)	0.67	7319	4630	4442	(−)	(−)	(−)	(−)	(+)	La, Sa, AIP, Ly	Thickening of renal pelvis	(−)/PSL40	0.59	
2	76/F	TIN	(−)/(−)	0.69	2721	769	267	(−)	(−)	(−)	(+)	(−)	La, Sa, Ly, Lu	Pa, pelvic tumor	(−)/PSL20	0.64	
3	76/M	TIN	(−)/(−)	0.71	3486	1030	NA	(+)	(−)	(+)	(−)	(−)	***Sa***, AIP	Pa	(−)/(−)	0.69	Saeki *et al.*[33,34]
4	40/F	TIN	(−)/(−)	0.74	3450	2400	272	(+)	(−)	(+)	(−)	(+)	La, Sa, Lu	Pa, S, calicectasis	(−)/Pulse+PSL60	0.58	Shimoyama *et al.*[35]
5	52/M	TIN	(−)/(−)	0.80	3180[a]	1430[a]	NA	(−)	(−)	(−)	(−)	(+)	***Sa, AIP, Lu***	Pa	PSL2.5/PSL10	0.7	Nakamura *et al.*[36]
6	56/M	TIN+mesPGN	(+)/(−)	0.9	5680	1920	248	(+)	(+)	(+)	(+)	(−)	La, Sa, Ly	Pa	(−)/PSL50	0.9	Yamamoto *et al.*[37]
7	70/M	TIN	(±)/(−)	0.9	3496	623	NA	(+)	(+)	(+)	(+)	(−)	AIP	S	(−)/PSL30	0.8	
8	61/M	TIN	(−)/(−)	1.09	6569	730[a]	1049[a]	(+)	(+)	(+)	(+)	NA	***Sa, AIP, ITP***, Ly	Pa	(−)/PSL60[b]	0.97	Saeki *et al.*[14,33,34]
9	74/M	TIN	(+)/(−)	1.1	4387	1320	560	(+)	(+)	(+)	(+)	(+)	***Sa***	S	(−)/PSL30	0.9	Nakada *et al.*[38]
10	58/M	TIN	(−)/(−)	1.15	2850	1470	456	(−)	(−)	(−)	(+)	NA	Pseudo-tumor (liver)	Pa	(−)/PSL30	1.06	
11	62/M	TIN+IgAGN	(−)/(±)	1.3	8194	NA	704	(+)	(+)	(+)	(+)	(+)	La, Sa, AIP, Ly	S	(−)/Pulse+PSL30	1.1	
12	75/M	TIN	(+)/(−)	1.34	5380	587	NA	(+)	(+)	(+)	(+)	(+)	Sa, Ly, Lu	Pa	(−)/PSL30	1.14	
13	68/M	TIN	(−)/(−)	1.37	2995	670	2323	(+)	(+)	(+)	(+)	(−)	Sa	Normal	(−)/PSL40	1.19	Saeki *et al.*[14,33]
14	83/M	TIN+MN	2.3 g/day/(3+)	1.48	3144	924	32	(+)	(+)	(+)	(+)	NA	(−)	Normal	(−)/PSL40	1.39	Saeki *et al.*[39]
15	60/M	TIN	(−)/(−)	1.7	8841	1028	NA	(+)	(+)	(+)	(+)	(−)	***Ly, Lu***, Sa	Normal	(−)/PSL30	NA	Takamura *et al.*[40]
16	60/M	TIN+mesPGN	(+)/(±)	1.75	5188	305[a]	NA	(+)	(+)	(+)	(+)	(+)	Sa, Ly	S	(−)/PSL50	1.55	Saeki *et al.*[14,33,34]
17	61/M	TIN	NA	2.0	8005	2390	858	(+)	(+)	(+)	(+)	(−)	Sa, Ly	S	(−)/unknown	NA	
18	55/M	TIN	(+)/(−)	2.1	5040	1780	NA	(−)	(−)	(−)	(+)	(+)	***AIP***, Sa	Pa	(−)/PSL40	1.3	Saeki *et al.*[33]
19	69/M	TIN	0.25 g/day/(±)	2.36	4001	1340	NA	(+)	(+)	(+)	(−)	NA	***AIP***	Normal	(−)/PSL30[b]	2.1	
20	64/M	TIN	NA	2.9	5100	1360	92	(−)	(+)	(−)	(+)	(−)	La, Sa	Normal	(−)/PSL50	NA	
21	76/M	TIN	0.3 g/day/(+)	5.4	2963	1800	125	(−)	(+)	(−)	(−)	(−)	***Sa***	Normal (nonenhanced)	(−)/PSL40	2.9	
22	78/M	TIN+MN	1.4 g/day/(2+)	6.17	3731	1860	NA	(+)	(+)	(−)	(−)	(−)	***Sa, AIP***	Pa	(−)/PSL20[b]	HD	Saida *et al.*[10]
23	68/M	TIN+endocap	(2+)/(2+)	6.87	4661	1120	335	(+)	(+)	(+)	(+)	(−)	La, Sa, Ly, Lu, P	S (nonenhanced)	(−)/PSL30	1.45	

Abbreviations: ANA, antinuclear antibody; Cr, serum creatinine (mg/dl); CT, computed tomography; endocap, endocapillary hypercellularity; IgAGN, IgA nephropathy; IgG, serum IgG (mg/dl); IgE, serum IgE (IU/ml); IgG4, serum IgG4 (mg/dl); Low CH50,C3,C4, low titer of serum CH50,C3,C4; M, month; mesPGN, mesangioproliferative glomerulonephritis; MN, membranous nephropathy; NA, not available; pre-/post-biopsy, pre-renal biopsy/post-renal biopsy; RF, rheumatoid factor; TIN, tubulointerstitial nephritis; Tx, treatment; U-B, hematuria; U-Pr, proteinuria.

[a]Value under steroid therapy. AIP, autoimmune pancreatitis; ITP, idiopathic thrombocytic purpura; La, dacryoadenitis; Lu, lung lesion; Ly, lymphadenitis; P, prostatitis; Pa, patchy lesion; PSL, prednisolone mg/day; S, diffuse swelling; Sa, sialadenitis (bold and italic extrarenal lesions were diagnosed before diagnosis of renal lesion).

[b]History of steroid treatment. HD, hemodialysis.

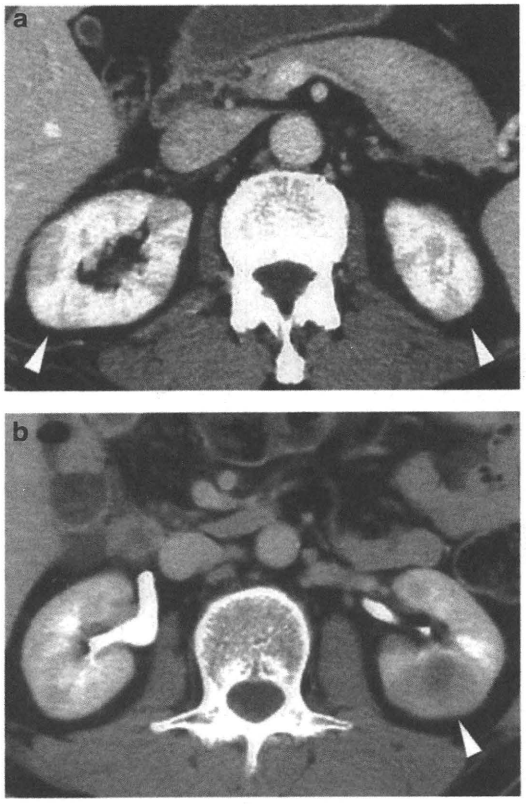

Figure 1 | Contrast-enhanced renal CT findings in patients with IgG4-related nephropathy. (**a**) Multiple wedge-shaped low-attenuation lesions in both renal cortices are evident (arrows, patient 3); (**b**) A tumor-like less-enhanced mass (arrow) is evident in the left kidney (patient 8). CT, computed tomography.

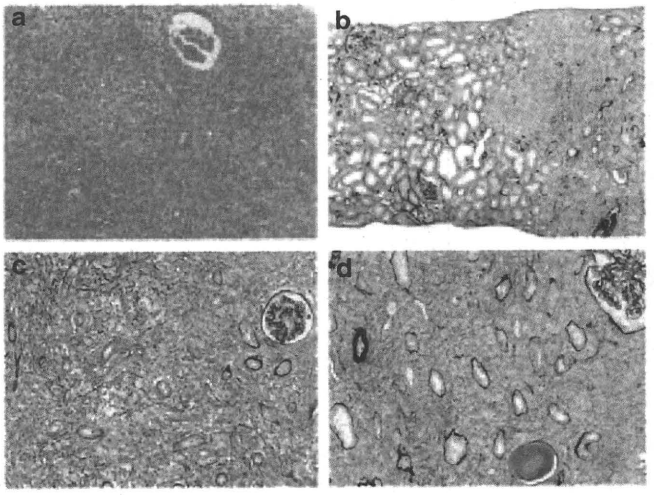

Figure 2 | Light microscopy findings in the renal tissues. (**a**) Diffuse marked renal interstitial inflammation consisting of lymphocytes and plasma cells with fibrosis is demonstrated, and most tubules are diminished (patient 8, HE; original magnification × 200); (**b**) Patchy distribution of dense lymphoplasmacytic infiltrates with irregular fibrosis, showing a clear margin (patient 16, PAM-Masson; original magnification × 80); (**c**, **d**) Collagen fibers exhibit a swirling pattern or an arabesque outline in a PAM-stained preparation, and the inflammatory cells show infiltration into the collagen fibers (panel **c**; patient 13, PAM-Masson; original magnification × 200, panel **d**; patient 21, PAM-Masson; original magnification × 250). HE, hematoxylin and eosin; PAM, periodic acid-methenamine.

parenchymal lesion was evident. Gallium citrate scintigraphy was performed before therapy in 17 patients (nos 2–9, 11–18, and 21), and all but 2 (nos 2 and 14) showed gallium-67 accumulation in both the kidneys. Gallium citrate scintigraphy performed during therapy showed no gallium-67 accumulation in the kidneys (patients 10 and 21).

Renal pathology

Tubulointerstitial nephritis was a dominant feature in all patients (Table 1). Light microscopy demonstrated dense cell infiltration with fibrosis and tubular atrophy. The infiltrate was predominantly composed of plasma cells and lymphocytes, and also eosinophils in some patients. Distribution of the lymphoplasmacytic infiltration was not only diffuse but also patchy, sometimes with a clear margin (Figure 2a and b). Tubule atrophy or diminishment developed according to the severity of cell infiltration and fibrosis. In the fibrotic interstitium, collagen fibers exhibited a swirling pattern or an arabesque outline in periodic acid-methenamine silver-stained preparations, and inflammatory cells infiltrated the collagen fibers, producing a characteristic pattern (Figure 2c and d). On light microscopy, the glomeruli were unremarkable in 18 patients, except for evidence of focal global sclerosis. One patient (no. 14) showed membranous nephropathy. Mild mesangioproliferative glomerulonephritis was evident in three patients (nos 6, 11, and 16), and focal segmental endocapillary hypercellularity was observed in one (no. 23). Direct immunofluorescence was evaluated in 14 patients (nos 1, 2, 5, 6, 9, 10, 11, 13, 14, 16, 18, 19, 21, and 22). In three patients (nos 1, 18, and 21), deposition of immunoglobulins and complement components was not confirmed in the glomeruli or the interstitium. In the patient with membranous nephropathy (no. 14), diffuse and global IgG and C3 deposits along the glomerular capillary walls, and focal deposits of IgG and C3 along the tubular basement membranes were observed. In patient 22, no deposition was observed in the glomeruli, and parts of the tubular basement membranes were positive for C3 deposition. Although the glomeruli on light microscopy were also unremarkable in the patient, electron microscopy revealed segmental subepithelial deposits on the glomerular basement membranes, and therefore, we diagnosed the patient as having tubulointerstitial nephritis with membranous nephropathy.[10] In three patients with mild mesangioproliferative glomerulonephritis (nos 6, 11, and 16), no apparent deposit was evident on the tubular basement membranes. In two of these patients (nos 6 and 16), mild IgG and IgA deposits (IgA was not dominant) were observed in the mesangial areas. In the other patient (no. 11), dominant IgA deposition was observed in the mesangial area, and we diagnosed the patient as having tubulointerstitial nephritis with IgA nephropathy. In four of

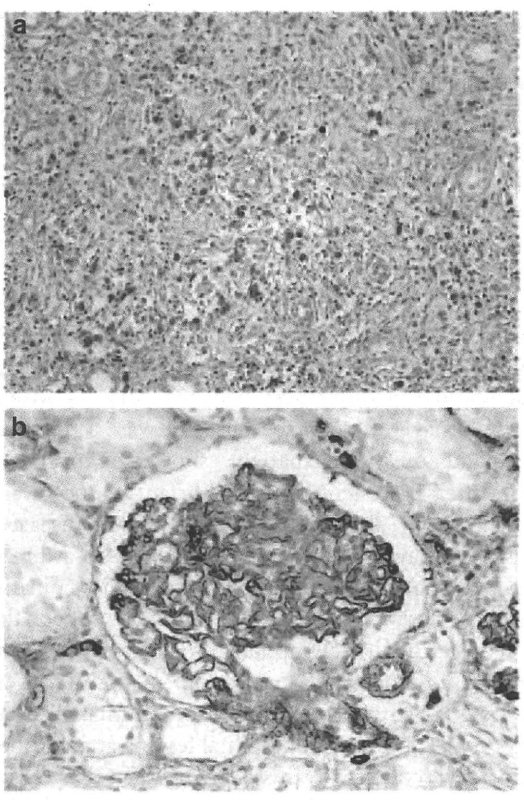

Figure 3 | Immunostaining of IgG4 in renal tissues.
(**a**) Numerous IgG4-positive plasma cells are evident in the renal interstitium (patient 13, original magnification × 250); (**b**) In the patient with membranous nephropathy (patient 14), diffuse staining of glomerular capillary walls is also shown in addition to the IgG4-positive infiltrating plasma cells in the renal interstitium (original magnification × 400).

the other six patients (nos 5, 9, 13, and 19), segmental staining for IgG or C3 was shown in the glomeruli, with no deposits on the tubular basement membrane; faint or mild segmental mesangial staining for IgG in patients 5, 9, and 19, and mild segmental staining for C3 along the glomerular capillary wall in patient 13. In the other two patients (nos 2 and 10), nonspecific faint segmental staining for C3 was evident on the tubule basement membranes, without any deposits in the glomeruli.

Immunostaining for IgG4 revealed infiltration of numerous IgG4-positive plasma cells (IgG4-positive plasma cells/IgG-positive plasma cells >40%; IgG4-positive plasma cells >10 per h.p.f.) into the renal interstitium (Figure 3a). In the patient with membranous nephropathy (no. 14), IgG4 immunostaining also showed diffuse reactivity in the glomerular capillary walls, and focal staining of the tubule basement membrane, in addition to the infiltrating plasma cells (Figure 3b).

Treatment and course

The treatment regimen was decided according to the opinion of each attending physician (Table 1). Of the 23 patients, 21 were treated with prednisolone (initial dose 10–60 mg/day) for renal lesions. Intravenous methylprednisone pulse therapy (500–1000 mg for 3 days) was also conducted in three patients. One patient (no. 3) was followed up without therapy because both AIP and renal lesions improved spontaneously. Among patients treated with corticosteroid, the clinical data after 4 weeks were evaluable in 19. In 18 of those patients, abnormalities of renal function, complement level, and imaging features were all improved by therapy. Extrarenal lesions were also improved. In one patient with renal failure (no. 22), renal function did not recover, and maintenance hemodialysis became necessary.

DISCUSSION

Lesions of the kidney associated with IgG4-related disease include those affecting the renal parenchyma, and lesions of other areas.[7,11] Renal parenchymal lesions associated with IgG4-related disease were first described in 2004 as case reports of tubulointerstitial nephritis associated with AIP.[12,13] Thereafter, the number of case reports of tubulointerstitial nephritis associated with IgG4-related disease has been increasing,[14–18] although most of such cases have been accompanied by AIP and their number is still limited.

The clinicopathological features of IgG4-related disease have been described most extensively for cases with pancreatic involvement (that is, AIP).[19–22] It predominantly affects middle-aged to elderly men. The clinical symptoms are mild and the condition usually comes to clinical attention because of imaging abnormalities of the pancreas or obstructive jaundice. In most patients, extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, lymphadenopathy, and retroperitoneal fibrosis occur during the clinical course, sometimes simultaneously and often metachronously.[23] Laboratory examinations reveal characteristically increased levels of serum IgG and IgG4 (increased serum IgG4 is observed in 68–90% of Japanese patients with AIP),[22] and a recent study has shown that the serum IgE level is also frequently elevated.[24] Antinuclear antibodies and rheumatoid factor are often positive, but anti-SSA and anti-SSB antibodies are negative,[21] and hypocomplementemia is observed in 17–36% of the patients.[25] CT scan or magnetic resonance imaging shows diffuse pancreatic enlargement or focal masses. Corticosteroid therapy is usually quite effective for normalizing pancreatic lesions, and also the clinical and laboratory parameters. Histopathologically, dense lymphoplasmacytic infiltration, swirling or storiform fibrosis, and abundant IgG4-positive plasma cell infiltration revealed by IgG4 immunostaining are characteristic, and several studies have shown that IgG4-related disease shows similar pathological features, regardless of the specific type of organ involved.[8,26] In this study, we diagnosed 23 patients as having renal parenchymal lesions associated with IgG4-related disease on the basis of the renal pathological features described above, in addition to high levels of serum IgG4. As an elevated serum IgG4 level is characteristic of IgG4-related disease, but not diagnostic (sensitivity 75%, specificity 93% in AIP),[20] histopathological examination including IgG4