

Distinct cytokine mRNA expression pattern in immunoglobulin G4-related kidney disease associated with renal cell carcinoma

Renya Watanabe¹, Tetsuhiko Yasuno¹, Satoshi Hisano², Yoshie Sasatomi¹ and Hitoshi Nakashima¹

¹Division of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University, Fukuoka, Japan and ²Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

Correspondence and offprint requests to: Tetsuhiko Yasuno; E-mail: yasuno9584@fukuoka-u.ac.jp

Abstract

We treated a 61-year-old man with immunoglobulin (Ig)G4-related kidney disease (IgG4-RKD). He had a history of allergic diseases and an allergic reaction and had received a diagnosis of autoimmune pancreatitis (AIP). He had also received a diagnosis of renal cell carcinoma (RCC) and had undergone segmental resection of the left kidney at 59 years of age. His serum amylase level and number of peripheral eosinophils increased after RCC development. We hypothesized that the RCC may have induced AIP and IgG4-RKD and we therefore examined the excised RCC tissue; typical findings of IgG4-RKD associated with RCC were recognized. We next evaluated the mRNA expression of cytokines in the excised tissues of this case and ten other ordinary RCC cases. In all cases, notable levels of IL-10 mRNA and high levels of TGF- β mRNA were seen. Although prominent differences were not observed in the mRNA expression of Th1, Th17 and Treg cytokines in all cases, the present case alone showed increased production of the Th2 cytokines IL-4 and IL-5, which were not detected in ordinary RCC cases. Although the mechanism underlying IgG4-RKD development has not yet been determined, Th2 and Treg cells are thought to play a prominent role in the pathogenesis. It is therefore likely that in this case, the association of these two diseases was not coincidental, and a distinct immune response against RCC may trigger IgG4-RKD development.

Keywords: IgG4-related kidney disease; renal cell carcinoma; tubulointerstitial nephritis

Background

Recently, immunoglobulin (Ig)G4-related diseases (IgG4-RDs) have been recognized as multi-organ disorders clinically characterized by high serum IgG4 concentration and diffuse lymphoplasmacytic infiltration with marked interstitial fibrosis [1]. The most common feature of renal involvement in IgG4-RD is tubulointerstitial nephritis with abundant IgG4-positive plasma cells, but glomerular lesions such as membranous glomerulonephritis have also been described [2–5]. Therefore, kidney lesions associated with IgG4-RD are referred to as IgG4-related kidney disease (IgG4-RKD) and include radiologically identified renal lesions in the setting of other forms of organ involvement confirmed histopathologically [3, 6]. Although recent studies have shown that IgG4-RD primarily affects middle-aged-to-elderly men, a few studies have reported concomitant malignant neoplasms. Herein, we describe a case of renal cell carcinoma (RCC) accompanied by IgG4-related disease. The role of IgG4 in disease pathogenesis remains unclear. Therefore, we tried to characterize the peculiar immunological response in this unusually complicated case in comparison with ordinary RCC and also to determine the mechanism underlying IgG4-RKD pathogenesis.

Case report

A 61-year-old man was admitted to our hospital with proteinuria and renal dysfunction. In 2006, he received the diagnosis of mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach on the basis of biopsy results obtained at the age of 55. The patient did not have *Helicobacter pylori* infection. Urine occult blood and an abnormal serum creatinine level were detected at that time. In November 2009, when the patient was 58 years old, abdominal computed tomography (CT) showed a nodular mass (diameter, 2 cm) in the left kidney; this finding was confirmed by magnetic resonance imaging. He received the diagnosis of RCC, and segmental resection of the left kidney was performed in April 2010. The postoperative pathologic finding was T1a N0 M0. In June 2012, when the patient was 61 years old, a high serum amylase level and pancreatic enlargement were detected, and autoimmune pancreatitis (AIP) was diagnosed. However, he did not receive any treatment for AIP. The patient had some allergic history, including bronchial asthma, pollinosis and contrast media allergy. In September 2012, a physical examination on admission showed hypertension (164/89 mmHg) and swelling of the left submandibular lymph node. There was no abnormality in the lungs, heart,

abdomen or central nervous system. Although cardiomegaly was not seen on the chest radiograph, left ventricular hypertrophy was recognized on ECG. The laboratory findings showed an increased erythrocyte sedimentation rate (34 mm/h), and the C-reactive protein level was 0.49 mg/dL. The hemoglobin concentration was 17.3 g/dL, the white blood cell count was $4000/\text{mm}^3$ (neutrophils, 62%; lymphocytes, 20.5%; monocytes, 5.0%; eosinophils, 10.0% and basophils, 2.5%) and the platelet count was $16.7 \times 10^4/\text{mm}^3$. Proteinuria and hematuria were also detected. The amount of urine protein was 0.5 g/dL. The number of RBCs in the urine sediment was 5–10/HPF. The results of the serum chemistry analyses were as follows: serum creatinine level, 1.32 mg/dL (normal range, 0.4–1.2 mg/dL); blood urea nitrogen level, 25 mg/dL; total serum protein level, 7.1 g/dL (normal range, 6.5–8.2 g/dL) and serum albumin level, 3.4 g/dL (normal range, 3.7–5.2 g/dL). Serum transaminase, amylase and lactate dehydrogenase levels were within normal limits. The immunological tests were positive for antinuclear antibody at a titer of 40 dil, and the immunofluorescence patterns were speckled and homogeneous. Anti-double-stranded DNA antibodies, rheumatoid factor, anti-Sjögren's syndrome A (anti-SS-A) antibodies and anti-SS-B antibodies were all absent. The serum IgG level (1876 mg/dL) was abnormally high, and the IgG4 level was 628 mg/dL, but the IgA and IgM levels were within normal limits (230 and 40 mg/dL, respectively); the serum IgE level was elevated (2045 U/mL). Serum protein electrophoresis showed polyclonal hypergammaglobulinemia. The serum levels of C3 and C4 and total serum hemolytic activity (CH50) were 89 mg/dL (normal range, 86–160 mg/dL), 24 mg/dL (normal range, 17–45 mg/dL) and 58 U/mL (normal range, 25–48 U/mL), respectively. Negative results were obtained for myeloperoxidase-antineutrophil cytoplasmic antibody and proteinase-3 antineutrophil cytoplasmic antibody. Recurrence of lymphoma in the stomach was excluded by endoscopic examination. Abdominal CT showed hyperplasia of the aortic wall and pancreatic diffuse enlargement, but tumor formation in the kidneys was not seen. Positron emission tomography showed abnormal accumulation in the left submandibular lymph node, pancreas, aortic wall and

right kidney. These laboratory data and the history of AIP suggested IgG4-RKD development, and echo-guided percutaneous kidney biopsy was performed on the 7th hospital day to determine the cause of renal dysfunction. The biopsy specimen showed severe fibrous intimal thickening in the interlobular arteries, in addition to glomerulosclerosis and tubulointerstitial fibrosis. These findings were compatible with hypertensive renal sclerosis. Lymphoplasmacytic and eosinophilic infiltration was observed in the interstitium. Immunostaining indicated diffuse infiltration of IgG4-positive plasma cells, with an IgG4-positive/IgG-positive plasma cell ratio of 44.2%. There was evidence of a storiform pattern and fibrosclerotic lesions with a bird's-eye-like appearance. Regarding the clinical course, a high serum amylase level and increased eosinophil count in the peripheral blood were noted following RCC development (Figure 1). We therefore hypothesized that RCC may have induced AIP and IgG4-RKD development.

Materials and methods

In addition to the present case, ten other ordinary RCC cases were evaluated. This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Fukuoka University. Written informed consent was obtained from the patients.

Histopathological and immunohistopathological studies of extracted RCC tissues

Paraffin sections (4 μm) were stained with H&E, periodic acid Schiff stain or periodic acid-methenamine silver. For immunohistochemical staining, kidneys were snap-frozen in optimal cutting temperature compound (Sakura Finetek Japan Co. Ltd, Tokyo, Japan). The sections were immunostained using mouse monoclonal antibodies against human IgG4 (Zymed Laboratory, San Francisco, CA, USA, or The Binding Site, Birmingham, UK), anti-CD38 antibody

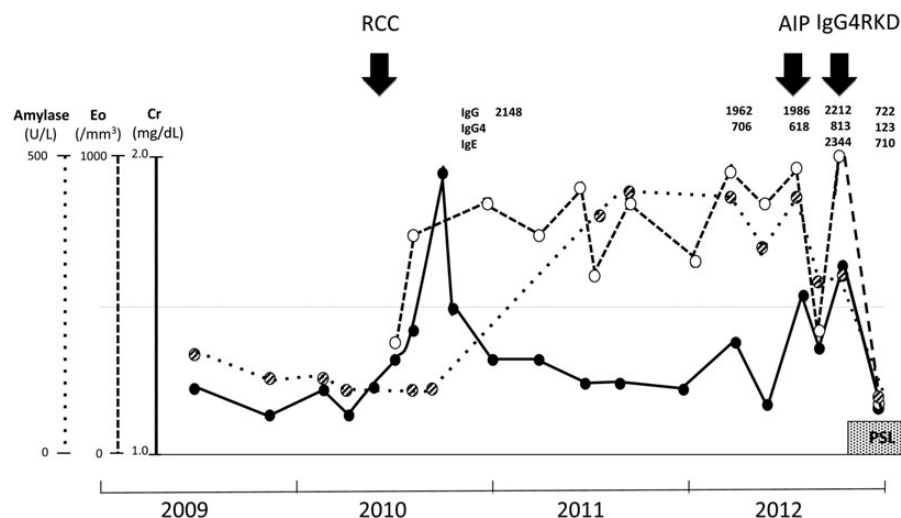


Fig. 1. Clinical course and laboratory findings in the present case. After the patient developed RCC, he received a diagnosis of AIP and IgG4-RKD. The serum amylase level and number of peripheral eosinophils increased after RCC development. RCC, renal cell carcinoma; AIP, autoimmune pancreatitis; IgG4-RKD, IgG4-related kidney disease; Eo, eosinophilic leukocyte; Cr, creatinine; PSL, prednisolone.

(Dako, Glostrup, Denmark) and anti-Foxp3 antibody (Abcam, Cambridge, UK).

Real-time quantitative PCR and TaqMan primers and probes

Total RNA was prepared from the area surrounding the RCC using an RNeasy Mini Kit (QIAGEN, Valencia, CA, USA), and quantitative cDNA amplification was performed according to the manufacturer's instructions. All samples were stored at -80°C before use. The cytokines examined were IFN- γ , IL-12p35, IL-4, IL-5, IL-10, TGF- β 1, IL-17A and IL-6. Foxp3 is a transcription factor specific for CD4+CD25+Foxp3+ Tregs, and the mRNA level for Foxp3 was also evaluated. To provide a meaningful comparison among different samples, we calculated the amount of PCR products relative to the amount of β -actin in each sample. Cytokine levels were measured using TaqMan PCR and an ABI Prism 7700 sequence detection system (PerkinElmer Japan Co. Ltd, Tokyo, Japan), according to the manufacturer's instructions (Applied Biosystems Japan Ltd, Tokyo, Japan). Oligonucleotide primers and probes were designed using the Primer Express program (Applied Biosystems Japan Ltd). The primer and probe sequences were as follows: IFN- γ , Mm99999071_m1; IL-12, Mm00434165_m1; IL-4, Mm00445260_m1; IL-5, Mm00439646_m1; IL-6, Mm99999064_m1; IL-17, Mm00439619_m1; IL-10, Mm00439616_m1; TGF- β , Mm00441726_m1 and Foxp3, Mm00475157_g1.

Results

Histopathological findings for the RCC tissue

Tubular atrophy associated with RCC was observed at low magnification (Masson's trichrome stain) (Figure 2A).

Figure 2B shows a schematic diagram of Figure 2A. Severe fibrosis with a storiform pattern was present (Figure 2C), and eosinophilic infiltration in the tubulointerstitium was also recognized in the tissue obtained from the patient. These findings were specific to this particular patient; no other ordinary RCC samples showed similar features. Immunostaining showed IgG4-positive plasma cell infiltration with 41.6% IgG4-positive plasma cells among CD38-positive plasma cells (Figure 2D). These results indicate that typical IgG4-RKD was associated with RCC. Immunostaining for Foxp3 showed that numerous Tregs had invaded the tissue from the present patient. However, this finding was not only observed in the present case; similar results were also found in ordinary RCC tissues (Figure 3).

Comparison of cytokine mRNA expression patterns between the present case and ordinary RCC cases

Th1 and Th17 cytokine mRNA levels in the present case were not different from those in ordinary RCC samples. With regard to Treg cytokines, TGF- β was highly expressed in the present case, but the levels were not different from those of ordinary RCC samples. IL-10 and Foxp3 mRNA levels were also similar to those of ordinary RCC samples. In contrast, IL-4 and IL-5 production was observed in the present case but not in ordinary RCC samples (Figure 4).

Discussion

We observed tumefactive lesions, a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells and storiform fibrosis around the RCC in the present case. Although it remains unclear whether the serum IgG4 level was high at the time of RCC resection, typical findings for IgG-RKD

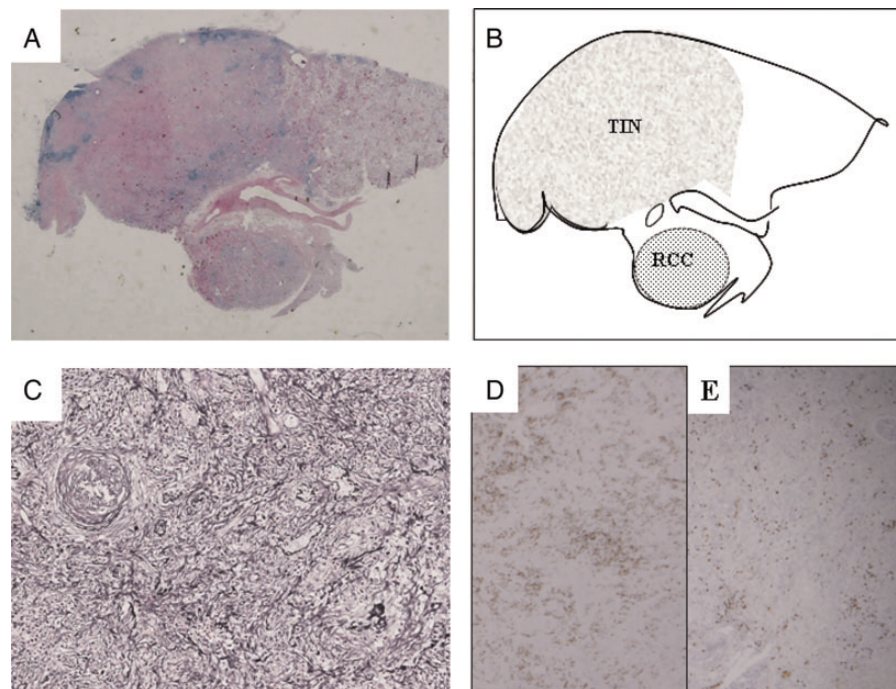


Fig. 2. Histopathological findings for the RCC tissue. (A) A low-magnification view showing tubular atrophy associated with RCC (Masson's trichrome stain). (B) Schematic diagram of A. (C) Storiform interstitial fibrosis and a chronic inflammatory infiltrate were present (periodic acid-silver methenamine-HE stain, 20 \times). (D) Immunohistochemistry. Left side, CD38-positive plasma cells; right side, IgG4-positive cells.

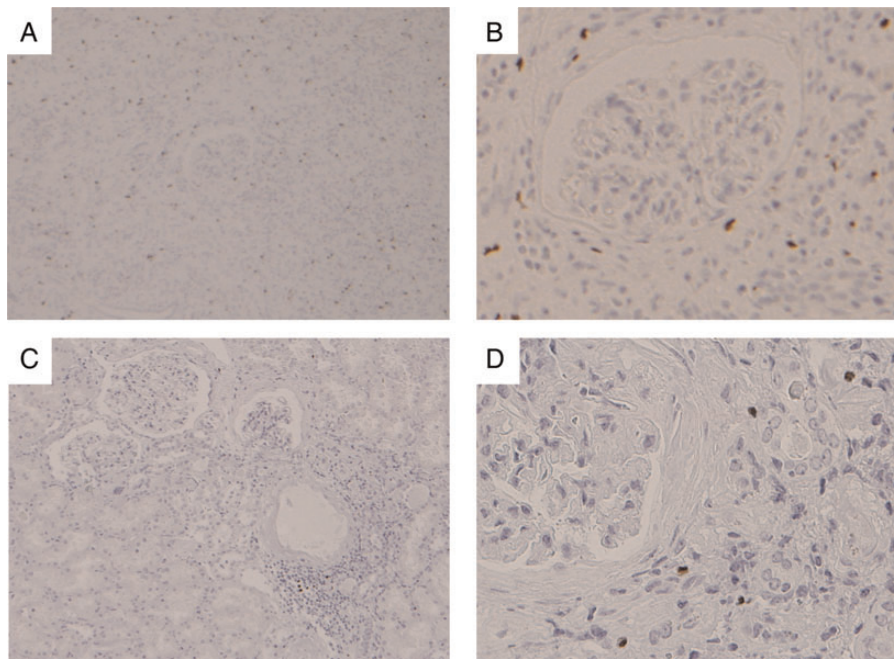


Fig. 3. Immunohistochemistry for Fcpx3 in the present case and in ordinary RCC tissue. (A and B) Fcpx3 in the present case. (C and D) Fcpx3 in ordinary RCC tissues. (A and C) 40 \times . (B and D) 20 \times . Fcpx3⁺Treg cells are present in the surroundings of RCC in most cases and are thus not particularly associated with IgG4-RKD.

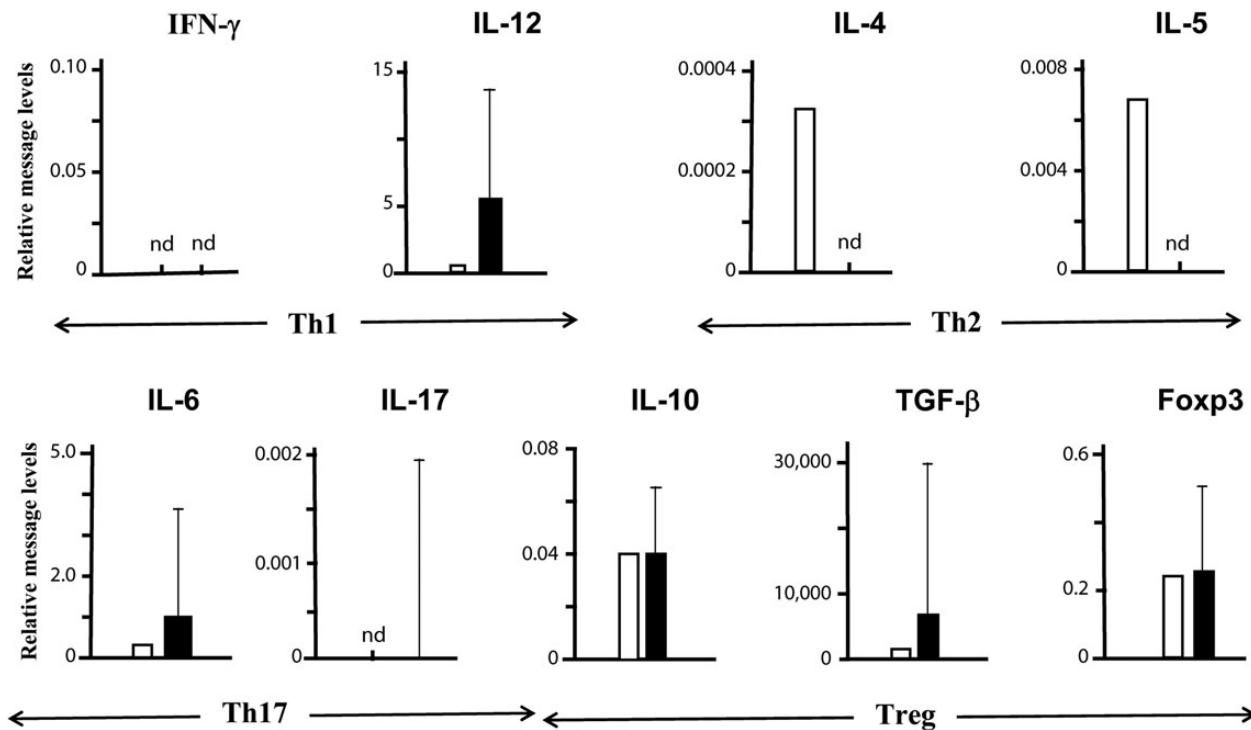


Fig. 4. Comparison of cytokine mRNA expression patterns between the present case (white bars) and ordinary RCC cases (black bars). Bars show the median and quartile deviation values for relative β -actin mRNA levels in ten ordinary RCC cases. We calculated the amount of the PCR products relative to the amount of β -actin PCR products in each sample.

were observed in association with RCC. To the best of our knowledge, no previous case report has described IgG4-RKD accompanied by RCC, although some studies have reported a malignant neoplasm concomitant with IgG4-RD. Two case reports of IgG4-related lung disease with lung cancer have been published [7, 8], and several case

reports of AIP associated with pancreatic cancer have been published [9-14]. These reports suggested that pancreatic cancer may develop in AIP patients, that K-ras mutations may be frequently occur in AIP patients and that AIP may be a risk factor for gastric and colonic cancers [15]. There have also been a few reports of

IgG4-RD associated with MALT lymphoma or other B-cell lymphoma [16, 17]. In fact, the present patient also received a diagnosis of gastric MALT lymphoma prior to RCC development. Although the biopsied gastric tissue specimen was immunohistochemically examined, diffuse lymphoplasmacytic infiltration and IgG4-producing plasma cells were not recognized (data not shown).

We evaluated the immune response against ordinary RCCs and compared it with that in the present case to clarify the distinct mechanism for IgG4-RKD development. Immunohistological analysis demonstrated that Foxp3⁺ Treg cells were present in the vicinity of RCC in most cases and were thus not particularly associated with IgG4-RKD (Figure 4). mRNA expression of Treg cytokines also increased (Figure 4), which suggests that Treg cells infiltrated and produced the immunosuppressive cytokine IL-10 around the RCC to inhibit antitumor immunity at the tumor site. This phenomenon has been observed in a murine pancreatic cancer model, which suggests that the tumor actively promotes the accrual of Treg cells through several mechanisms involving the activation of naturally occurring Treg cells and conversion of non-T cells into T cells [18, 19]. Analysis of tumor-draining LNs demonstrated that both antitumor effector T cells and Foxp3⁺ Treg cells are primed in the same LNs during tumor progression. These tumor antigen-specific Treg cells possessed the same functional properties as Treg cells that arise naturally in the thymus [20, 21]. Similarly, a larger study concluded that the prevalence of CD4⁺CD25⁺ Treg cells is increased not only in the tumor microenvironment of patients with invasive breast or pancreatic cancer but also in the peripheral blood, suggesting that the increase in Treg cells is a generalized phenomenon [21].

It is notable that mRNA expression of Th2 cytokines (IL-4 and IL-5) was detected in the present case but not in ordinary RCCs (Figure 4). This finding may indicate that the antitumor immunity produced by the Th2 response in the present case was particularly dominant compared with that in the ordinary RCC cases. It has been demonstrated that Th2 and Treg cell responses play a central role in the pathogenesis of IgG4-RKD [22] and in AIPC [23] and Mikulicz disease [24]; therefore, immune responses by Th2 and Treg cells may be a common feature among IgG4-RDs, and an imbalance of Th2 cells providing antitumor immunity and Treg cells suppressing antitumor immunity may be responsible for the pathogenesis of IgG4-RKD. Treg cells are activated by excessive immune responses to prevent a Th2-type immune response in allergic disease [25, 26]. Treg cells produce regulatory cytokines, including IL-10 and TGF- β . IL-10 directs B cells to switch from IgE to IgG4 production. Therefore, IgG4 behaves as a suppressive antibody in allergic diseases [27, 28]. This Th2 response, which suppresses the allergic Th2 response, has been called a 'modified Th2 response' [29]. These mechanisms support the hypothesis that in the current case, the IgG4-RKD may have developed under an imbalance between the antitumor Th2 immune response against RCC and the immunosuppressive response by Treg cells.

Tregs can be broadly classified into two major types: endogenous T cells (naturally occurring regulatory T cells, nTregs) and inducible T cells with low autologous recognition (inducible regulatory T cells, iTregs), which differentiate from naive CD4-positive T cells. Endogenous Tregs are produced in the thymus, along with autoreactive T cells. When inducible Tregs are antigen-stimulated in the presence of TGF- β , their differentiation from naive T cells in the peripheral blood is induced. Both of these cell types

are involved in the mechanism of immune tolerance. Tregs are present around cancer cells, suggesting that cancer cells may induce the formation of Tregs.

IgG4-RKD may be associated with allergic manifestations and may be driven by enhanced Th2 responses. Recent studies from the Stone laboratory suggest that the Th2 responses that have been reported in IgG4-RKD may result from concomitant atopic manifestations in disease subjects [30, 31]. Because cytokine expression at disease onset was not examined in detail in this case, pathogenic involvement remains unclear.

We experienced a rare case of IgG4-RKD associated with RCC. Analysis of cytokine mRNA production suggested that the association of these two diseases may not be coincidental and that a distinct immune response against RCC may trigger IgG4-RKD development. IgG4-RDs have been described only recently; therefore, further accumulation of such cases is necessary to elucidate the mechanism underlying IgG4-RD development.

Acknowledgements. Investigators and Departments were Watanebe M. and Saito T. from Division of Nephrology and Rheumatology, Department of Internal Medicine; Wada K. from Department of Urology; Aoyagi K. from Department of Urology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan.

Conflict of interest statement. None declared.

References

1. Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroent* 2006; 41: 613–625
2. Saeki T, Nishi S, Imai N et al. Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. *Kidney Int* 2010; 78: 1016–1023
3. Kawano M, Saeki T, Nakashima H et al. Proposal for diagnostic criteria for IgG4-related kidney disease. *Clin Exp Nephrol* 2011; 15: 615–626
4. Raissian Y, Nasr SH, Larsen CP et al. Diagnosis of IgG4-related tubulointerstitial nephritis. *J Am Soc Nephrol* 2012; 22: 1343–1352
5. Cornell LD. IgG4-related kidney disease. *Cur Opin Nephrol Hypertens* 2012; 21: 279–288
6. 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
7. Zen Y, Inoue D, Kitao A et al. IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases. *Am J Surg Pathol* 2009; 33: 1886–1893
8. Inoue T, Hayama M, Kobayashi S et al. Lung cancer complicated with IgG4-related disease of the lung. *Ann Thorac Cardiovasc Surg* 2013; doi: 10.5761/actcs.cr.12.02208
9. Inoue H, Miyatani H, Sawada Y et al. A case of pancreas cancer with autoimmune pancreatitis. *Pancreas* 2006; 33: 208–209
10. Kamisawa T, Chen PY, Tu Y et al. Pancreatic cancer with a high serum IgG4 concentration. *World J Gastroenterol* 2006; 12: 6225–6228
11. Ghazale A, Chari S. Is autoimmune pancreatitis a risk factor for pancreatic cancer? *Pancreas* 2007; 35: 376
12. Witkiewicz AK, Kennedy EP, Kenyon L et al. Synchronous autoimmune pancreatitis and infiltrating pancreatic ductal adenocarcinoma: case report and review of the literature. *Human Pathol* 2008; 39: 1548–1551
13. Fukui T, Mitsuyama T, Takaoka M et al. Pancreatic cancer associated with autoimmune pancreatitis in remission. *Intern Med* 2008; 47: 151–155

14. Loos M, Esposito I, Hedderich DM et al. Autoimmune pancreatitis complicated by carcinoma of the pancreatobiliary system: a case report and review of the literature. *Pancreas* 2011; 40: 151–154
15. Kamisawa T, Horiguchi S, Hayashi Y et al. K-ras mutation in the major duodenal papilla and gastric and colonic mucosa in patients with autoimmune pancreatitis. *J Gastroent* 2010; 45: 771–778
16. Sato Y, Ohshima K, Ichimura K et al. Ocular adnexal IgG4-related disease has uniform clinicopathology. *Pathol Int* 2008; 58: 465–470
17. Cheuk W, Yuen HK, Chu SY et al. Lymphadenopathy of IgG4-related sclerosing disease. *Am J Surg Pathol* 2008; 32: 671–681
18. Linehan DC, Goedegebuure PS. CD25⁺ CD4⁺ regulatory T-cells in cancer. *Immunol Res* 2005; 32: 155–168
19. Sasada T, Kimura M, Yoshida Y et al. CD4⁺CD25⁺ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. *Cancer* 2003; 98: 1089–1099
20. Hiura T, Kagamu H, Miura S et al. Both regulatory T cells and antitumor effector T cells are primed in the same draining lymph nodes during tumor progression. *J Immunol* 2005; 175: 5058–5066
21. Liyanage UK, Moore TT, Joo HG et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* 2002; 169: 2756–2761
22. Nakashima H, Miyake K, Moriyama M et al. An amplification of IL-10 and TGF-beta in patients with IgG4-related tubulointerstitial nephritis. *Clin Nephrol* 2010; 73: 385–391
23. 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
24. Tanaka A, Moriyama M, Nakashima H et al. Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. *Arthritis Rheum* 2012; 64: 254–263
25. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005; 5: 271–283
26. Robinson DS, Larche M, Durham SR. Tregs and allergic disease. *J Clin Invest* 2004; 114: 1389–1397
27. Ebner C, Siemann U, Bohle B et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997; 27: 1007–1015
28. Wachholz PA, Soni NK, Till SJ et al. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; 112: 915–922
29. Erwin EA, Wickens K, Custis NJ et al. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. *J Allergy Clin Immunol* 2005; 115: 74–79
30. Mattoo H, Della-Torre E, Mahajan VS et al. Circulating Th2 memory cells in IgG4-related disease are restricted to a defined subset of subjects with atopy. *Allergy* 2013. doi:10.1111/all.12342
31. Della Torre E, Mattoo H, Mahajan VS et al. Prevalence of atopy, eosinophilia, and IgE elevation in IgG4-related disease. *Allergy* 2014; 69: 269–272

Received for publication: 6.12.13; Accepted in revised form: 21.2.14