

Exceptional Case

Evidence of follicular T-cell implication in a case of IgG4-related systemic disease with interstitial nephritis

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Abstract

IgG4-related systemic disease is a protean disorder that covers a wide variety of lesions. We report on a patient with tubulointerstitial nephritis, lymphadenopathies, sialadenitis and retroperitoneal fibrosis. The salivary gland and kidney interstitium were infiltrated with B lymphocytes and T lymphocytes and IgG3⁺ and IgG4⁺ plasma cells. The overexpression of IgG1 and IgG3, in addition to IgG4, the unusual abundance of interfollicular plasma cells and CD4⁺ T cells in germinal centres of lymph nodes, and the dramatic response to rituximab point to possible roles of follicular helper T cells in enhancing a skewed B-cell terminal maturation and of CD20⁺ B cells in disease progression.

Keywords: follicular helper T cells; IgG4-related systemic disease; IgG subclasses; tubulointerstitial nephritis

Background

IgG4-related systemic disease (IgG4-RSD) is a protean disorder that covers a wide variety of lesions [1, 2]. Renal involvement mainly features interstitial nephritis. Little is known on the underlying mechanisms, and few reports have studied all IgG subclasses and suggested a possible implication of T cells. We report on a case of IgG4-RSD with interstitial nephritis that raises new hypotheses on underlying mechanisms.

Case report

An 83-year-old Chinese man, with a history of pulmonary tuberculosis and 60-pack/year smoking, was hospitalized for progressive dyspnea, a 12-kg weight loss, abdominal pain and anicteric cholestasis. Abdominal computed tomography scan and endoscopic ultrasonography showed

bile duct enlargement with a stricture just above the papilla. A sphincterotomy was performed. No definite diagnosis was retained. During the following months, serum creatinine increased to 140 µmol/L (estimated glomerular filtration rate according to the four-variable Modification of Diet in Renal Disease study equation, 42 mL/min/1.73 m²). Examination disclosed a further 7-kg weight loss and axillary and inguinal lymphadenopathies. Renal ultrasound showed normal-sized kidneys but mild left hydronephrosis related to local retroperitoneal fibrosis, which did not require ureteral endoprosthesis. Laboratory data are summarized in Table 1. Urinalysis revealed a tubular proteinuria (1.5 g/day) but neither haematuria nor leukocyturia. Antinuclear antibodies were positive (>1:1280). However, testing for antibodies to double-stranded DNA, extractable nuclear antigens including Sjögren's syndrome antigens and SSB, as well as rheumatoid factor and cryoglobulin, was negative. The haemolytic complement activity and components C3 and C4 were decreased to 3% (normal values > 50%), 0.25 mg/mL (Normal range (NR) = 0.9–1.74 mg/mL) and <0.03 mg/mL (NR = 0.22–0.51 mg/mL), respectively. Testing for HIV, hepatitis B and C viruses, human T-lymphotropic viruses and human herpes virus-8 was negative. There were striking elevations of serum IgG (81 g/L, NR = 6.6–12.4 g/L) and immunoglobulin free light chains *kappa* (1080 mg/L, NR = 3.3–19.4 mg/L) and *lambda* (372 mg/L, NR = 5.7–26.3 mg/L). Serum IgG subclasses measured by competitive enzyme-linked immunosorbent assay with monoclonal antibodies showed increased IgG1 (6000 mg/dL), IgG3 (1950 mg/dL) and IgG4 levels (1750 mg/dL) and normal IgG2 (330 mg/dL). Serum IgE was increased (1295 U/mL, NR < 150 U/mL), serum IgA normal (2.4 g/L, NR = 1.0–3.2 g/L) and IgM in the lower normal range (0.52 g/L, NR = 0.48–1.37 g/L).

The axillary lymph node biopsy (Figure 1A) exhibited lymphoid follicular hyperplasia, and the remarkable presence of numerous plasma cells mainly in the interfollicular zones, most

Table 1. Weight and laboratory data before (M 0) and 1, 6, 12 and 18 months following rituximab administration^a

	Reference range	Before treatment M 0	Time after rituximab administration			
			M 1	M 6	M 12	M 18
Weight (kg)	75	55	56	66	70	70
Biological findings						
Haemoglobin (g/dL)	12 to 18	8.9	10.3	12.9	13.4	12.8
Alkaline phosphatase (U/L)	30 to 115	369	255	86	116	91
Gamma-glutamyltransferase (U/L)	<50	207	157	24	22	24
TSH (mIU/L)	0.3 to 3.6	8.1	-	4.2	1.96	-
Serum creatinine (μmol/L)	45 to 90	140	93	90	95	88.5
eGFR ^b (mL/min/1.73m ²)	>60	42	67	70	66	71
Proteinuria (g/day)	<0.15	1.5–2.5	1.5	0.28	0.35	<0.15
Serum electrophoresis						
Proteins (g/L)	65 to 75	119	95	83	87	80
Gammaglobulins (g/L)	5.5 to 11	84	51.7	26.5	26.4	25.3
Albumin (g/L)	38 to 45	22	-	39.3	41.4	38.5
Serum IgG levels ^c (g/L)	6.6 to 12.4	81	51	27.8	24.7	23.9
IgE levels (IU/mL)	<150	1295	-	-	-	744
Serum IgG subclass ^d						
IgG1 (mg/dL)	400 to 980	6000	4200	2320	1740	-
IgG2 (mg/dL)	60 to 90	330	440	425	340	-
IgG3 (mg/dL)	18 to 80	1950	1530	115	100	-
IgG4 (mg/dL)	< 1 to 160	1750	830	140	56	-

^aeGFR, estimated glomerular filtration rate; TSH, thyroid-stimulating hormone.

^beGFR was calculated using four-variable Modification of Diet in Renal Disease Study equation.

^cSerum IgG levels were measured by nephelometry.

^dSerum IgG subclass levels were measured by competitive enzyme-linked immunosorbent assay with monoclonal antibodies. IgG subclass ranges are given for healthy adults. The range levels of IgG4 are given for males.

of which were IgG4⁺ (57%) or IgG3⁺ (42.8%). Another striking feature was the abundance of CD4⁺ T cells in light zones of germinal centres. CD8⁺ cells were also found in germinal centers. The kidney biopsy (Figure 1B) displayed a heavy interstitial infiltrate including B lymphocytes and T lymphocytes together with plasma cells. A minor salivary gland biopsy (Figure 1C) also revealed abundant infiltrates of B lymphocytes, T lymphocytes and plasma cells. The ratios of IgG3⁺/IgG⁺ and IgG4⁺/IgG⁺ cells were 60.7 and 32.5% in the kidney and 42 and 44% in the salivary gland, respectively.

The diagnosis of IgG4-RSD was retained because of dense IgG4⁺ plasma cell infiltration of mildly swollen lymph nodes with follicular hyperplasia, tubulointerstitial nephritis, lymphoplasmacytic sialadenitis, retroperitoneal fibrosis and bile duct involvement. The patient did not receive steroids considering the risk of tuberculosis reactivation. Instead, he was administered 4 weekly infusions of rituximab (375 mg/m²/week). Clinical and biological response to treatment was prompt. At last follow-up, the patient was still in remission (Table 1).

Discussion

IgG4-RSD includes a broad spectrum of inflammatory and sclerosing lesions (Table S1) [1, 2]. Renal involvement occurs in 10–30% of cases and mainly relates to tubulointerstitial nephritis with infiltration of lymphocytes and plasma

cells [1–3]. Glomerular involvement, particularly membranous nephropathy with IgG4 deposits, can occur in association with plasma-cell tubulointerstitial nephritis [3]. It is noteworthy that IgG4 also predominates in idiopathic membranous nephropathy-related deposits. Kidney tumour-like lesions with dense IgG4⁺ plasma-cell infiltrate and retroperitoneal fibrosis have also been reported [1–3].

Our patient displayed some unusual features for IgG4-RSD. First, he exhibited neither obstructive jaundice nor radiological features of autoimmune pancreatitis (AIP) [1, 2]. However, many cases of IgG4-RSD without AIP have already been reported [1–3]. Second, IgG1 and IgG3 were elevated together with IgG4 and IgE, and tissue-infiltrating plasma cells mostly expressed IgG3 in addition to IgG4. Patients with IgG4-RSD often have high serum levels of other IgG subclasses, while some of them (~30%) do not even have elevated IgG4 [1, 2].

Steroid therapy is the mainstay of IgG4-RSD treatment [4]. The long-term outcome remains uncertain and relapse occurs in 30–40% of cases [4]. In the present case, rituximab was preferred to minimize the risk of tuberculosis reactivation. As reported in other cases, our patient dramatically improved under anti-CD20 therapy [5, 6]. This observation points to a possible key role of CD20⁺ infiltrating B lymphocytes that likely mature into short-lived plasma cells and could participate in the sclerosing process.

While increased serum IgG4 levels and infiltrating IgG4⁺ plasma cells are significant diagnostic markers of IgG4-RSD, their pathophysiological implication remains uncertain. The present observation of abundant T cells in germinal centres is quite remarkable as compared with other cases of follicular hyperplasia. Follicular helper T cells (Tfh) are essential for B-cell terminal maturation and immunoglobulin class switch [7–9]. In this case, because most germinal centre T cells were CD4⁺, it is likely that enhanced Tfh activity participates in follicular hyperplasia, excess of plasma cells and hypergammaglobulinaemia [7–9]. The occurrence of germinal centre CD8⁺ cells is more puzzling, and might, for instance, relate to a recently proposed regulatory T-cell subset [10]. A specific commitment to IgG1, IgG3 and IgG4, as observed, may result from cytokines such as IL-4, IL-10 or IL-21, which are typically produced by Tfh and are also known as isotype switch factors [7–9, 10]. Alternately, or in addition, other yet undefined anomalies upstream of B-cell maturation events could also explain the bias in Ig isotypes [11].

In conclusion, this case of IgG4-RSD with interstitial nephritis illustrates the broad spectrum of this protean disorder, and points to some aspects that raise hypotheses on its pathophysiology. Further studies are required to better assess the role of lymphoid cells in the pathogenesis of IgG4-RSD and identify the factors that attract these cells to the involved organs.

Supplementary data

Supplementary data is available online at <http://ndt.oxfordjournals.org>.

Conflict of interest statement. None declared.

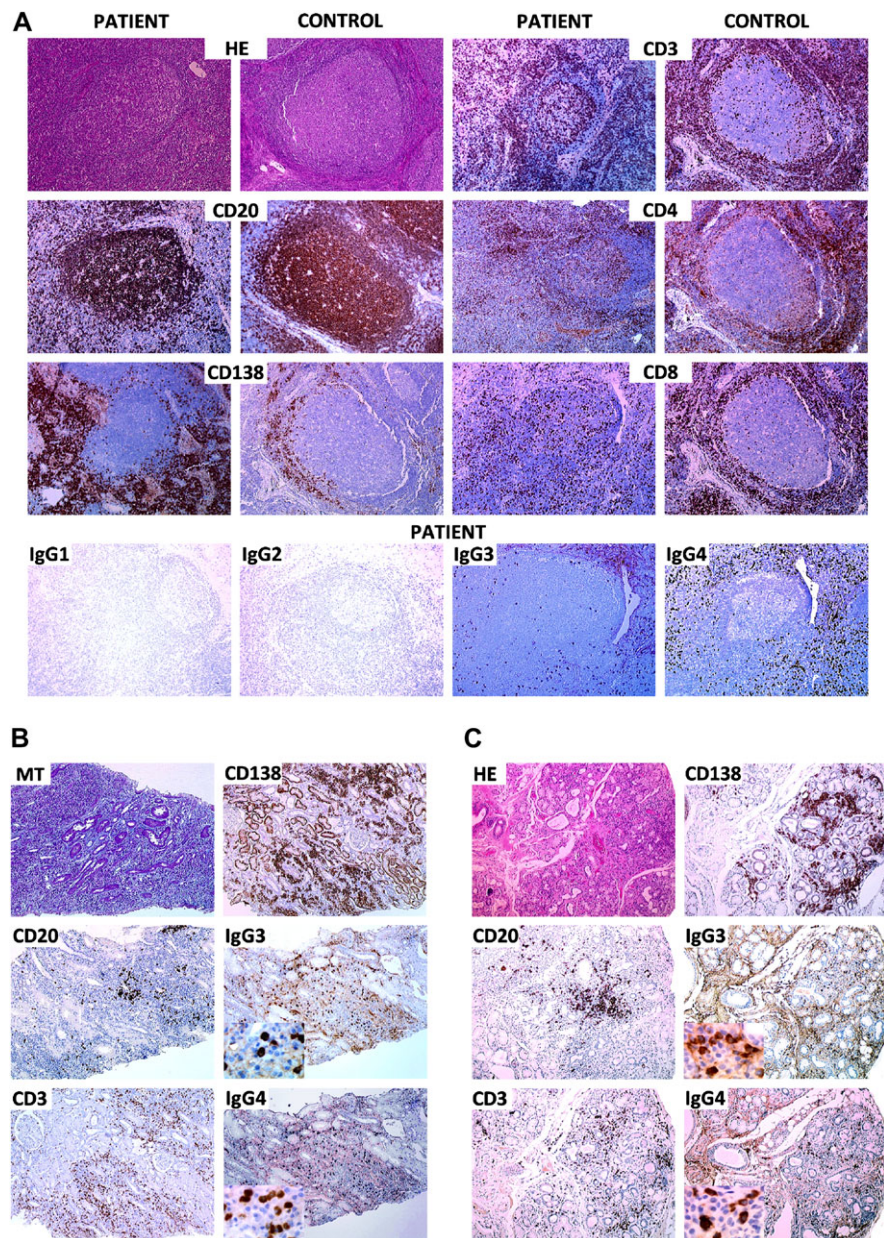


Fig. 1. (A) Histological and immunohistochemical analyses of lymph nodes from the patient and a control with lymphoid follicular hyperplasia. Haematoxylin and eosin staining (HE, original magnification, $\times 200$) showed lymphoid follicular hyperplasia in the patient's axillary lymph node and in the lymph node of a control patient with HIV infection. Two other control cases, featuring follicular hyperplasia secondary to colon carcinoma and systemic lupus erythematosus, yielded similar results. B lymphocytes, plasma cells and T lymphocytes were labeled for CD20, CD138 and CD3, respectively, and for CD4 and CD8. Immunostaining for intracytoplasmic IgG subclasses was performed using monoclonal antibodies to human IgG1, IgG2, IgG3 and IgG4 (clones 4E3, HP6014, ZG4 and MC011, diluted 1:10, 1:10, 1:10 000 and 1:200, respectively). Numerous CD138⁺ plasma cells were present, as compared with controls, most of which were located in the interfollicular regions (64% of nucleated cells). Immunostaining for intracytoplasmic IgG subclasses demonstrated that most plasma cells in the interfollicular zones contained either IgG3 (42.8%) or IgG4 (57%), which was not observed in the controls. The topography of the patient's T cells was quite unusual as compared with controls, with mostly CD4⁺ T cells (62% of total T cells) infiltrating the light zones of the germinal centres. CD8⁺ T cells were also found in the germinal centres. These findings were not seen in the three control patients with follicular hyperplasia. (B) Histological and immunohistochemical findings in the renal tissue. Light microscopy (Masson's trichrome; original magnification, $\times 100$) revealed 16 glomeruli, three of which were fibrotic. The remaining 13 glomeruli were normal. Only 10–20% of tubules showed atrophic changes but no marked tubulitis. The interstitium showed diffuse and marked renal interstitial Mononuclear cell infiltration consisting of CD3⁺ T lymphocytes and CD20⁺ B lymphocytes, and numerous CD138⁺ plasma cells representing 33.5, 12 and 54% of the cells, respectively. Immunofluorescence study revealed no extracellular immunoglobulin deposits. Immunostaining for intracytoplasmic IgG1 (data not shown), IgG2 (data not shown), IgG3 (inset: original magnification, $\times 1000$) and IgG4 (inset: original magnification, $\times 1000$) subclasses, performed as in the axillary lymph node, revealed that most plasma cells stained for IgG3 (60.7%) and IgG4 (32.5%). (C) Histological and immunohistochemical findings in the minor salivary gland tissue. Light microscopy (HE, original magnification, $\times 100$) revealed periductal fibrosis with a similar infiltration of mononuclear cells consisting of CD3⁺ T lymphocytes and CD20⁺ B lymphocytes and plasma cells (staining for both *kappa* and *lambda* light chains, data not shown), which represented 40, 10 and 51% of the cells, respectively. Immunostaining for intracytoplasmic IgG1 (data not shown), IgG2 (data not shown), IgG3 (inset: original magnification, $\times 1000$) and IgG4 (inset: original magnification, $\times 1000$) subclasses revealed that most plasma cells stained for IgG3 (42%) and IgG4 (44%).

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References

1. Kamisawa T, Okamoto A. IgG4-related sclerosing disease. *World J Gastroenterol* 2008; 14: 3948–3955
2. Cheuk W, Chan JK. IgG4-related sclerosing disease: a critical appraisal of an evolving clinicopathologic entity. *Adv Anat Pathol* 2010; 17: 303–332
3. Saeki T, Nishi S, Imai N *et al.* Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. *Kidney Int* 2010; 78: 1016–1023
4. Kamisawa T, Shimosegawa T, Okazaki K *et al.* Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; 58: 1504–1507
5. Khosroshahi A, Bloch D, Deshpande V *et al.* Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. *Arthritis Rheum* 2010; 62: 1755–1762
6. Topazian M, Witzig T, Smyrk T *et al.* Rituximab therapy for refractory biliary strictures in immunoglobulin G4-associated cholangitis. *Clin Gastroenterol Hepatol* 2008; 6: 364–366
7. Gatto D, Brink R. The germinal center reaction. *J Allergy Clin Immunol* 2010; 126: 898–907
8. McHeyzer-Williams LJ, Pelletier N, Mark L *et al.* Follicular helper T cells as cognate regulators of B cell immunity. *Curr Opin Immunol* 2009; 21: 266–273
9. Yu D, Vinuesa CG. The elusive identity of T follicular helper cells. *Trends Immunol* 2010; 31: 377–383
10. Kim H, Verbinnen B, Tang X *et al.* Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. *Nature* 2010; 467: 328–332
11. Boulanger E, Fuentes V, Meignin V *et al.* Polyclonal IgG4 hypergammaglobulinemia associated with plasmacytic lymphadenopathy, anemia and nephropathy. *Ann Hematol* 2006; 85: 833–840

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SUPPLEMENTARY MATERIAL FOR ONLINE PUBLICATION ONLY

THE HALLMARKS OF IgG4-RSD [1–3]	
1- Histological hallmarks	– pronounced IgG4+ polyclonal plasma cells and T-lymphocytes infiltration of various organs – sclerosis and obliterative phlebitis.
2- Biological hallmarks	– high IgG4 serum level (≥ 135 mg/dL) – increased serum IgG level (≥ 18 g/L) – hypergammaglobulinemia (≥ 20 g/L) without monoclonal component – +/- increased serum IgG
3- Presence of auto-antibodies (including antinuclear antibodies and rheumatoid factor)	– without definite auto-immune disorder – anti-SS-A/SS-B antibodies are almost always negative
4- Marked improvement with steroid therapy	
THE CLINICAL SPECTRUM OF IgG4-RSD [1–3]	
Pancreas	– Autoimmune pancreatitis (type 1), chronic sclerosing pancreatitis.
Hepatobiliary system	– Stricture of the bile ducts, Sclerosing cholangitis – Sclerosing cholecystitis – Hepatic “inflammatory” pseudotumor”, IgG4-hepatopathy
Lymph nodes	– Lymphadenopathy with at least five histological subtypes: – Castleman’s disease-like morphology – Reactive follicular hyperplasia – Interfollicular plasmacytosis and immunoblastosis – Progressive transformation of germinal center-like – Inflammatory pseudotumor-like
Salivary and lacrimal gland	– Sclerosing sialadenitis (Rüttner tumor), Mikulicz disease – Sclerosing dacryoadenitis
Kidney	– Tubulo-interstitial nephritis – Pseudo-tumors of the kidney – Membranous nephropathy
Retroperitoneum	– Retroperitoneal fibrosis with obstructive uropathy
Thyroid gland	– Chronic thyroiditis, subclinical hypothyroidism
Mesentery, Aorta, and Mediastinum	– Multifocal fibrosclerosis – Sclerosing mesenteritis, mediastinal fibrosis – Periaortitis, Inflammatory aortic aneurysm
Lung and Pleura	– Interstitial pneumonia, nodular lesions
Stomach and Colon	– Chronic gastritis – Chronic (+/- ulcerative) colitis
Prostate	– Sclerosing prostatitis
Central nervous system	– Sclerosing Hypophysitis, pachymeningitis 1
Other lesions	– Inflammatory and sclerosing pseudo-tumors (liver, kidney, orbit, breast, lung, and brain...)

Table S1. The hallmarks and clinical spectrum of IgG4-related systemic disease (IgG4-RSD) [1–3].