4. 涙腺と末梢血中に関連クローンが検出されたため，IgG4 クローンは局所と末梢血間を行き来する可能性が考えられました[4].

MDでは何らかの免疫反応が強く起きていますが，現時点では，ある特定の抗原により病変が作られるのではないと考えます.

村上　質疑応答をお願いします.

フロア　対応抗原は推測できますか．抗原が一つでも，antigenicityの場所が多様で，多種類のCDR3 が使われており，一つに絞り込めていない可能性はないでしょうか.

川野　現時点で見つけたのは 2 クローンだけです．一個ずつクローンを作り，対応抗原を探す方法がありますが，一つの抗体は，いろいろな抗原と交差反応する可能性があります．IgG4 陽性の血清で免疫染色した結果，染まった導管に対応抗原がある，という論文がありますが，追試がありません．このような抗体全体からのアプローチ法もありますが，労力にみあう成果が得られるかは不明です.

「IgG4＋多臓器リンパ増殖症候群～64 例の臨床的検討」

正木康史（金沢医大血液免疫制御学）

IgG4 関連疾患やMDは稀な病態で，少数の報告例にとどまります．菅井先生の呼びかけにより，日本のSS研究会の分科会として 2004 年 9 月から，IgG4＋MOLPS(multiorgan lymphoproliferative syndrome)/MD研究会を発足し，多数例を検討することになりました．全国 10 施設から計 85 症例をご登録いただき，典型的な 64 例をIgG4＋MOLPSと診断しました.

診断基準は，(1) 血清IgG4 は 135 mg/dl以上．(2) 組織でIgG4 陽性の形質細胞浸潤がある（病理組織にてIgG4 陽性形質細胞/IgG陽性形質細胞が 50% 以上であり，さらにHE染色で典型的線維化や硬化性病変を有する）です.

IgG4＋MOLPSの特徴（典型的SSとの比較）：

1. アレルギー性鼻炎，自己免疫性膵炎の合併が有意に多く認められました.

2. ほとんどの症例が抗SS-A抗体および抗SS-B抗体が陰性で，RF（rheumatoid factor）およびANA（antimuclear antibody）陽性率が有意に低率でした.

3. IgG4＋MOLPS群では，血清total IgG, IgE, IgG2，およびIgG4 が有意にSSより高値であり，IgA，IgM，IgG1，IgG3 は有意に低値でした.

4. IgG4＋MOLPSの組織ではIgG4 陽性形質細胞浸潤を認めましたが，典型的SSでは認められませんでした.

5. IgG4＋MOLPS/Mikulicz病の一部はSSの診断基準を満たす場合もありますが，典型的SSとは異なる疾患群です.

6. ステロイド治療が著効します[5].

どのような時にIgG4＋MOLPSを疑うか：

SS/自己免疫性疾患の立場からは，非典型的なSS（男性例，抗SS-A抗体，抗SS-B抗体陰性例）の場合疑います．また，リンパ腫/腫瘤性病変の立場からは，あらゆる腫瘤性病変で疑いますが，特にリンパ腫様ですが，クロナリティがはっきりせず，硬化像，線維化を伴うもの（炎症性偽腫瘍）で強く疑います．また，両者の立場からは，多クローン性高γグロブリン血症（＋高IgE血症）の場合です．このような患者さんでは，血清IgGサブクラス測定や，IgG4，IgG免疫染色を考慮します.

全体のまとめ

臨床像：IgG4 関連疾患は，下垂体，涙腺，唾液腺，甲状腺，膵臓を含む消化器領域，肺，泌尿器生殖器，大動脈など，全身の組織で生じえる．IgG4 関連疾患の肝胆道病変は，PSCや胆管癌との鑑別が重要である[2]．肺病変では様々な形態をとりうる[3]．また，IgG4 関連疾患は典型的SSとは全く異なる疾患であり，ステロイドが著効する[5].

病態：IgG4 関連疾患の発症にはTh2 優位の免

疫応答と，制御性T細胞の活性化が関与する可能性がある．また，IgG4関連疾患であるMDでは，局所の体細胞高頻度突然変異が生じており，IgG4クローンが局所（涙腺）と末梢血間を行き来する可能性が考えられた[4]．

文　献

1) 厚生労働省難治性膵疾患調査研究班・日本膵臓学会：自己免疫性膵炎臨床診断基準2006. 膵臓　21：395–397, 2006.
2) Zen Y, et al : IgG4-related sclerosing cholangitis with and without hepatic inflammatory pseudotumor, and sclerosing pancreatitis-associated sclerosing cholangitis:do they belong to a spectrum of sclerosing pancreatitis? Am J Surg Pathol 28 : 1193–1203, 2004.
3) 松井祥子：IgG4関連疾患の肺病変．日胸臨　67：378–386, 2008.
4) Yamada K, et al : Clonal relationship between infiltrating immunoglobulin G4 (IgG4)-positive plasma cells in lacrimal glands and circulating IgG4-positive lymphocytes in Mikuliz's disease. Clin Exp Immunol 152 : 432–439, 2008.
5) Masaki Y, et al : Proposal for a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome : analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis 2008（in press）.

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Membranous nephropathy associated with IgG4-related systemic disease and without autoimmune pancreatitis

T. Saeki[1], N. Imai[2], T. Ito[1], H. Yamazaki[1] and S. Nishi[2]

[1]Department of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka, and [2]Divisions of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Key words
IgG4 – membranous nephropathy – tubulo-interstitial nephritis – autoimmune pancreatitis

Abstract. We describe an elderly man with membranous nephropathy and lymphoplasmacytic infiltration into the renal interstitium who presented with a high serum IgG4 concentration and no organ involvement in the pancreatobiliary system. Although the patient had hypocomplementemia and was positive for antinuclear antibodies, he was negative for antibodies against Sm, SS-A, SS-B and RNP, and his anti-DNA antibody titer was not elevated. Immunohistochemistry demonstrated that the infiltrated plasma cells in the renal interstitium and glomerular capillary walls were strongly positive for IgG4. Electron microscopy showed electron-dense deposits on the glomerular basement membranes and tubular basement membranes. The present case suggests that membranous nephropathy, like tubulo-interstitial nephritis, is one of the renal features of IgG4-related systemic disease.

Introduction

Recently, autoimmune pancreatitis (AIP) has been recognized as a pancreatic manifestation of a systemic fibroinflammatory disease that affects not only the pancreas, but also a variety of other organs including the bile duct, salivary glands, retroperitoneum, lymph nodes, lungs and kidneys [Chari 2007, Kamisawa and Okamoto 2006]. In this condition, elevation of the serum IgG4 concentration and abundant IgG4-positive plasma cell infiltration of the affected organs are characteristic features, and steroid therapy is quite effective. Among the renal lesions associated with AIP, tubulo-interstitial nephritis (TIN) is especially noteworthy. Recently, we reported TIN patients without AIP, but in whom the clinicopathological features were quite similar to those of TIN associated with AIP, suggesting that TIN is one of the renal features of this systemic disease, irrespective of the presence of AIP [Saeki et al. 2007b]. In this report, we present a non-AIP patient with membranous nephropathy associated with IgG4-related systemic disease.

Case report

An 83-year-old Japanese man was admitted to a local hospital with leg edema, proteinuria and renal insufficiency. He had been treated for a renal stone 10 years previously, and undergone surgery for chronic subdural hematoma 5 years before presentation. His medical records had shown no abnormalities except for mild glucose intolerance for several years. He had not taken any drugs and had no adverse lifestyle habits. Recently he had undergone transurethral resection of the prostate (TUR-P) because of bilateral hydronephrosis caused by benign prostatic hypertrophy. Although the leg edema and bilateral hydronephrosis improved, proteinuria and renal insufficiency persisted after TUR-P. Therefore, he was referred to our hospital 5 weeks after TUR-P. On admission, no characteristic physical findings or sicca complex were evident. His blood pressure was 156/72 mmHg. Laboratory findings were as follows: Hb 11.1 g/dl, Ht 34.1%, WBC 4000/μl (St 2, Seg 47, Mono 7, Ly 43, Ba 1), platelets 32.7 · 10^4/μl, serum creatinine 1.48 mg/dl, blood urea nitrogen 21.4 mg/dl, total protein 7.8 g/dl, serum albumin 2.8 g/dl, and HbA1c 5.9%. Serum electrolytes, and the results of liver function stud-

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Correspondence to
Dr. T. Saeki, MD, PhD
Department of Internal Medicine, Nagaoka Red Cross Hospital, Senshu 2-297-1, Nagaoka, Niigata 940-2085, Japan
saekit@nagaoka.jrc.or.jp

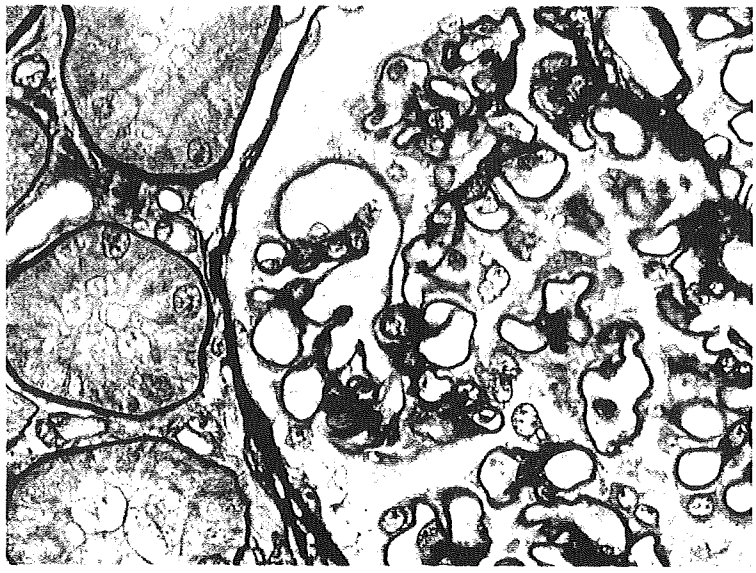

Figure 1A.

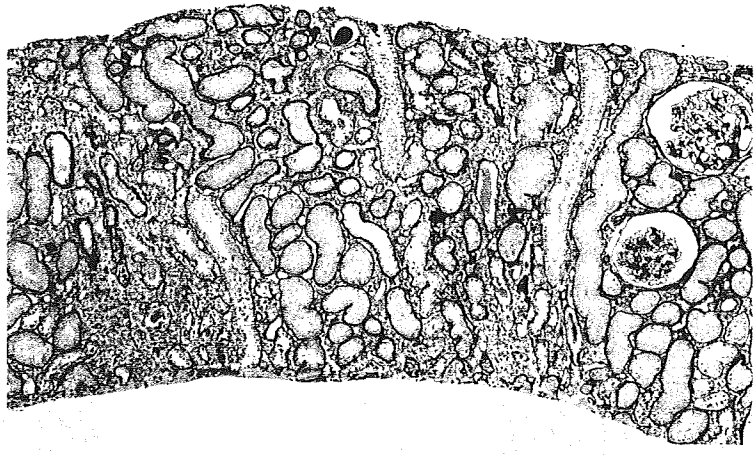

Figure 1B.

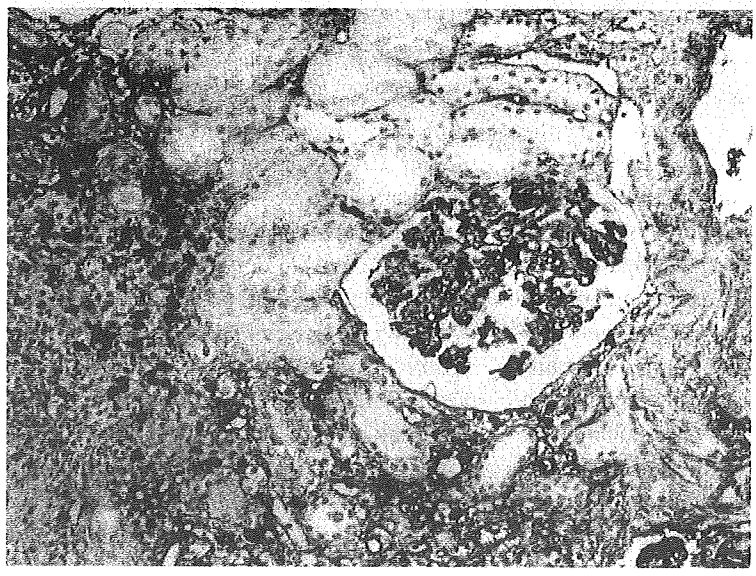

Figure 2.

Figure 1. Light microscopy findings of the renal biopsy. (A) Glomeruli showed segmental spike formation and subepithelial deposits on the glomerular capillary walls (PAM-Masson trichrome × 800); (B) Interstitium showed mononuclear cell infiltration and interstitial fibrosis with tubule atrophy (PAM-Masson trichrome × 80).

Figure 2. Immunostaining of IgG4 showed diffuse staining of the glomerular capillary walls, and strongly positive IgG4 staining on the infiltrated plasma cells in the renal interstitium. IgG4 staining was also shown on the tubular basement membranes focally (× 300).

ies including serum amylase, were all within the normal ranges. Urinalysis revealed 3+ proteinuria and 3+ occult blood, although hematuria had not been evident before TUR-P. 24-hour urine collection yielded 2.3 g of protein and showed a creatinine clearance of 44.4 ml/min. The levels of urinary β2-microglobulin and N-acetyl-β-D-glucosaminidase (NAG) were 10.7 mg/ day and 10.3 U/l, respectively. Serum IgG, IgA, IgM and IgE levels were 3144 mg/dl, 385 mg/dl, 79 mg/dl and 32.1 IU/ml, respectively. Serum IgG subclass concentrations and % IgG subclass studies showed IgG1 = 1890 mg/dl (46.2%), IgG2 = 1110 mg/dl (27.1%), IgG3 = 170 mg/dl (4.1%) and IgG4 = 924 mg/dl (22.5%) (normal range of IgG4 < 105 mg/dl, 3 – 6%). C-reactive protein level was 0.47 mg/dl. Although antinuclear antibody (ANA) was positive (× 2,560), anti-DNA antibody was 6 IU/ml (normal < 6 IU/ml), and anti-Sm, anti-SS-A, anti-SS-B, and anti-RNP antibodies were not detected. Lupus anticoagulant, anti-cardiolipin antibody, myeloperoxidase-antineutrophil cytoplasmic antibody and poteinase-3-antineutrophil cytoplasmic antibody were all negative. Complement studies showed CH50 = 16 U/ml (normal 30 – 45), C3 = 56 mg/dl (normal 65 – 135), and C4 = 6 mg/dl (normal 13 – 35). Hepatitis B surface antigen, hepatitis C antibody, and cryoglobulin were not found. Serum M protein was not detected. Chest X-ray, whole-body CT examination and gallium citrate scintigraphy showed no abnormal findings.

Because of a suspected autoimmune disease, renal biopsy was conducted. The specimen for light microscopy contained 18 glomeruli, of which 5 were globally sclerotic. Fine subepithelial deposits on the glomerular

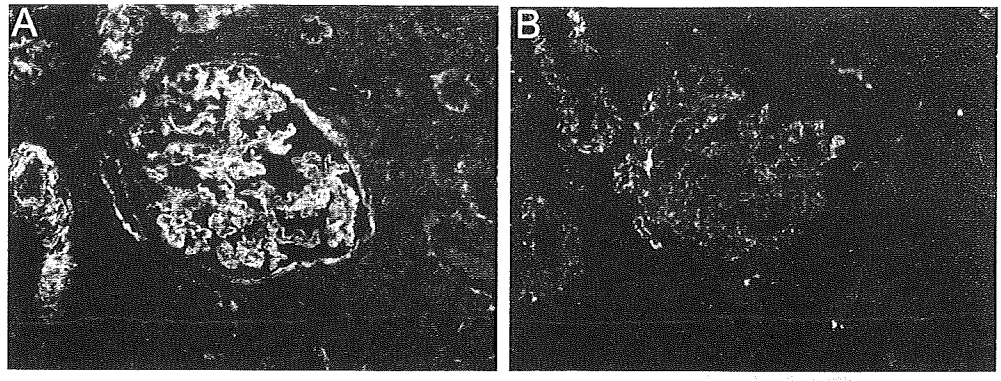

Figure 3. Immunofluorescence study revealed diffuse and global IgG and C3 deposition along the glomerular capillary walls. IgG and C3 depositions were also observed partially on the tubular basement membranes. (A) IgG (× 400); (B) C3 (× 400).

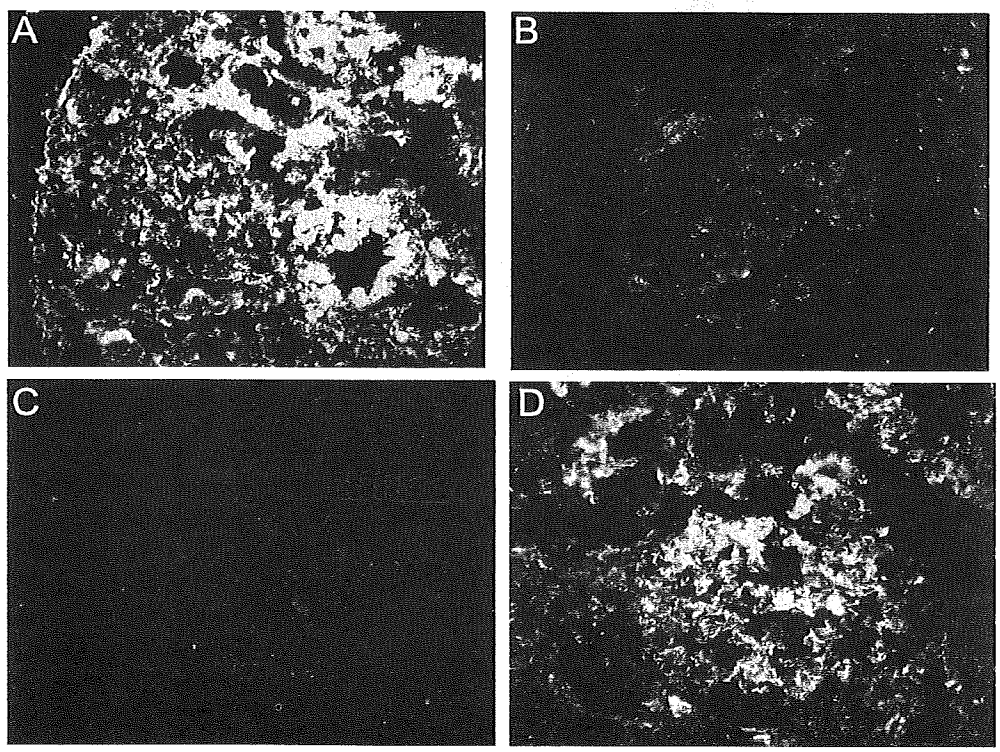

Figure 4. Immunofluorescence staining for IgG subclasses showed diffuse and global IgG1 and IgG4 deposition and weak IgG2 deposition in the glomeruli and the interstitium. (A) IgG1 (× 400); (B) IgG2 (× 400); (C) IgG3 (× 400); (D) IgG4 (× 400).

capillary walls were noticed on segmental glomerular basement membranes by PAM-Masson trichrome staining (Figure 1A). Interstitial cell infiltration consisting of lymphocytes and plasma cells and atrophic tubules adjoining interstitial fibrosis were also recognized in focal areas of the renal interstitium (Figure 1B). Immunohistochemistry demonstrated strongly positive IgG4 staining on the infiltrated plasma cells in the renal interstitium, and diffuse staining of the glomerular capillary walls. IgG4 staining was also shown on a part of the tubular basement membranes (TBMs) (Figure 2). Routine immunofluorescence study revealed diffuse and global IgG and C3 deposition along the glomerular capillary walls. IgG and C3 depositions were also observed partially on the TBMs (Figure 3). There was no apparent deposition of IgA, IgM, C1q or fibrinogen in the glomeruli or interstitium. Although precise evaluation was difficult because the specimens were inadequate, immunofluorescence staining for IgG subclasses showed diffuse and global deposition of IgG1 and IgG4 and weak deposition of IgG2 in the glomeruli and

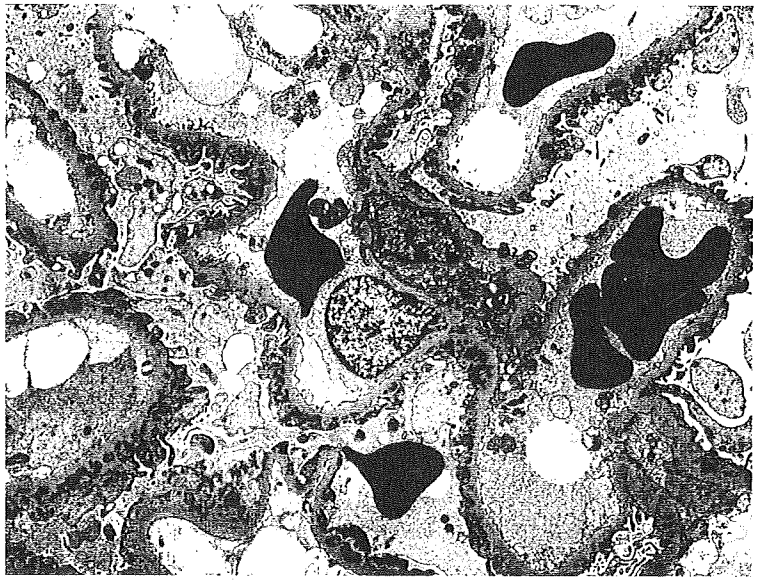

Figure 5A.

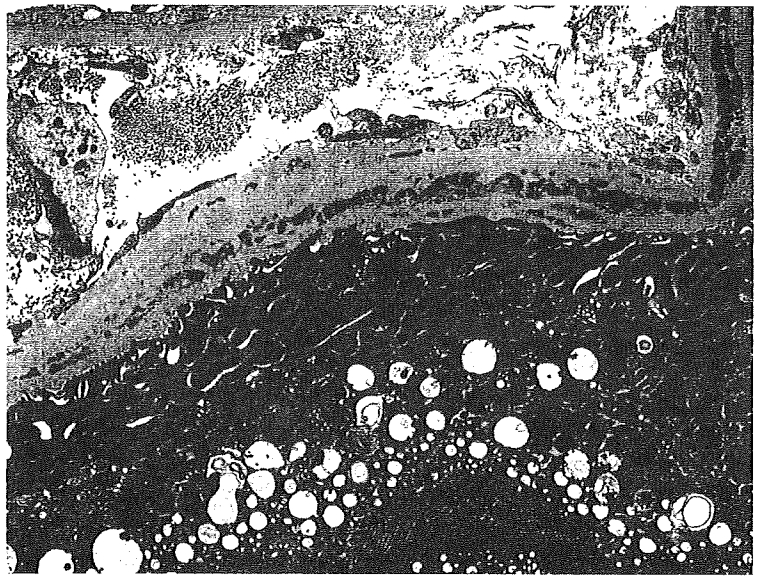

Figure 5B.

Figure 5. Electron microscopy showed electron-dense subepithelial deposits on the glomerular basement membranes (A) and the tubular basement membranes (B).

the interstitium (Figure 4). Electron microscopy showed electron-dense subepithelial deposits on the glomerular basement membranes and the TBMs (Figure 5). In the mesangial area, a slightly expanded mesangial matrix and tiny electron-dense deposits were noticed. No microtubular structures were found in the glomerular endothelial cells. After daily administration of oral prednisolone at 40 mg, the hypocomplementemia and elevated IgG4 soon ameliorated. 4 months later, the serum creatinine level had decreased to 1.22 mg/dl, and proteinuria to 1.6 g/day.

Discussion

Although the name and definition of the disease have not been established, the concept of IgG4-related systemic disease has been recognized recently [Kamisawa and Okamoto 2006]. Although AIP is a representative form of IgG4-related systemic disease, there has been a recent increase in the number of articles dealing with various organ involvements associated with IgG4-related systemic disease [Nakanuma and Zen 2007, Yamamoto et al. 2006], irrespective of the presence of AIP, and the condition is now recognized as a multi-organ disease. We recently described that renal parenchymal lesions in IgG4-related systemic disease are due to dense lymphoplasmacytic infiltration of the renal interstitium, and that the lesions vary from diffuse TIN to tumor-like masses according to the distribution patterns of the infiltrating cells [Saeki et al. 2007a]. Indeed, many case reports of TIN associated with IgG4-related systemic disease including AIP have been reported [Cornell et al. 2007, Yoneda et al. 2007]. In most of them, marked infiltration of lymphocytes and IgG4-positive plasma cells into the renal interstitium with interstitial fibrosis were characteristic, and the glomerular lesions were minor. However, there are a few case reports of IgG4-related disease showing glomerular changes in addition to TIN [Katano et al. 2007, Uchiyama-Tanaka et al. 2004, Watson et al. 2006]. Watson and colleagues [2006] described a patient with AIP who developed focal sclerosing lymphoplasmacytic TIN and concurrent membranous nephropathy. The clinicopathological features of the patient were quite similar to those in the present case, although our patient did not have associated AIP. Both of the patients were elderly men and showed markedly elevated serum IgG4 levels. Membranous nephropathy with dense infiltration of plasma cells and lymphocytes into the renal interstitium were demonstrated by renal biopsies. Immunofluorescence studies revealed global capillary wall deposits of IgG and granular peritubular immunopositivity for

IgG. Electron microscopy demonstrated subepithelial deposits on the glomerular basement membrane and in the TBMs. Immunohistochemistry for IgG4 showed numerous IgG4-positive plasma cells infiltrating the renal cortex and immunoreactivity for IgG4 in the glomerular capillary walls and renal interstitium. An increased percentage of serum IgG4 and predominant IgG4 staining of glomerular capillary walls have also been documented in patients with idiopathic membranous nephropathy [Kuroki et al. 2002]. However, in both patients, the percentage of IgG4 was quite high (30% in Watson's case and 22.6% in ours, respectively) in comparison to patients with primary membranous nephropathy (8 ± 6.4%) [Kuroki et al. 2002]. Abundant IgG4-positive plasma cell infiltration in the renal interstitium and electron-dense deposits in the TBMs are frequently described in TIN associated with IgG4-related systemic diseases [Cornell et al. 2007, Saeki et al. 2007b]. However, they have not been documented in idiopathic membranous nephropathy. Furthermore, our patient showed hypocomplementemia often observed in IgG4-related disease [Muraki et al. 2006, Saeki et al. 2007a,b], but not in idiopathic membranous nephropathy. Taken together, the present case was diagnosed as membranous nephropathy associated with IgG4-related systemic disease.

Although high serum IgG4 and IgG4-positive plasma cell infiltration are characteristic, the pathogenesis of the disease has not been elucidated. IgG4 is an unusual antibody. It is the rarest of IgG subclasses and is unable to bind C1q complement [van der Zee et al. 1986]. High serum IgG4 concentrations have been reported in a limited number of diseases, including atopic dermatitis [Aalberse et al. 1993] and pemphigus vulgaris and pemphigus foliaceus [Futei et al. 2001]. IgG4 behaves as a pathogenic antibody in pemphigus [Rock et al. 1989], and as a suppressive antibody in allergic diseases [Platts-Mills et al. 2001]. Zen and colleagues [2007] demonstrated that Th2 and regulatory immune reactions are up-regulated in the affected tissues of patients with IgG4-related sclerosing pancreatitis and cholangitis and suggested that an allergic nature might be associated with the pathogenesis of IgG4-related disease.

On the other hand, Hamano and colleagues [2001] showed that IgG4-type immune complexes are closely associated with the disease activity in patients with AIP, suggesting that immune complexes are related to the pathogenesis of AIP. Cornell and coworkers [2007] demonstrated IgG4 immune-complex deposition in the renal TBMs of patients with TIN associated with AIP, also suggesting an immune complex mechanism. Muraki and colleagues [2006] demonstrated that AIP showed high levels of serum circulating immune complex in its active state, linking it to a complement activation system with the classical pathway. Interestingly, high circulating immune complex levels determined by C1q assay were significantly associated with elevated serum IgG1, and not with serum IgG4. It was considered that, in the pathogenesis of AIP, an IgG1-type immune complex might induce complement activation through the classical pathway. Van der Zee and coworkers [1986] demonstrated that IgG4 antibodies protect against the biological effects of the complement-fixing IgG subclasses under conditions of high IgG4 antibody titers resulting from prolonged exposure to antigens. IgG4 might act as a blocking antibody under such conditions.

Membranous nephropathy is caused by immune complex localization in the subepithelial zone of glomerular capillaries. Because the target antigen in human idiopathic membranous nephropathy has not been elucidated, the pathogenic mechanisms of human idiopathic membranous nephropathy remain incompletely understood. As mentioned above, the significance of IgG4 has been documented in idiopathic membranous nephropathy [Kuroki et al. 2002]. Several hypothetical roles of IgG4 in the pathogenesis of idiopathic membranous nephropathy have been discussed, but nothing definitive has yet emerged. Concerning clinical and histological features, there are many differences between IgG4-related systemic disease and idiopathic membranous nephropathy. However, predominance of Th2 cytokines is common [Kuroki et al. 2005] and IgG4 seems to play an important role in both conditions. Further examinations are therefore needed.

In conclusion, the present case suggests that membranous nephropathy is one of the renal features of IgG4-related systemic disease, and can develop irrespective of the presence of AIP. IgG4-related systemic disease

should be considered as an additional pathogenesis of membranous nephropathy.

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References

Aalberse RC, Van Milligen F, Tan KY, Stapel SO. Allergen-specific IgG4 in atopic disease. Allergy. 1993; *48:* 559-569.

Chari ST. Diagnosis of autoimmune pancreatitis using its five cardinal features: introducing the Mayo Clinic's HISTORt criteria. J Gastoenterol. 2007; *42 (Suppl 18):* 39-41.

Cornell LD, Chicano SL, Deshpande V, Collins AB, Selig MK, Lauwers GY, Barisoni L, Colvin RB. Pseudotumors due to IgG4 immune-complex tubulo-interstitial nephritis associated with autoimmune pancreatocentric disease. Am J Surg Pathol. 2007; *31:* 1586-1597.

Futei Y, Amagai M, Ishii K, Kuroda-Kinoshita K, Ohya K, Nishikawa T. Predominant IgG4 subclass in autoantibodies of pemphigus vulgaris and foliaceus. J Dermatol Sci. 2001; *26:* 55-61.

Hamano H, Kawa S, Horiuchi A, Unmo H, Furuya N, Akamatsu T, Fukushima M, Nikaido T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001; *344:* 732-738.

Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. J Gastroenterol. 2006; *41:* 613-625.

Katano K, Hayatsu Y, Matsuda T, Miyazaki R, Yamada K, Kawano M, Takahashi N, Kimura H, Yoshida H. Endocapillary proliferative glomerulonephritis with crescent formation and concurrent tubulo-interstitial nephritis complicating retroperitoneal fibrosis with a high serum level of IgG4. Clin Nephrol. 2007; *68:* 308-314.

Kuroki A, Shibata T, Honda H, Totsuka D, Kobayashi K, Sugisaki T. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. Intern Med. 2002; *41:* 936-942.

Kuroki A, Iyoda M, Shibata T, Sugisaki T. Th2 cytokines increase and stimulate B cells to produce IgG4 in idiopathic membranous nephropathy. Kidney Intern. 2005; *68:* 302-310.

Muraki T, Hamano H, Ochi Y, Komatsu K, Komiyama Y, Arakura N, Yoshizawa K, Ota M, Kawa S, Kiyosawa K. Autoimmune pancreatitis and complement activation system. Pancreas. 2006; *32:* 16-21.

Nakanuma Y, Zen Y. Pathology and immunopathology of immunoglobulin G4-related sclerosing cholangitis: the latest addition to the sclerosing cholangitis family. Hepatol Res. 2007; *3 (Suppl):* S478-S486.

Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet. 2001; *357:* 752-756.

Rock B, Martins CR, Theofilopoulos AN, Balderas RS, Anhalt GJ, Labib RS, Futamura S, Rivitti EA, Diaz LA. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). N Engl J Med. 1989; *320:* 1463-1469.

Saeki T, Nishi S, Ito T, Yamazaki H, Miyamura S, Emura I, Imai N, Ueno M, Saito A, Gejyo F. Renal lesions in IgG4-related systemic disease. Intern Med. 2007a; *46:* 1365-1372.

Saeki T, Saito A, Yamazaki H, Emura I, Imai N, Ueno M, Nishi S, Miyamura S, Gejyo F. Tubulo-interstitial nephritis associated with IgG4-related systemic disease. Clin Exp Nephrol. 2007b; *11:* 168-173.

Uchiyama-Tanaka Y, Mori Y, Kimura T, Sonomura K, Umemura S, Kishimoto N, Nose A, Tokoro T, Kijima Y, Yamahara H, Nagata T, Masaki H, Umeda Y, Okazaki K, Iwasaka T. Acute tubulo-interstitial nephritis associated with autoimmune-related pancreatitis. Am J Kidney Dis. 2004; *43:* 18-25.

Watson SJ, Jenkins DA, Bellamy CO. Nephropathy in IgG4-related systemic disease. Am J Surg Pathol. 2006; *30:* 1472-1477.

Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, Shinomura Y, Imai K. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol. 2006; *16:* 335-340.

Yoneda K, Murata K, Katayama K, Ishikawa E, Fuke H, Yamamoto N, Ito K, Shiraki K, Nomura S. Tubulo-interstitial nephritis associated with IgG4-related autoimmune disease. Am J Kidney Dis. 2007; *50:* 455-462.

van der Zee JS, van Swieten P, Aalberse RC. Inhibition of complement activation by IgG4 antibodies. Clin Exp Immunol. 1986; *64:* 415-422.

Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, Nakamura Y. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology. 2007; *45:* 1538-1546.

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ORIGINAL ARTICLE

Hypocomplementemia of unknown etiology: an opportunity to find cases of IgG4-positive multi-organ lymphoproliferative syndrome

Takako Saeki · Tomoyuki Ito · Hajime Yamazaki · Naofumi Imai · Shinichi Nishi

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Abstract Recently, a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome (IgG4+ MOLPS), characterized by hyper-IgG4 gammaglobulinemia and IgG4-positive plasma cell tissue infiltration, has been proposed. It includes autoimmune pancreatitis (AIP), Mikulicz's disease, and many other inflammatory conditions affecting multiple organs. However, diagnosis is difficult if the disease is not suspected because serum IgG subclasses are not measured routinely and the affected organs vary. Because hypocomplementemia is often observed in this condition, we investigated the serum subclasses of IgG in patients with hypocomplementemia, especially of unknown etiology. We found 6 patients with high serum IgG4 levels among 10 patients with hypocomplementemia of unknown etiology who visited our hospital between December 2004 and September 2007. The results of additional pathological and imaging examinations in the 6 patients with high serum IgG4 levels were compatible with IgG4+ MOLPS. Our results suggest that hypocomplementemia of unknown etiology offers an opportunity to find cases of IgG4+ MOLPS.

Keywords IgG4 · Complement · Hypocomplementemia · Autoimmune pancreatitis · Mikulicz's disease

T. Saeki (✉) · T. Ito · H. Yamazaki
Department of Internal Medicine, Nagaoka Red Cross Hospital,
2-297-1 Senshu, Nagaoka, Niigata 940-2085, Japan
e-mail: saekit@nagaoka.jrc.or.jp

N. Imai · S. Nishi
Division of Clinical Nephrology and Rheumatology,
Graduate School of Medical and Dental Sciences,
Niigata University, 1-757, Asahimachi-dori,
Niigata 951-8500, Japan

Introduction

Autoimmune pancreatitis (AIP) is a chronic fibroinflammatory condition characterized by an elevated serum IgG4 concentration, abundant IgG4-positive plasma cell infiltration of the pancreas, and a favorable response to steroids [1–3]. Because increased numbers of IgG4-positive plasma cells have been documented not only within the pancreatobiliary system but also in other organs, a concept of IgG4-related systemic disease has been proposed [1]. On the other hand, Mikulicz's disease has been shown to be a spectrum of IgG4-related systemic disease, rather than a part of Sjögren's syndrome [4], and currently, various conditions associated with IgG4-related disease are being discussed [4–9]. Recently, a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome (IgG4+ MOLPS), characterized by hyper-IgG4 gammaglobulinemia and IgG4-positive plasma cell tissue infiltration, has been proposed [10]. IgG4+ MOLPS includes AIP, Mikulicz's disease, sclerosing cholangitis, Küttner's tumor, inflammatory pseudotumor of the lung, liver, and breast, retroperitoneal and mediastinal fibrosis, interstitial nephritis, autoimmune hypophysitis, and many other inflammatory conditions affecting multiple organs [10]. Although the distribution of the involved organs in IgG4+ MOLPS is similar to that in Sjögren's syndrome, there are obvious differences in clinical and pathological features between them, and IgG4+ MOLPS is an entirely new systemic disease [10]. However, diagnosis is difficult in the absence of AIP or Mikulicz's disease, which is the representative condition of IgG4+ MOLPS, because serum IgG subclasses are not measured routinely and the affected organs vary. Although the pathogenesis has not been elucidated, hypocomplementemia has often been documented in IgG4+ MOLPS [11, 12]. This situation prompted us to examine the serum subclasses of

IgG in patients with hypocomplementemia, especially that of unknown etiology.

Patients and methods

Between December 2004 and September 2007, serum C3, C4, and CH50 levels were measured in 5,826 serum samples (some from the same patients) at the Department of Internal Medicine, Nagaoka Red Cross Hospital, at the request of the attending physicians. In 121 patients, the CH50 level was found to be decreased, together with a decrease in the serum level of C3, C4, or both. Among these patients, the etiology of the hypocomplementemia was clarified in 111 (systemic lupus erythematosus in 72 patients, parvovirus infection in 9, rheumatoid arthritis with vasculitis in 8, autoimmune hemolytic anemia in 6, liver failure in 6, cryoglobulinemia in 3, membranoproliferative glomerulonephritis in 3, acute glomerulonephritis in 2, and AIP in 2). In the remaining 10 patients, the etiology of the hypocomplementemia was initially unclear, and we measured the serum subclasses of IgG in 8 of them.

Among the 8 patients with hypocomplementemia of unknown etiology, 3 male patients (patients 1 to 3) were diagnosed as having tubulointerstitial nephritis; in which two of them (patients 1 and 2) had accompanying systemic lymphadenopathy, and one (patient 1) had a history of chronic pancreatitis, sialadenitis, and idiopathic thrombocytic purpura. One male patient (patient 4) was referred to us because of proteinuria, and a renal biopsy showing membranous nephropathy with lymphoplasmacytic infiltration into the renal interstitium and another male patient (patient 6) was referred to us because of asymptomatic hypergammaglobulinemia. One female patient (patient 5) was referred to us because of edema, eosinophilia, and lymphadenopathy. The other 2 patients (patients 7 and 8) were diagnosed as having pleuritis and pneumosilicosis, respectively. They did not have AIP or Mikulicz's disease at the time when hypocomplementemia was diagnosed. Laboratory examinations demonstrated hypergammaglobulinemia in all of them. Although 6 out of the 8 patients were positive for antinuclear antibodies, they were negative for antibodies against Sm, SS-A, SS-B, Scl-70, Jo-1, and RNP, and their anti-DNA antibody titers were not elevated. All of them were negative for cryoglobulin, lupus anticoagulant, anti-cardiolipin antibody, perinuclear and cytoplasmic antineutrophil cytoplasmic antibodies, and M-protein.

We also measured the serum subclasses of IgG in 11 of the patients with systemic lupus erythematosus (SLE) and 2 of the patients with cryoglobulinemia, which are representative conditions characterized by hypocomplementemia. SLE was diagnosed in accordance with the 1982 SLE classification criteria. In the 2 patients with cryoglobulinemia, one case was idiopathic, and the other was associated with chronic hepatitis C. The 13 patients with SLE or cryoglobulinemia had various degrees of organ involvement. Among a total of 21 patients for whom the serum subclasses of IgG were measured, 3 were receiving steroid treatment and the others were seen before steroid therapy. We conducted additional imaging and histological studies of the patients with high serum IgG4 levels because of suspected IgG4+ MOLS.

Results

Among the 8 patients with hypocomplementemia of unknown etiology, 6 patients (patients 1 to 6) showed both markedly high serum IgG4 concentrations (305–2340 mg/dl; normal: <105) and percentage IgG4 levels (IgG4/total IgG) (16.7–35.5%; normal: 3–6%; Table 1). In patient 7, the serum IgG4 level was slightly increased, but pleuritis, hypocomplementemia and the increased serum IgG4 level ameliorated without therapy, and the significance of this feature was obscure. All of the 13 patients with a definitive diagnosis of SLE or cryoglobulinemia had normal serum IgG4 concentrations and percentage IgG4 levels (Table 2).

In the 6 patients with high serum concentrations of IgG4 (patients 1 to 6), we conducted additional imaging and histological studies because of suspected IgG4+ MOLPS. Immunohistological studies of tissues obtained by biopsy revealed abundant infiltration of IgG4-positive plasma cells in the renal interstitium in patients 1 to 3, and in the salivary gland in patient 2. In patients 2 and 3, the results of gallium citrate scintigraphy demonstrated gallium-67 accumulation, although salivary gland swelling was not evident. (We have previously reported patients 1 to 3 in references [7–9]). In patient 4 with membranous nephropathy, glomerular capillary walls and infiltrated plasma cells in the renal interstitium were strongly positive for IgG4. An increased percentage of serum IgG4 and predominant IgG4 staining of glomerular capillary walls have also been documented in patients with idiopathic membranous nephropathy [13]. However, the percentage of IgG4 was quite high in comparison with patients having primary membranous nephropathy [13], and abundant IgG4-positive plasma cell infiltration in the renal interstitium has not been documented in idiopathic membranous nephropathy, although it has been frequently described in tubulointerstitial nephritis associated with IgG4-related systemic diseases [8]. Therefore, the patient was diagnosed as having membranous nephropathy associated with IgG4+ MOLPS [14]. In patient 5, minor salivary gland biopsy revealed IgG4-positive plasma cell infiltration, although the salivary glands were not swollen. Therefore, we diagnosed patients 1 to 5 as having IgG4+ MOLPS, also taking into account the

Table 1 Profiles in patients with hypocomplementemia of unknown etiology

Patient	Age/Sex	Clinical features	IgG (mg/dl)	IgG1 (mg/dl) IgG1/IgG (%)	IgG2 (mg/dl) IgG2/IgG (%)	IgG3 (mg/dl) IgG3/IgG (%)	IgG4 (mg/dl) IgG4/IgG (%)	ANA	C3 (mg/dl)	C4 (mg/dl)	CH50 (U/l)	Affected organs	IgG4+ plasma infiltration
1	61/M	Lymphadenopathy	6569	1160*	609*	149*	730*	+	24	1	13	K, Ly, (P, Sa)	K
		TIN		43.8*	23.0*	5.6*	27.6*						
2	60/M	Lymphadenopathy	5188	942*	555*	26*	305*	+	27	1	<10	K, Ly, Sa	K, Sa
		TIN		51.5*	30.3*	1.4*	16.7*						
3	68/M	TIN	2995	1940	1050	98	670	+	41	2	<10	K, Sa	K
				51.6	27.9	2.6	17.8						
4	83/M	Membranous	3144	1890	1110	170	924	+	56	6	16	K	K
		Nephropathy		46.1	27.1	4.1	22.6						
5	39/F	Edema, eosinophilia	4708	2460	1530	268	2340	–	39	2	<10	Sa, Ly	Sa
		Lymphadenopathy		37.2	23.1	4.1	35.5						
6	83/M	Hypergammaglobulinemia	5762	3150	1400	213	1720	–	37	2	<10	Biliary system, renal pelvis	NE
		Without symptom		48.6	21.6	3.3	26.5						
7	86/F	Pleuritis	2177	1360	570	92.9	159	+	21	2	<10	Lung	NE
				62.3	26.1	4.3	7.3						
8	49/M	Pneumosilicosis	2561	1780	747	215	21.6	–	67	2	<10	Lung	NE
				64.4	27.1	7.7	0.78						

TIN tubulointerstitial nephritis, *ANA* antinuclear antibody, *K* kidney, *Ly* lymph nodes, *P* pancreas, *Sa* salivary glands, *NE* not examined, * under treatment. Normal ranges of C3, C4, and CH50 are 65–135 mg/dl, 13–35 mg/dl and 30–45 U/ml, respectively

Table 2 Profiles of patients with SLE and cryoglobulinemia

Patient	Age/Sex	Diagnosis	Clinical features	IgG (mg/dl)	IgG1 (mg/dl)	IgG2 (mg/dl)	IgG3 (mg/dl)	IgG4 (mg/dl) (IgG4/IgG %)	C3 (mg/dl)	C4 (mg/dl)	CH50 (U/l)
1	45/F	SLE	Pancytopenia	1187	636	532	51	10.6 (0.9)	51	4	14
2	64/F	SLE	Erythema, pleuritis, lymphadenopathy	2390	1470	580	59	89.5 (4.1)	31	2	<10
3	17/F	SLE	Lymphadenopathy	2169	1410	293	151	36.5 (1.9)	40	2	<10
4	65/M	SLE	Lupus nephritis (WHO V)	1443	865	532	48.3	19 (1.3)	58	11	28
5	30/F	SLE	Erythema, arthritis	1476	899	436	67	25 (1.8)	50	10	24
6	53/M	SLE	Erythema, pancytopenia	3725	2700	168	137	17.6 (0.6)	40	9	18
7	24/F	SLE	Thrombocytopenia, eosinophilia	1476	577*	292*	17.5*	15.4*(1.7*)	66	10	20
8	32/F	SLE	Lupus nephritis (WHO V), AIHA	672	453	79	34	3 (0.5)	65	3	13
9	56/M	SLE	Lupus nephritis (WHO IV), thrombocytopenia	825	307	305	24	16 (2.5)	39	10	17
10	51/F	SLE	Lupus nephritis (WHO IV), AIHA	2387	1450	319	25	41 (2)	68	5	15
11	36/F	SLE	Pancytopenia	1493	880	508	28	32 (2.2)	39	2	2
12	52/M	Cryo	Purpura, arthritis	2186	1440	936	74	90 (3.5)	27	1	<10
13	71/F	Cryo, HCV hepatitis	Purpura, hematuria	2095	1020	306	175	0	10	NE	<10

SLE systemic lupus erythematosus, *cryo* cryoglobulinemia, *AIHA* autoimmune hemolytic anemia, * under treatment

effectiveness of corticosteroid therapy for the organ involvement. Although patient 6 had no symptoms, and liver and renal functions were normal, CT and magnetic resonance imaging scans showed intrahepatic biliary strictures resembling primary sclerosing cholangitis and wall thickening of the renal pelvis, which were compatible with IgG4+ MOLPS. This patient has been followed up without therapy.

Discussion

Hypocomplementemia is generally induced by consumption of, or a defect in complement. Various immune-complex-mediated conditions including SLE, post-streptococcal acute glomerulonephritis, membranoproliferative glomerulonephritis, cryoglobulinemia, and some complement deficiency states are well known etiologies of hypocomplementemia. Recently, a new concept of IgG4-related disease has been discussed, and hypocomplementemia in IgG4-related disease has been receiving attention. The levels of serum complement are decreased in 17–36% of patients with AIP when examined by different assays [11], and in 30% of patients with Mikulicz's disease [12].

In IgG4+ MOLPS, high levels of serum IgG4 and IgG4-positive plasma cell infiltration in tissues are the most important features for diagnosis [10]. However, as neither of the parameter is examined routinely, diagnosis is difficult if the disease is not suspected. Recently, it is becoming more widely known that AIP, Mikulicz's disease, some types of sclerosing cholangitis, and retroperitoneal fibrosis are the manifestations of IgG4+ MOLPS, although many other conditions could also potentially be involved [10]. In this study, we have shown that hypocomplementemia of unknown etiology offers an opportunity to find patients with IgG4+ MOLPS. We found 6 patients with high serum IgG4 levels among patients with hypocomplementemia of unknown etiology who visited our hospital during the last few years. Generally, the level of IgG4 does not vary with sex or age, and the quality of IgG4 and IgG4/total IgG ratio tends to remain constant [15]. Because in these 6 patients the IgG4/total IgG ratio was extremely high, in addition to the serum IgG4 concentration, the high serum IgG4 level must have been significant not because of hypergammaglobulinemia. The results of additional pathological and imaging examinations in these 6 patients were compatible with IgG4+ MOLPS. Abundant IgG4-positive plasma cell infiltration was confirmed in the tissues of 5 patients (patients 1 to 5). In patient 6, CT and magnetic resonance imaging scans showed intrahepatic biliary strictures resembling sclerosing cholangitis, and also wall thickening of the renal pelvis. As sclerosing cholangitis and pelvic lesions are often accompanied by AIP [1, 2, 5, 16], this case might fall within the spectrum of IgG4-related systemic disease, though IgG4-positive plasma cells were not verified histologically.

As the subjects of this study were limited in number, the frequency of IgG4+ MOLPS among patients with hypocomplementemia is unknown. As the serum IgG4 levels in the patients with definitively diagnosed SLE or cryoglobulinemia were all normal, IgG4+ MOLPS do not seem to be considerable in such a classical autoimmune disease.

However, a large-scale study is required to elucidate the relationship between IgG4 and hypocomplementemia. Although immune-complex mechanisms are suggested [3, 11, 12, 17], the pathogenesis of IgG4-related systemic disease is still unclear, and accumulation of further case reports will be essential for clarifying its nature. In a clinical setting, the levels of complement are measured routinely, and hypocomplementemia is observed in a relatively restricted proportion of patients in comparison with hypergammapathy, which is one of the characteristic features of IgG4+ MOLPS [10]. Of course, many patients with IgG4+ MOLPS show normal complement levels. Masaki et al. showed that serum CH50 levels were decreased in 57.8% of patients with IgG4+ MOLPS [10]. However, hypocomplementemia (especially that of unknown etiology) can be a key feature for differentiation of patients with IgG4+ MOLPS. Clinicians should be aware of this possibility when encountering such patients, in whom IgG subclasses should be determined and histological studies including immunohistochemistry and imaging studies should be considered if the IgG4 levels are found to be elevated.

References

1. Kamisawa T (2006) IgG4-related sclerosing disease. Intern Med 45:125–126. doi:10.2169/internalmedicine.45.0137
2. Chari ST, Smyrk TC, Levy MJ et al (2006) Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. Clin Gastroenterol Hepatol 4:1010–1016. doi:10.1016/j.cgh.2006.05.017
3. Hamano H, Kawa S, Horiuchi A et al (2001) High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med 344:732–738. doi:10.1056/NEJM200103083441005
4. Yamamoto M, Takahashi H, Ohara M et al (2006) A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol 16:335–340. doi:10.1007/s10165-006-0518-Y
5. Nakamura Y, Zen Y (2007) Pathology and immunopathology of immunoglobulin G4-related sclerosing cholangitis: the latest addition to the sclerosing cholangitis family. Hepatol Res Suppl 3:S478–S486
6. Kitagawa S, Zen Y, Harada K et al (2005) Abundant IgG4-positive plasma cell infiltration characterizes chronic sclerosing sialadenitis (Kuttner's tumor). Am J Surg Pathol 29:783–791. doi:10.1097/01.pas.0000164031.59940.fc
7. Saeki T, Saito A, Hiura T et al (2006) Lymphoplasmacytic infiltration of multiple organs with immunoreactivity for IgG4: IgG4-related systemic disease. Intern Med 45:163–167. doi:10.2169/internalmedicine.45.1431
8. Saeki T, Saito A, Yamazaki H et al (2007) Tubulointerstitial nephritis associated with IgG4-related systemic disease. Clin Exp Nephrol 11:168–173. doi:10.1007/s10157-007-0464-9
9. Saeki T, Nishi S, Ito T et al (2007) Renal lesions in IgG4-related systemic disease. Intern Med 46:1365–1372. doi:10.2169/internalmedicine.46.0183
10. Masaki Y, Dong L, Kurose N et al. (2008) Proposal for a new clinical entity, IgG4-positive multi-organ lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. Ann Rheum Dis Aug 13 [Epub ahead of print]
11. Muraki T, Hamano H, Ochi Y et al (2006) Autoimmune pancreatitis and complement activation system. Pancreas 32:16–21. doi:10.1097/01.mpa.0000188308.75043.e4
12. Yamamoto M, Takahashi H, Naishiro Y et al (2008) Mikulicz's disease and systemic IgG4-related plasmacytic syndrome (SIPS). Nihon Rinsho Meneki Gakkai Kaishi 31:1–8. doi:10.2177/jsci.31.1
13. Kuroki A, Shibata T, Honda H et al (2002) Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. Intern Med 41:936–942. doi:10.2169/internalmedicine.41.936
14. Saeki T, Imai N, Ito T, Yamazaki H, Nishi S (2009) Membranous nephropathy associated with IgG4-related systemic disease and without autoimmune pancreatitis. Clin Nephrol 71(2):173–178
15. Lock RJ, Unsworth DJ (2003) Immunoglobulins and immunoglobulin subclass in the elderly. Ann Clin Biochem 40:143–148. doi:10.1258/000456303763046067
16. Takahashi N, Kawashima A, Fletcher JG, Chari ST (2007) Renal involvement in patients with autoimmune pancreatitis: CT and MR imaging findings. Radiology 242:791–801. doi:10.1148/radiol.2423060003
17. Cornell LD, Chicano SL, Deshpande V et al (2007) Pseudotumors due to IgG4 immune-complex tubulointerstitial nephritis associated with autoimmune pancreatocentric disease. Am J Surg Pathol 31:1586–1597

Castleman's disease of the retroperineum. With special reference to IgG-4 related disorder.

Masaru Kojima,[a]Naoya Nakamura,[b] Tadashi Motoori,[c] Ken Shimizu,[d] Yoshiro Otuski,[e] Joji Haratake,[f] Akira Ogawa,[g] Tadahiko Igarashi,[h] Nobuhide Masawa,[a] Hiroshi Kobayashi,[e] Shigeo Nakamura.[i]

a. Department of Anatomic and Diagnostic Pathology, Dokkyo University School of Medicine, Mibu, Japan.
b. Department of Pathology, Tokai University School of Medicine, Isehara, Japan.
c. Department of Pathology, Kitasato University, Kitasato Institute Medical Center Hospital, Kitamoto, Japan.
d. Department of Pathology, Saitama Social Insurance Hospital, Saitama, Japan.
e. Department of Pathology, Hamamatsu Seirei Hospital, Hamamatsu, Japan.
f. Department of Pathology and Clinical Laboratories, Saiseikai Yahata Hospital, Kitakyushu, Japan.
g. Department of Pathology and Clinical Laboratories, National Takasaki Hospital, Takasaki, Japan.
h. Department of Hematology, Gunma Cancer Center Hospital, Ohta, Japan.
i. Department of Pathology and Clinical Laboratories, Nagoya University School of Medicine, Nagoya, Japan.

Address correspondence to; Masaru Kojima, M D, Anatomic and Diagnostic Pathology, Dokkyo University School of Medicine, 321-0293 Mibu, Japan.
TEL +81-282-86-1111 (ex2178) Fax +81-282-86-5171
E-mail; mkojima@dokkyomed.ac.jp

Running head: Castleman's disease
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Summary

Localized Castleman's disease (CD) has been divided two types, the classical hyaline vascular (HV) type and rare plasma cell (PC) type. Recently, we have reported two cases of IgG4-related disorder of the retroperitoneum showing PC type of CD. To further clarify the clinicopathological findings of CD of the retroperitoneum, eight such cases have been studied. A single lesion was located in the retroperitoneum (n=3), ureter (n=2) and renal hilum (n=2). One case had bilateral ureter lesions. The HV type of CD accounts for approximately 90% of cases. However, 50% (n=4) of our cases was PC type of CD. Three of the four lesions of HV type had lymph node lesions, whereas all four PC type of CD were soft tissue mass. These clinicopathologic findings appear quite different from previous description. Immunohistochemical study demonstrated numerous IgG4+ plasma cells accounting for more that 50% of IgG4+ cells in three cases of the four PC type of CD. Moreover, serum IgG4 concentration was increased in two of the four case of PC type of CD that was examined. The serum interleukin-6 level was within the normal range in two cases of PC type that was examined. Present study suggested that a majority of the PC type of CD arising in the retroperitoneum appears IgG4-related disorder.

Introduction

In 1956, Castleman et al described an entity involving localized mediastinal lymph node hyperplasia that resembled thymoma.[1] Since the original description, Castleman's disease (CD) has been extended to included two types, the classical hyaline vascular (HV) type and plasma cell (PC) type.[2-4] The HV type of CD accounts for approximately 90% of cases[2-4]. This type is usually located to a single lymph node, asymptomatic, and showing benign clinical course. Histologically, HV type of CD is characterized by abnormal lymphoid follicles and interfollicular vascularity. The PC type of CD is occasionally multifocal and may be associated with systemic problems such as fever, weight loss, hemolytic anemia and hypergammaglobulinemia.[2-4] Histologically, the PC type of CD is defined by numerous lymphoid follicles with an active germinal center (GC) and interfollicular polyclonal plasmacytosis.[2-4]

IgG4 is the least common subclass of IgG, normally accounting for only 3% to 6% in the serum (normal range; 4.8-105mg/dl). IgG4-related sclerosing disease is a recently recognized syndrome characterized clinically by tumor like enlargement of one or more exocrine glands or extranodal tissues and raised serum IgG4 level, and pathologically by lymphoplasmacytic infiltration and sclerosis, as well as increasing IgG4-secreting plasma cells.[5-8] IgG-related sclerosing disease is defined by elevated serum IgG4 level (> 135mg/dl) and/or numerous IgG4+ plasma cells accounting for more than 50% of IgG+ cells in the affected organs.[8]

Recently, we have reported two cases of IgG4-related disorder of the retroperitoneum showing histological findings of PC type of CD[9]. To further clarify the clinicopathological findings of CD of the retroperitoneum, we have examined eight such cases.

Materials and Methods

Eight cases were collected from a series by one of the authors (M.K) treated between 1988 and June 2009. Medical records of eight cases were extensively reviewed. Two cases (nos. 5 and 7) have been reported previously[9].

Surgical specimens were fixed in formalin, routinely processed and embedded in paraffin. For light microscopic examination, the sections were stained with hematoxylin-eosin (HE) and elastica van Gieson (EVG) stain.

Immunohistochemical studies were performed using the antigen retrieval method on the avidin-biotin- peroxidase method or Ventana automated (BenchMark^{TM}) stainer according to the manufacture's instructions.

The panel of antibodies included human immunoglobulin light chains (kappa and lambda)(Dako A/S, Glostrup, Denmark), Ig A (Dako), IgD (Dako), IgG (Novocastra), MCO011 (IgG4; Binding Site, Birmingham, UK), IgM (Dako), PS-1 (CD3; Immunotech, Marseille, France), 56C6 (CD10;Novocastra, Newcastle, UK), L26 (CD20; Dako), cocktail of 2G9 (CD21; Novocastra) and RB L25 (CD35; Novocastra) , DFT-1 (CD43; Dako), 1B16 (CD56; Novocastra), SP-4 (Cyclin D1; Nichirei Co. Tokyo, Japan), 124 (bcl-2; Dako) and 137B1 (human herpes virus type-8 [HHV-8]; Novocastra). Sections with known reactivity for antibodies assayed served as positive controls and sections treated with normal rabbit- and mouse serum served as negative controls.

In situ hybridization (ISH) with Epstein-Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed paraffin-embedded sections using a Ventana automated (BenchMarkTM) stainer or using the hybridization kit (Dako).

Paraffin-embedded tissues from the operatively resected specimen were prepared for polymerase chain reaction (PCR), and the rearranged immunoglobulin heavy-chain (IgH) genes were amplified using the seminested PCR method as described by Wan et al.[10]

Results

Main clinicopathological findings are shown in Table.

1. Clinical findings

The patients, three men and five women, ranged in age from 21 to 75 years with a median age of 58.5 years. "B" symptoms such as fever was recorded in only one patient (no. 7). The tumor was located in the retroperitoneum in three cases (nos. 1, 2 and 4), periureter tissue in three cases (nos. 3, 6 and 7) and in the renal hilum in two cases (nos. 5 and 8). Case 3 had bilateral periureter tissue tumors. The other

cases had a single solitary lesion.

In one patient (no. 3) an association of chronic sclerosing sialoadenitis which is belonged to the IgG4-related disease[11].

Postoperatively, the serum IgG4 level was examined in four cases (nos. 3, 5-7), and serum IgG4 level was increased in two cases (nos. 3 and 7). The serum interleukin-6 [IL-6] level was within normal range in two cases examined (nos. 3 and 7).

One patient (no. 3) was given prednisolone after the tumor biopsy, the remaining six patients did not receive medication. Follow-up data were obtained except one patient (no. 4). Seven patients were alive during follow-up period ranging four to 108 months (median 12 months).

2. Pathological, immunohistochemical and EBV findings

The size of the lesion is from 1.5 to 5 cm in diameter (mean= 3.6cm). Macroscopically, all eight lesions were solitary and firm which were relatively well circumscribed.

HV type

Under low magnification, the lesion in four cases (nos. 1, 2, 4 and 8) was found to contain numerous lymphoid follicles. Three types of the lymphoid follicles were delineated. (i) The lymphoid follicles with normal hyperplastic GCs. (ii) Large nodules of mantle cells contained multiple small atrophic GC with increased vascularity (multiple GC pattern) (Fig. 1a).[3] (iii) Large, often irregularly shaped nodules of mantle cells with inconspicuous GCs. Frequently, these nodules were radically penetrated by small vessels and somewhat resembled primary follicles (primary follicular pattern).[3] A portion of follicular dendritic cells (FDCs) in these GCs demonstrated enlarged nuclei with prominent nucleoli (Fig. 1b) The majority of the lymphoid follicles in all four cases showed primary follicular pattern and/or multiple GC pattern. There were no plasmacytoid dendritic cells in any of the four lesions. Marked small vessel proliferation and perivascular fibrous masses were observed in all four subjects (Fig. 1c). There was interfollicular sclerosis in all four lesions and that was prominent in one case (no.4) (Fig.1c).

The results of immunohistochemical study of these patients were similar to those described in previous reports[4,12].

Briefly, mantle cells in primary follicular pattern and multiple GC pattern were CD20+, sIgM+, sIgD+, CD3-, CD10-, CD43-, bcl-2+ and cyclinD1-[4, 12]. Staining with monoclonal antibodies cocktail of 2G9 and RB L25 highlighted the meshwork of FDCs. The FDC networks of the primary follicular pattern and multiple GC pattern showed a tight/concentric pattern or expanded/disrupted pattern as previously described by Nguyen et al.[13]. There were only a few IgG4-positive plasma cells in all four lesions.

There were no HHV-8 and EBER-positive cells in any of the four cases.

PC type

All four lesions were composed of a dense lymphoplasmacytic infiltration. The inflammatory process extended to the periureter adipose tissue. Numerous lymphoid follicles with active germinal centers were also observed (Fig.1d). The interfollicular area was characterized by sheets of proliferating mature plasma cells (Fig. 1e). A few immature plasma cells and immunoblasts were intermingled with mature plasma cells. Many plasma cells containing numerous basophilic rounded cytoplasmic inclusions (Mott cells) were seen in Case 7. However, there were no Dutcher bodies, centrocyte-like (CCL) cells or amyloid deposition in any of the four lesions. In the interfollicular area, there was no marked proliferation of blood vessels in any of the four lesions. However, in the interfollicular area, there were marked fibrous sclerosis in two lesions (nos. 3 and 6). Partially obstructive phlebitis was observed in the two lesions (nos. 5 and 6)(Fig. 1f)

The immunoglobulin light chain reactivity of plasma cells showed a polyclonal pattern in all four lesions (Figs. 2a and b).[9] There were numerous IgG-positive plasma cells with scattered IgA- or IgM-positive plasma cells in all four cases. IgG4-positive cells comprised 50-60% of IgG-positive plasma cells in three cases (nos. 3, 5, 7)(Figs. 2c and d), whereas Case 6 contained only a few IgG4-postitve plasma cells .

CD20 immunostain demonstrated that there were no intraepithelial B-lymphocytes in the renal pelvis or ureter mucosa. B-cells in the germinal centers were Bcl-2-in both lesions. There were no CD43+, cyclin D1+ small B-cells in either lesion. There were no CD56+ plasma cells in any of the four lesions.

There were no HHV-8 and EBER-positive cells in any of the four cases.

3. Genotypic study