2. Schaffer JM, Tilley FW. Further investigations of the relation between the chemical constitution and the germicidal activity of alcohols and phenols. J Bacteriol. 1927;14(4):259–73.
3. Sjogren H. Keratoconjunctivitis sicca and chronic polyarthritis. Acta Med Scand. 1948;30:484–8.
4. Morgan WS, Castleman B. A clinicopathologic study of Mikulicz's disease. Am J Pathol. 1953;29:471–503.
5. Tsubota K, Fujita H, Tadano K, Onoda N, Tsuzaka K, Takeuchi T. Abnormal expression and function of Fas ligand of lacrimal glands and peripheral blood in Sjögren's syndrome patients with enlarged exocrine glands. Clin Exp Immunol. 2002;129:177–82.
6. Yamamoto M, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, Takahashi H, et al. Elevated IgG4 concentrations in serum of patients with Mikulicz's disease. Scand J Rheumatol. 2004; 33:432–3.
7. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol. 2006;16(6):335–40.
8. Masaki Y, Sugai S, Umehara H. IgG4-related diseases including Mikulicz's disease and sclerosing pancreatitis: diagnostic insights. J Rheum. 2010;37:1380–5.
9. Masaki Y, Iwao H, Nakajima A, Miki M, Sugai S, Umehara H. IgG4-related disease (IgG4+MOLPS)—diagnostic criteria and diagnostic problems. Curr Immunol Rev. 2011;7:172–7.
10. Sarles H, Sarles JC, Muratore R, Guien C. Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease? Am J Dig Dis. 1961;6:688–98.
11. Kawaguchi K, Koike M, Tsuruta K, Okamoto A, Tabata I, Fujita N. Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. Hum Pathol. 1991;22(4):387–95.
12. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci. 1995;40:1561–8.
13. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001;344:732–8.
14. Okazaki K, Kawa S, Kamisawa T, Itoh T, Inui K, Irie H, et al. Japanese clinical guideline for autoimmune pancreatitis. Pancreas. 2009;38:849–66.
15. Kamisawa T, Nakajima H, Egawa N, et al. IgG4-related sclerosing disease incorporating sclerosing pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis with lymphadenopathy. Pancreatology. 2006;6:132–7.
16. Zen Y, Sawazaki A, Miyayama S, et al. A case of retroperitoneal and mediastinal fibrosis exhibiting elevated levels of IgG4 in the absence of sclerosing pancreatitis (autoimmune pancreatitis). Hum Pathol. 2006;37:239–43.
17. Zen Y, Kitagawa S, Minato H, Kuramaya H, Katayanagi K, Masuda S, et al. IgG4-positive plasma cells in inflammatory pseudotumor (plasma cell granuloma) of the lung. Hum Pathol. 2005;36:710–7.
18. Kitagawa S, Zen Y, Harada K, Sasaki M, Sato Y, Minato H, et al. Abundant IgG4-positive plasma cell infiltration characterizes chronic sclerosing sialadenitis (Küttner's tumor). Am J Surg Pathol. 2005;29:783–91.
19. Saeki T, Nishi S, Ito T, Yamazaki H, Miyamura S, Emura I, et al. Renal lesions in IgG4-related systemic disease. Intern Med. 2007;46(17):1365–71.
20. Kamisawa T, Funata N, Hayashi Y. Lymphoplasmacytic sclerosing pancreatitis is a pancreatic lesion of IgG4-related systemic disease. Am J Surg Pathol. 2004;28(8):1114.
21. Kamisawa T, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, et al. A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol. 2003;38(10):982–4.
22. Yamamoto M, Takahashi H, Hasebe K, Suzuki C, Naishiro Y, Hayashi T, et al. The analysis of interleukin-6 in patients with systemic IgG4-related plasmacytic syndrome–expansion of SIPS to the territory of Castleman's disease. Rheumatology (Oxford). 2009;48:860–2.
23. Masaki Y, Umehara H. IgG4-related disease–the diagnostic confusion and how to avoid it. Jpn J Clin Immunol. 2009;32:478–83.
24. Comings D, Skubi K, Van Eyes J, Motulsky A. Familial multifocal fibrosclerosis. Findings suggesting that retroperitoneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudotumor of the orbit may be different manifestations of a single disease. Ann Intern Med. 1967;66:884–92.
25. Kamisawa T, Chen PY, Tu Y, Nakajima H, Egawa N, Tsuruta K, et al. Pancreatic cancer with a high serum IgG4 concentration. World J Gastroenterol. 2006;12(38):6225–8.
26. Witkiewicz AK, Kennedy EP, Kennyon L, Yeo CJ, Hruban RH. Synchronous autoimmune pancreatitis and infiltrating pancreatic ductal adenocarcinoma: case report and review of the literature. Hum Pathol. 2008;39(10):1548–51.
27. Gill J, Angelo N, Yeong ML, McIvor N. Salivary duct carcinoma arising in IgG4-related autoimmune disease of the parotid gland. Hum Pathol. 2009;40(6):881–6.
28. Cheuk W, Yuen H, Chan A, Shih L, Kuo T, Ma M, et al. Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease. Am J Surg Pathol. 2008;32:1159–67.
29. Masaki Y, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, 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. 2009;63:1310–5.
30. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A case of Mikulicz's disease (IgG4-related plasmacytic disease) complicated by autoimmune hypophysitis. Scand J Rheumatol. 2006;35:410–1.
31. Yamamoto M, Takahashi H, Sugai S, Imai K. Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy). Autoimmun Rev. 2005;4(4):195–200.
32. Dahlgren M, Khosroshahi A, Nielsen GP, Deshpande V, Stone JH. Riedel's thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum. Arthritis Care Res (Hoboken). 2010;62(9):1312–8.
33. Okazaki K, Uchida K, Miyoshi H, Ikeura T, Takaoka M, Nishio A. Recent concepts of autoimmune pancreatitis and IgG4-related disease. Clin Rev Allergy Immunol 2011. doi:10.1007/s12016-010-8214-2.
34. Okazaki K, Uchida K, Koyabu M, Miyoshi H, Takaoka M. Recent advances in the concept and diagnosis of autoimmune pancreatitis and IgG4-related disease. J Gastroenterol. 2011;46(3):277–88.
35. Notohara K, Burgart LJ, Yadav D, Chari S, Smyrk TC. Idiopathic chronic pancreatitis with periductal lymphoplasmacytic infiltration: clinicopathologic features of 35 cases. Am J Surg Pathol. 2003;27(8):1119–27.
36. Zamboni G, Luttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P, et al. Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens. Virchows Arch. 2004;445(6): 552–63.
37. Hamano H, Arakura N, Muraki T, Ozaki Y, Kiyosawa K, Kawa S. Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. J Gastroenterol. 2006;41(12): 1197–205.

38. Hamano H, Kawa S, Uehara T, Ochi Y, Takayama M, Komatsu K, et al. Immunoglobulin G4-related lymphoplasmacytic sclerosing cholangitis that mimics infiltrating hilar cholangiocarcinoma: part of a spectrum of autoimmune pancreatitis? Gastrointest Endosc. 2005;62(1):152–7.
39. Zen Y, Harada K, Sasaki M, Sato Y, Tsuneyama K, Haratake J, 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. 2004;28(9):1193–203.
40. Kamisawa T, Okamoto A. IgG4-related sclerosing disease. World J Gastroenterol. 2008;14(25):3948–55.
41. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology. 2008;134(3): 706–15.
42. Uchiyama-Tanaka Y, Mori Y, Kimura T, Sonomura K, Umemura S, Kishimoto N, et al. Acute tubulointerstitial nephritis associated with autoimmune-related pancreatitis. Am J Kidney Dis. 2004;43(3):e18–25.
43. Takeda S, Haratake J, Kasai T, Takaeda C, Takazakura E. IgG4-associated idiopathic tubulointerstitial nephritis complicating autoimmune pancreatitis. Nephrol Dial Transplant. 2004;19(2):474–6.
44. Saeki T, Saito A, Yamazaki H, Emura I, Imai N, Ueno M, et al. Tubulointerstitial nephritis associated with IgG4-related systemic disease. Clin Exp Nephrol. 2007;11(2):168–73.
45. Saeki T, Nishi S, Imai N, Ito T, Yamazaki H, Kawano M, et al. Clinicopathological characteristics of patients with IgG4-related tubulointerstitial nephritis. Kidney Int. 2010;78(10):1016–23.
46. Saeki T, Imai N, Ito T, Yamazaki H, Hishi S. Membranous nephropathy associated with IgG4-related systemic disease and without autoimmune pancreatitis. Clin Nephrol. 2009;2:173–8.
47. Saeki T, Nishi S, Ito T, Yamazaki H, Miyamura S, Emura I, et al. Renal lesion in IgG4-related systemic disease. Intern Med. 2007;46:1365–72.
48. Zen Y, Inoue D, Kitao A, Onodera M, Abo H, Miyayama S, et al. IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases. Am J Surg Pathol. 2009;33(12):1886–93.
49. Inoue D, Zen Y, Abo H, Gabata T, Demachi H, Kobayashi T, et al. Immunoglobulin G4-related lung disease: CT findings with pathologic correlations. Radiology. 2009;251(1):260–70.
50. Saegusa H, Momose M, Kawa S, Hamano H, Ochi Y, Takayama M, et al. Hilar and pancreatic gallium-67 accumulation is characteristic feature of autoimmune pancreatitis. Pancreas. 2003;27(1):20–5.
51. Takato K, Yasui M, Ichikawa Y, Fujimura M, Nakao S, Zen Y, et al. Nonspecific interstitial pneumonia with abundant IgG4-positive cells infiltration, which was thought as pulmonary involvement of IgG4-related autoimmune disease. Intern Med. 2008;47:291–4.
52. Li Y, Bai Y, Liu Z, Ozaki T, Taniguchi E, Mori I, et al. Immunohistochemistry of IgG4 can help subclassify Hashimoto's autoimmune thyroiditis. Pathol Int. 2009;59(9):636–41.
53. Kakudo K, Li Y, Hirokawa M, Ozaki T. Diagnosis of Hashimoto's thyroiditis and IgG4-related sclerosing disease. Pathol Int. 2011;61(4):175–83.
54. Kojima M, Hirokawa M, Kuma H, Nishihara E, Masawa N, Nakamura N, et al. Distribution of IgG4- and/or IgG-positive plasma cells in Hashimoto's thyroiditis: an immunohistochemical study. Pathobiology. 2010;77(5):267–72.
55. Cheuk W, Yuen HK, Chu SY, Chiu EK, Lam LK, Chan JK. Lymphadenopathy of IgG4-related sclerosing disease. Am J Surg Pathol. 2008;32(5):671–81.
56. Sato Y, Kojima M, Takata K, Morito T, Asaoku H, Takeuchi T, et al. Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease. Mod Pathol. 2009;22(4):589–99.
57. Sato Y, Notohara K, Kojima M, Takata K, Masaki Y, Yoshino T. IgG4-related disease: historical overview and pathology of hematological disorders. Pathol Int. 2010;60(4):247–58.
58. Hamano H, Kawa S, Ochi Y, Unno H, Shiba N, Wajiki M, et al. Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis. Lancet. 2002;359(9315):1403–4.
59. Neild GH, Rodriguez-Justo M, Wall C, Connolly JO. Hyper-IgG4 disease: report and characterisation of a new disease. BMC Med. 2006;4:23.
60. Zen Y, Onodera M, Inoue D, Kitao A, Matsui O, Nohara T, et al. Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4. Am J Surg Pathol. 2009;33(12):1833–9.
61. Khosroshahi A, Stone JH. A clinical overview of IgG4-related systemic disease. Curr Opin Rheumatol. 2011;23(1):57–66.
62. Kasashima S, Zen Y, Kawashima A, Konishi K, Sasaki H, Endo M, et al. Inflammatory abdominal aortic aneurysm: close relationship to IgG4-related periaortitis. Am J Surg Pathol. 2008;32(2):197–204.
63. Stone JH, Khosroshahi A, Hilgenberg A, Spooner A, Isselbacher EM, Stone JR. IgG4-related systemic disease and lymphoplasmacytic aortitis. Arthritis Rheum. 2009;60(10):3139–45.
64. Ishida F, Kitano K, Kobayashi H, Saito H, Kiyosawa K. Elevated IgG4 levels in a case with multicentric Castleman's disease. Br J Haematol. 1997;99(4):981–2.
65. Khosroshahi A, Bloch DB, Deshpande V, Stone JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. Arthritis Rheum. 2010;62(6):1755–62.
66. Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology. 2002;105(1):9–19.
67. van der Nuet Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science. 2007;317(5844):1554–7.
68. Aoki S, Nakazawa T, Ohara H, Sano H, Nakao H, Joh T, et al. Immunohistochemical study of autoimmune pancreatitis using anti-IgG4 antibody and patients' sera. Histopathology. 2005; 47(2):147–58.
69. Miyake K, Moriyama M, Aizawa K, Nagano S, Inoue Y, Sadanaga A, et al. Peripheral CD4+ T cells showing a Th2 phenotype in a patient with Mikulicz's disease associated with lymphadenopathy and pleural effusion. Mod Rheumatol. 2008;18(1):86–90.
70. Akitake R, Watanabe T, Zaima C, Uza N, Ida H, Tada S, et al. Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease. Gut. 2010;59(4):542–5.
71. Suzuki K, Tamaru J, Okuyama A, Kameda H, Amano K, Nagasawa H, et al. IgG4-positive multi-organ lymphoproliferative syndrome manifesting as chronic symmetrical sclerosing dacryosialadenitis with subsequent secondary portal hypertension and remarkable IgG4-linked IL-4 elevation. Rheumatology (Oxford). 2010;49(9):1789–91.
72. Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology. 2007;45(6):1538–46.
73. Miyoshi H, Uchida K, Taniguchi T, Yazumi S, Matsushita M, Takaoka M, et al. Circulating naive and CD4+CD25 high regulatory T cells in patients with autoimmune pancreatitis. Pancreas. 2008;36(2):133–40.
74. Zen Y, Nakanuma Y. Pathogenesis of IgG4-related disease. Curr Opin Rheumatol. 2011;23(1):114–8.
75. Nakashima H, Miyake K, Moriyama M, Tanaka A, Watanabe M, Abe Y, et al. An amplification of IL-10 and TGF-beta in patients

with IgG4-related tubulointerstitial nephritis. Clin Nephrol. 2010;73(5):385–91.
76. van der Vliet HJ, Perenboom RM. Multiple pseudotumors in IgG4-associated multifocal systemic fibrosis. Ann Intern Med. 2004;141(11):896–7.
77. Zen Y, Fujii T, Sato Y, Masuda S, Nakanuma Y. Pathological classification of hepatic inflammatory pseudotumor with respect to IgG4-related disease. Mod Pathol. 2007;20(8):884–94.
78. Geyer JT, Ferry JA, Harris NL, Stone JH, Zukerberg LR, Lauwers GY, et al. Chronic sclerosing sialadenitis (Kuttner tumor) is an IgG4-associated disease. Am J Surg Pathol. 2010;34(2):202–10.
79. Okazaki K, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I, et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol. 2006;41(7):626–31.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases

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Abstract
Objectives Patients with autoimmune pancreatitis (AIP) characteristically show elevated serum levels of immunoglobulin G4 (IgG4) and abundant infiltration of IgG4-positive plasmacytes in the involved organs. The most common involved organ showing extrapancreatic lesions is the bile duct, which exhibits sclerosing cholangitis (SC). However, the role of IgG4 in the development of IgG4-related SC (IgG4-SC) remains unclear. To clarify the role of IgG4 in IgG4-SC, we have performed an immunohistochemical analysis of the bile duct.
Methods Laboratory and immunohistochemical findings of liver biopsy specimens obtained from patients with IgG4-SC, primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH), and primary biliary cirrhosis (PBC) were compared. The biopsy specimens were first stained with anti-IgG1, anti-IgG4, and anti-Foxp3 (forkhead box P3) antibodies, and the ratio of IgG4-, IgG1-, and Foxp3-positive cells, respectively, to infiltrated mononuclear cells (IgG4/Mono, IgG1/Mono, Foxp3/Mono) was assessed.
Results The ratio of IgG4/IgG1-positive plasma cells was significantly higher in specimens obtained from patients with IgG4-SC than in those from patients with PSC, AIH, and PBC. The Foxp3/Mono ratio in patients with PBC was significantly higher than that in patients with IgG4-SC and PSC. In patients with IgG4-SC, the number of Foxp3-positive cells was significantly correlated with the number of IgG4-positive cells; in the other patient groups, there was no correlation.
Conclusions The IgG4/IgG1 ratio in the liver may be a useful marker for differential diagnosis of IgG4-SC and PSC. In IgG4-SC, abundant infiltration of regulatory T cells (Tregs) may affect the switching of B cells to IgG4-producing plasmacytes, and there is a possibility that the function of Tregs is different in IgG4-SC and other liver diseases (PSC, AIH, and PBC).

Keywords Autoimmune pancreatitis · IgG4 · IgG4-related diseases · Regulatory T cell · Sclerosing cholangitis

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Introduction

Sarles et al. [1] reported the first case of pancreatitis with hypergammaglobulinemia in 1961. This was followed by the occasional report of the coexistence of pancreatitis with other autoimmune diseases. In 1995, Yoshida et al. [2] proposed the concept of autoimmune pancreatitis (AIP): a clinical condition in which patients show a diffusely enlarged pancreas, narrowing pancreatogram, increased serum immunoglobulin (Ig)G, presence of autoantibodies, fibrotic changes with lymphocytic infiltration, and steroidal

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efficacy. Many AIP cases have since been reported by Japanese investigators, and AIP has been accepted as a new clinical entity [3, 4]. Patients with AIP often show discomfort in the epigastrium, obstructive jaundice due to bile-duct stricture, and diabetes mellitus. It is more common among middle-aged and elderly men. Patients with AIP often also have extrapancreatic lesions, such as biliary lesions, sialoadenitis, retroperitoneal fibrosis, enlarged celiac and hilar lymph nodes, chronic thyroiditis, and interstitial nephritis [5–8], which suggests that AIP may be a systemic disorder. In 2001, Hamano et al. [9] reported that patients with AIP have high serum IgG4 concentrations. In 2006, Kamisawa et al. [10] proposed the existence of IgG4-related sclerosing disease, which has recently led to immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) being recognized as a disease entity characterized by sclerosing inflammation with abundant IgG4-positive plasma cells; most cases reported to date have been associated with AIP [11]. The cholangiographic findings on radiological images of patients with IgG4-SC and primary sclerosing cholangitis (PSC) are similar [8, 12]. However, while IgG4-SC responds dramatically to steroid therapy, PSC, even in patients receiving medication, remains a progressive disease that involves the intra- and extra-hepatic bile ducts and leads to biliary cirrhosis. Therefore, it is important that the clinician is able to distinguish between IgG4-SC and PSC.

Attention has recently focused on the relationship between various autoimmune diseases and regulatory T cells (Tregs), which are present in human peripheral blood [13–15], intestinal lamina propria [16], and the thymus [15]. $CD4^{+}CD25^{high}$ Tregs, which are characterized by the expression of a specific transcription factor, forkhead box P3 (Foxp3), play a key role in the autoimmune diseases. We have previously reported that increased numbers of $CD4^{+}CD25^{high}$ Tregs may influence IgG4 production and that naive Tregs may be involved in the pathogenesis of AIP [17]. However, the relationship between Tregs in the peripheral blood and the liver remains unclear. The aim of the study reported here was to clarify the role of IgG4 in IgG4-SC by comparing the immunohistochemical features of the liver in patients with IgG4-SC, PSC, autoimmune hepatitis (AIH), and primary biliary cirrhosis (PBC).

Patients and methods

Subjects

The patient cohort consisted of 16 IgG4-SC patients of the Kansai Medical University and its affiliated hospitals (16 patients untreated with corticosteroids; 15 men and one woman; mean age 63 years; range 31–81 years). Of these 16 patients, 14 had SC with AIP based on the Japanese clinical diagnostic criteria for AIP proposed by the Research Committee of Intractable Diseases of the Pancreas and accepted by both the Japanese Ministry of Health, Labor, and Welfare and the Japan Pancreas Society in 2006 [5] and on Asian criteria [18]. Two patients were diagnosed according to the Mayo criteria for IgG4-associated cholangitis [19].

We classified the IgG4-SC patients into two groups, intra-/extra-hepatic (intra-IgG4-SC) and extra-hepatic (extra-IgG4-SC), based on the nature of the biliary strictures as characterized by endoscopic retrograde cholangiopancreatography (ERCP) (Fig. 1). In those patients with intra-IgG4-SC ($n = 8$), strictures were present in the intra-hepatic or in both the intra-hepatic and extra-hepatic bile ducts. In those patients with extra-IgG4-SC ($n = 8$), the strictures were present only in the extra-hepatic bile duct. In our study, we analyzed only the eight intra-IgG4-SC patients. Prior to biliary drainage, we performed an intraductal ultrasonography (IDUS) in all patients to evaluate thickening of the bile duct wall. The IDUS demonstrated

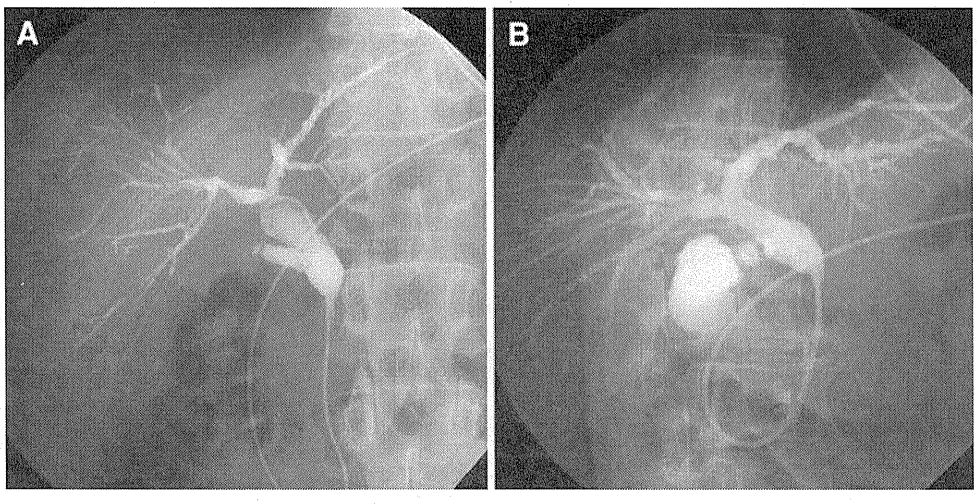

Fig. 1 Endoscopic nasobiliary drain (ENBD) cholangiogram of immunoglobulin G4-related sclerosing cholangitis (IgG4-SC). The IgG4-SC patients were classified into two groups (intra- and extra-IgG4-SC, respectively) based on the nature of the biliary strictures. **a** Stricture of the intra-hepatic and extra-hepatic bile duct (intra). **b** Stricture of the extra-hepatic bile duct (extra)

that it was the thickening of the bile-duct wall itself that caused the biliary strictures in all patients—and not extrinsic compression from inflammatory pancreatic tissue. We consequently diagnosed all patients with IgG4-SC.

The control group consisted of patients with PSC (n = 26; all patients treated with ursodeoxycholic acid; 15 men and 11 women; mean age 36 years; range 6–77 years) (Ludwig's stage 2, n = 4; stage 3, n = 8; stage 4, n = 14), AIH (n = 9; five patients untreated and four patients treated with corticosteroids; nine women; mean age 53 years; range 33–70 years), and PBC (n = 9; all patients untreated with ursodeoxycholic acid; nine women; mean age 53 years; range 35–67 years) (Table 1). All patients with AIH were diagnosed as definitely having AIH based on the scoring system established by the International Autoimmune Hepatitis Group and classified as having type 1 AIH [20, 21]. The diagnosis of PBC was based on internationally accepted criteria, and the antimitochondrial antibody status of each patient was verified [22]. Patients with overlap syndrome were excluded from this study. The diagnosis of PSC was based on typical cholangiographic and liver biopsy criteria [23]. Liver biopsy was performed in all IgG4-SC, AIH, and PBC patients and in three of the 26 PSC patients. In the remaining 23 PSC patients, a liver specimen was obtained during liver transplantation surgery. This study was approved by the Kansai Medical University's Ethics Committee.

Histopathology and immunohistochemistry

Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid–Schiff after diastase digestion, Azan–Mallory, reticulin, or orcein. The remaining material was used for immunohistochemical analysis. The immunostaining of IgG4 was performed using a monoclonal antibody for human IgG4 (ZYMED Laboratories, San Francisco, CA) and that of IgG1 and Foxp3 using the avidin–biotin complex (ABC) method with reagents obtained from Vector Laboratories (Burlingame, CA). The antibodies used to identify the inflammatory cells in the liver were the IgG1 antibody (Binding Site, Birmingham, UK) and Foxp3 (eBioscience, San Diego, CA). The deparaffinized sections were pretreated in ethylenediaminetetraacetic acid buffer (pH 8.0) in a pressure cooker at 100°C for 5 min. Following incubation with the first antibody at 4°C overnight, biotinylated rabbit anti-sheep serum IgG (Vector Laboratories) was used as the secondary antibody (sections for IgG1), and immunoreactive deposits were visualized with 3,3′-diaminobenzidine tetrahydrochloride. To correct for differences in the sizes of the portal tracts, we counted the numbers of immunohistochemically identifiable IgG1-, IgG4-, and Foxp3-positive cells and mononuclear cells contained within the portal tracts selected in each specimen under five different high power fields (hpf); two pathologists subsequently calculated the ratio between IgG1-, IgG4-, Foxp3-positive cells and infiltrated mononuclear cells in each case.

Table 1 Clinical profile and characteristics of the patient and control groups

Clinical profile	n	Sex (male/female)	Age (years)[a]
IgG4-SC	16	15/1	63 ± 3 (31–81)
Intra	8	7/1	59 ± 6 (31–75)
Extra	8	8/0	67 ± 3 (54–81)
PSC	26	15/11	36 ± 4 (6–77)*, **
AIH	9	0/9	53 ± 4 (33–70)
PBC	9	0/9	53 ± 4 (35–67)

IgG4-SC Immunoglobulin G4-related sclerosing cholangitis, *Intra* intra-hepatic or both intra-hepatic and extra-hepatic biliary strictures, *Extra* extra-hepatic biliary strictures, *PSC* primary sclerosing cholangitis, *AIH* autoimmune hepatitis, *PBC* primary biliary cirrhosis

* $p < 0.01$ vs. AIH and PBC; ** $p < 0.001$ vs. IgG4-SC, intra- and extra-hepatic

[a] Values are given as the mean ± standard error of the mean (SEM), with the range in parenthesis

Statistical analysis

For all studies, data are expressed as mean ± standard error of the mean (SEM). Differences were analyzed using the nonparametric Mann–Whitney rank test and Fisher's exact test, where p values <0.05 were considered to be significant.

Results

Patients profile

Patient age was significantly lower in the PSC group than in the other groups. There was one peak in the age distribution of the IgG4-SC patients between 60 and 70 years and two peaks in the age distribution of the PSC patients (one between 20 and 30 years, and the other between 40 and 50 years). Of the eight intra-IgG4-SC patients, seven were male; all AIH and PBC patients were female (Table 1).

Laboratory findings

The serum asparate aminotransferase and alanine aminotransferase values were elevated in all groups, with no difference in laboratory values among the groups. The

serum alkaline phosphatase level was significantly higher in the PSC group than in the AIH group, and the serum γ-glutamyl transpeptidase level was significantly higher in the PBC group than in the AIH group. The serum total bilirubin was significantly higher in the PSC group. The eosinophil level was significantly higher in the PBC and intra-IgG4-SC groups than in the PSC and AIH groups. There were no significant differences in the serum levels of IgA. The serum IgG4 values were elevated in patients with intra-IgG4-SC. Antinuclear antibodies were positive in 38% of the intra-IgG4-SC patients, 48% of the PSC patients, 78% of the AIH patients, and 22% of the PBC patients. Antimitochondrial antibodies were positive in 0% of the intra-IgG4-SC and PSC patients, 22% of the AIH patients, and 78% of the PBC patients (Table 2).

Immunohistochemical findings of IgG1 and IgG4

As shown in Figs. 2 and 3, the ratio of IgG4-positive plasma cells to infiltrated mononuclear cells (IgG4/Mono) was significantly higher in patients with intra-IgG4-SC (0.121 ± 0.069) than in those with PSC (0.02 ± 0.003; $p = 0.002$), AIH (0.013 ± 0.004; $p = 0.0052$), and PBC (0.013 ± 0.002; $p = 0.0052$; Fig. 3a). The ratio of IgG1-positive plasma cells to infiltrated mononuclear cells (IgG1/Mono) was significantly lower in patients with intra-IgG4-SC (0.041 ± 0.009) than in those with AIH (0.084 ± 0.014; $p = 0.0161$; Fig. 3b). The ratio of IgG4/Mono to IgG1/Mono (IgG4/G1) was significantly higher in patients with intra-IgG4-SC (3.084 ± 1.824) than in those with PSC (0.424 ± 0.068; $p = 0.0018$), AIH (0.169 ± 0.042; $p = 0.004$), and PBC (0.196 ± 0.02; $p = 0.0044$; Fig. 3c). The intra-IgG4-SC patients were found to have an IgG4/G1 ratio >1.

Immunohistochemical findings of Foxp3

As shown in Figs. 4 and 5, patients with PBC has a significantly higher ratio of Foxp3-positive cells to infiltrated mononuclear cells (Foxp3/Mono) (0.042 ± 0.008) than those with intra-IgG4-SC (0.013 ± 0.006; $p = 0.0007$) and PSC (0.006 ± 0.001; $p < 0.0001$). Patients with AIH (0.027 ± 0.009) had a significantly higher Foxp3/Mono ratio than those with PSC ($p = 0.0016$). The Foxp3/Mono ratio was significantly higher in patients with intra-IgG4-SC than in those with PSC ($p = 0.0314$; Fig. 5, dotted line).

Correlation between the Foxp3/Mono and IgG4/Mono ratios in patients with IgG4-SC

The Foxp3/Mono and IgG4/Mono ratios were found to be positively correlated in the group of patients with intra-IgG4-SC ($R = 0.75$), but there was no correlation found in the other patient groups (PSC, $R = 0.05$; AIH, $R = 0.07$; PBC, $R = 0.11$; Fig. 6).

Table 2 Laboratory findings of AIP-SC, PSC, AIH, and PBC patients

Laboratory parameters[a]	Intra-IgG4-SC ($n = 8$)	PSC ($n = 26$)	AIH ($n = 9$)	PBC ($n = 9$)	Statistical significance[b]
AST (13–35 U/l)	57 ± 15	135 ± 14	152 ± 69	144 ± 96	NS
ALT (5–35 U/l)	52 ± 17	93 ± 16	185 ± 77	132 ± 91	NS
ALP (107–340 U/l)	837 ± 313	1284 ± 228*	360 ± 57	626 ± 66	$p < 0.05$
γ-GTP (11–64 U/l)	242 ± 59	211 ± 34	136 ± 31	330 ± 99*	$p < 0.05$
T-Bil (0.2–1.2 mg/dl)	2.7 ± 1.4	15.8 ± 2.5***	2.5 ± 1.1	1.0 ± 0.3	$p < 0.001$
ALB (3.8–5.0 g/dl)	3.3 ± 0.2	3.2 ± 0.1	3.6 ± 0.2‡	3.8 ± 0.1**, †	$p < 0.05$, 0.01
Eosinophils (%)	6.2 ± 0.9*	2.7 ± 0.7	1.0 ± 0.3	9.3 ± 2.5***	$p < 0.05$, 0.001
IgM (33–190 mg/dl)	97 ± 22 (6)	154 ± 24 (17)	316 ± 126	558 ± 132**, ***	$p < 0.01$, 0.001
IgA (110–410 mg/dl)	307 ± 85 (6)	509 ± 112 (16)	281 ± 44 (8)	280 ± 44 (8)	NS
IgG (870–1700 mg/dl)	2585 ± 345 (7)*	1780 ± 156 (20)	2323 ± 457	1685 ± 168	$p < 0.05$
IgG4 (4.8–105 mg/dl)	556 ± 224	–	–	–	–
ANA-positive	38% (3/8)	48% (10/21)	78% (7/9)*	22% (2/9)	$p < 0.05$
AMA-positive	0% (0/6)	0% (0/14)	22% (2/9)	78% (7/9)***	$p < 0.001$

Values are given as the mean ± SEM

[a] *AST* Aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *γ-GTP* γ-glutamyl transpeptidase, *T-Bil* total bilirubin, *ALB* albumin, *ANA* antinuclear antibody, *AMA* antimitochondrial antibody

[b] Significance: *NS* not significant; ALP, *$p < 0.05$ vs. AIH; γ-GTP, *$p < 0.05$ vs. AIH; T-Bil, ***$p < 0.001$ vs. intra-IgG4-SC, AIH, and PBC; ALB, **$p < 0.01$ vs. PSC, †$p < 0.05$ vs. intra-IgG4-SC, ‡$p < 0.05$ vs. PSC; eosinophils, ***$p < 0.001$ vs. PSC and AIH, *$p < 0.05$ vs. AIH and PSC; IgM, **$p < 0.01$ vs. intra-IgG4-SC, ***$p < 0.001$ vs. PSC; IgG, *$p < 0.05$ vs. PSC and PBC; ANA, *$p < 0.05$ vs. PBC; AMA, ***$p < 0.001$ vs. intra-IgG4-SC, PSC, and AIH

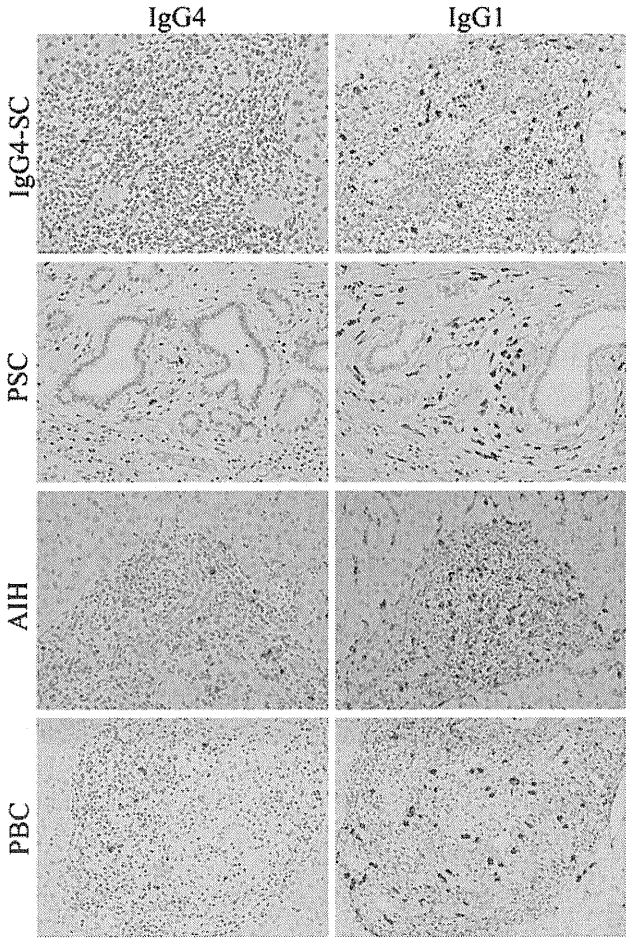

Fig. 2 Immunostaining of formalin-fixed, paraffin-embedded liver sections obtained from patients with IgG4-SC, primary sclerosing cholangitis (*PSC*), autoimmune hepatitis (*AIH*), and primary biliary cirrhosis (*PBC*). Representative liver sections of IgG4-SC, PSC, AIH, and PBC patients show immunostaining of IgG4 and IgG1. The density of IgG4-positive cells is higher than that of IgG1-positive cells in the IgG4-SC liver sections. In the liver sections of PSC, AIH and PBC patients, the density of IgG1-positive cells is higher than that of IgG4-positive cells (×200)

Comparison between immunohistochemical findings in the liver specimens of AIH patients with and without steroid therapy

In terms of the Foxp3/Mono, IgG4/Mono, IgG1/Mono, and IgG4/G1 ratios, AIH patients not receiving steroid therapy showed an increasing tendency relative to those receiving steroid therapy, but the differences were not significant.

Discussion

In general, AIP is currently accepted to be a unique distinctive disease in which histopathological findings show an abundant infiltration of IgG4-positive plasma cells and fibrosis, a condition denoted as lymphoplasmacytic

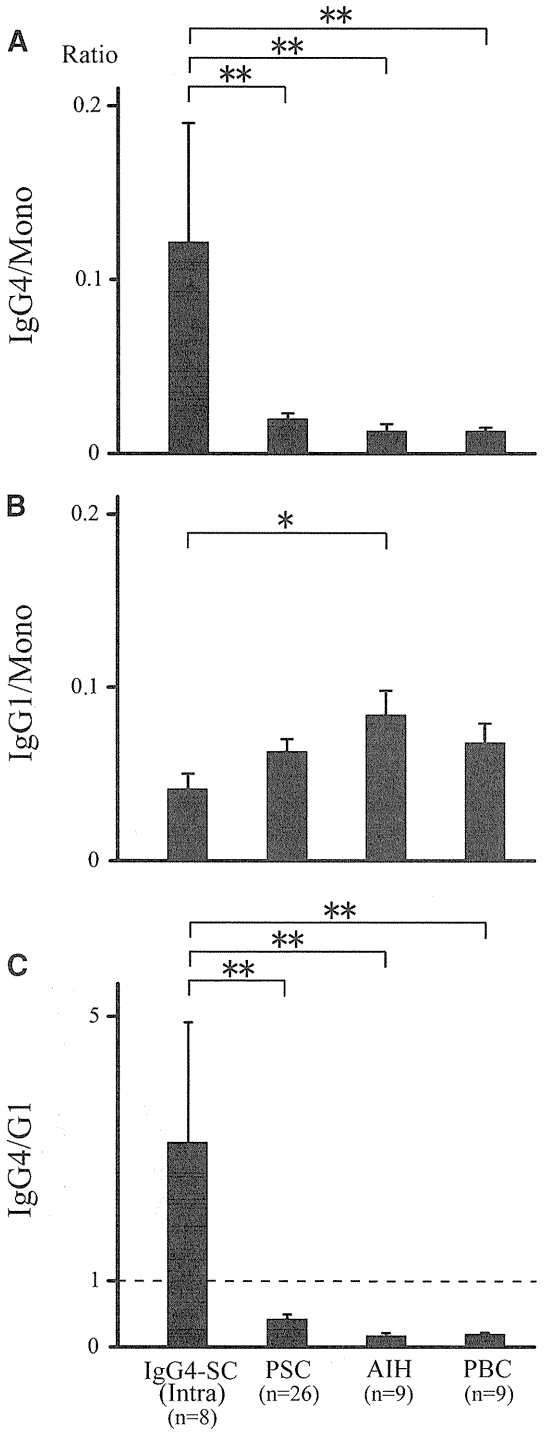

Fig. 3 Ratios of IgG4-positive plasma cells/infiltrated mononuclear cells (*IgG4/Mono*), IgG1-positive plasma cells/infiltrated mononuclear cells (*IgG1/Mono*), and IgG4/Mono to IgG1/Mono (*IgG4/G1*) in the liver of patients with intra-IgG4-SC in comparison with those with other liver diseases. **a** The IgG4/Mono ratio was significantly higher in patients with intra-IgG4-SC than in those with other liver diseases. **b** The IgG1/Mono ratio was significantly lower in patients with intra-IgG4-SC than in those with AIH. **c** The IgG4/G1 was significantly higher in patients with intra-IgG4-SC than in those with other liver diseases. Data are expressed as the mean ± standard error of the mean (SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, *NS* not significant

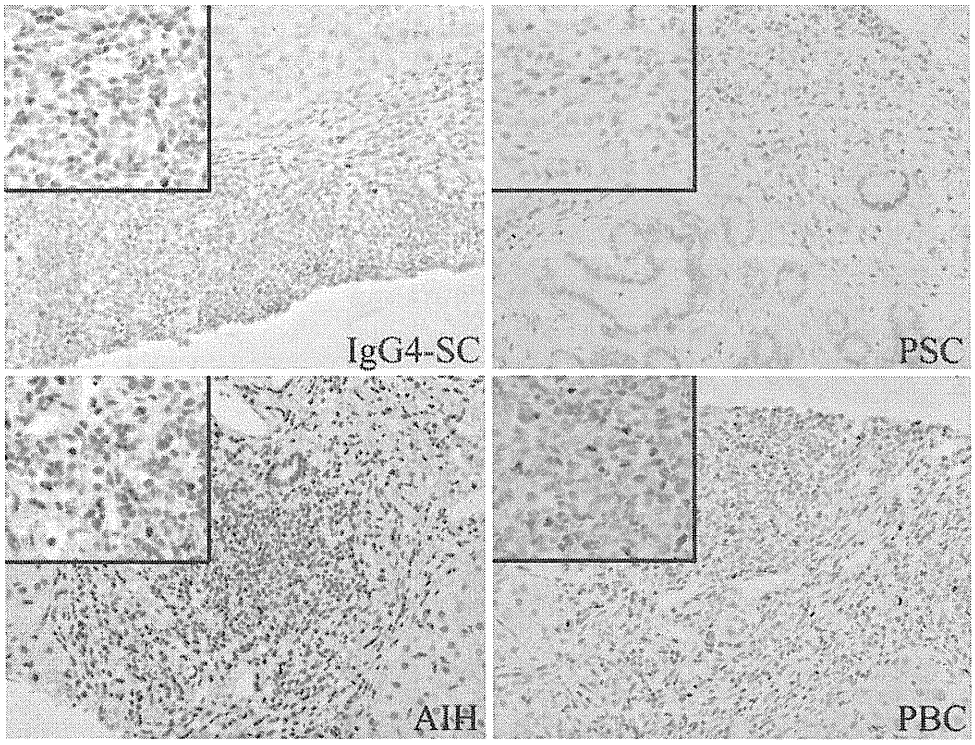

Fig. 4 Immunostaining of forkhead box P3 (Foxp3) in liver sections from IgG4-SC, PSC, AIH, and PBC patients. Foxp3-positive cells were found to be scattered among the lymphoid infiltrates in the portal tracts of the IgG4-SC, AIH, and PBC sections but among those of the PSC sections (×200)

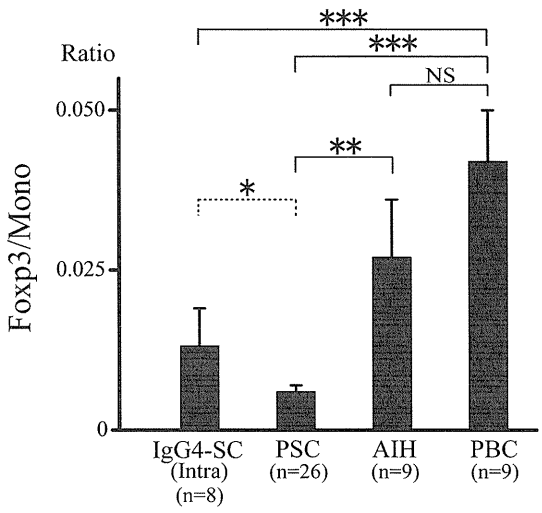

Fig. 5 Ratios of Foxp3 to infiltrated mononuclear cells (*Foxp3/Mono*) in the liver sections of patients with intra-IgG4-SC in comparison with those with other liver diseases. Patients with PBC had a significantly larger Foxp3/Mono ratio than those with intra-IgG4-SC and PSC. The Foxp3/Mono ratio was significantly decreased in PSC patients compared with those with intra-IgG4-SC, AIH, and PBC. *Dotted line* Comparison between intra-IgG4-SC and PSC patients. Data are expressed as the mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, *NS* not significant

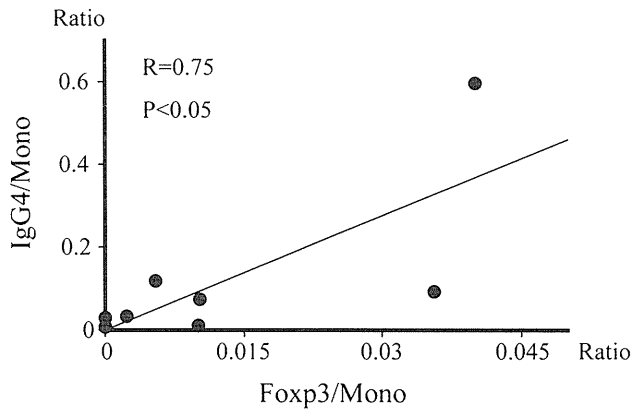

Fig. 6 Correlation between the Foxp3/Mono and IgG4/Mono ratio in patients with intra-IgG4-SC. The Foxp3/Mono and IgG4/Mono ratios are positively correlated in the intra-IgG4-SC patients ($R = 0.75$), but there is no correlation in the other patient groups

sclerosing pancreatitis (LPSP), and the clinical manifestations dramatically respond to steroid treatment. In addition to pancreatic lesions, patients with AIP have occasional extrapancreatic lesions, such as SC, sclerosing sialoadenitis, and retroperitoneal fibrosis (all of which are similar to LPSP). The novel features of this systemic disease have been proposed to be IgG4-related sclerosing disease [10], systemic IgG4-related plasmacytic disease [24], and IgG4-positive multi-organ lymphoproliferative syndrome [25]. Among these, IgG4-SC is the most common and serious extrapancreatic lesion resulting in obstructive jaundice. Prior to the concept of AIP being established, IgG4-SC was often misdiagnosed as PSC complicating chronic pancreatitis. Therefore, differential diagnosis between IgG4-SC and PSC is important. Although IgG4-SC is usually associated with pancreatic lesions, some patients with IgG4-SC have no apparent pancreatic changes or other organ

involvement [26, 27]. The cholangiographic findings in patients with IgG4-SC and PSC have been compared and summarized in a number of studies [12, 28, 29]; the following are only found in PSC patients: a band-like stricture, a beaded appearance, a pruned-tree appearance, and a diverticulum-like formation. Long stenosis, segmental stricture, and a long stricture with prestenotic dilatation are significantly more common in IgG4-SC patients. A shaggy appearance and stricture of the hilar region are occasionally present in both groups. Histological studies have revealed that IgG4-SC patients have fibrosis with lymphoplasmacytic and eosinophil infiltration with mild fibrosis and a significantly increased number of IgG4-positive plasma cells than PSC patients [30, 31]. In one recent study, the immunohistochemical analysis of the liver biopsy specimens revealed that IgG4-positive plasma cell infiltration was significantly more severe in IgG4-SC than in PSC patients [28, 32]. Our immunohistochemical findings on IgG4 are consistent with the results of these earlier studies. However, we did not observed abundant IgG4-positive plasma cell infiltration in all our cases of IgG4-SC. In our study, we classified IgG4-SC patients into two groups based on the intra-/extra-hepatic (intra-IgG4-SC) or extrahepatic (extra-IgG4-SC) nature of the biliary strictures. The infiltration of IgG4-positive plasma cells was more severe in patients with intra-IgG4-SC than in those with extra-IgG4-SC (data not shown). In terms of IgG4 serum levels, there were no significant differences between intra- (556 ± 224; $n = 8$) and extra-IgG4-SC (341 ± 61; $n = 8$). Moreover, IgG4-positive plasma cell infiltration in both classes of IgG4-SC was high compared to that of IgG1-positive plasma cell infiltration, whereas in other liver diseases (PSC, AIH, PBC), IgG1-positive plasma cell infiltration was higher than that of IgG4. Taken together, our study shows that the ratio of IgG4/Mono to IgG1/Mono (IgG4/G1) in intra-IgG4-SC patients was significantly higher than that in patients with other liver diseases (PSC, AIH, and PBC). In recent studies, IgG4-associated AIH was differentiated from other recognized types of AIH [33, 34]. Chung et al. [34] reported that patients with IgG4-associated AIH showed increased IgG serum levels and a marked response to prednisolone therapy. In our study, one of the nine AIH patients showed infiltration of abundant IgG4-positive plasma cells in the liver (>10 IgG4-positive plasma cells/hpf) and increased serum levels of IgG (2886 mg/dl).

In our study, we also examined the local infiltration of $Foxp3^+$ Tregs in the liver of intra-IgG4-SC, AIH, PSC, and PBC patients because $Foxp3^+$ Tregs have been reported to be involved in the development of various autoimmune diseases. Several recent studies have demonstrated the presence of $CD4^+CD25^{high}$ Tregs in patients with autoimmune liver diseases, such as AIH and PBC [35–39], but these results are still open to discussion in terms of PBC [35–37]. Lan et al. [35] recently reported a decrease in the level of Tregs in PBC patients and suggested that Tregs may play a role in the loss of immune tolerance in PBC; however, other investigators [36, 37] have reported a relative increase of Tregs in PBC patients. Sasaki et al. [36] reported that the level of Foxp3, interleukin (IL)-10, and transforming growth factor beta (TGF-β) mRNA expression was higher in the livers of PBC patiens than in normal healthy livers and that the amount of infiltrating $Foxp3^+$ Tregs in portal tracts paralleled the degree of portal inflammation. In contrast, the level of $CD4^+CD25^+$ Tregs suppressing the effector Th1 and Th2 responses were decreased in peripheral blood samples from AIH patients [39]. Our data show a significantly increased infiltration of $Foxp3^+$ Tregs in the liver of intra-IgG4-SC, AIH, and PBC patients compared with PSC patients and a significantly increased number of $Foxp3^+$ Tregs in PBC patients than in those with intra-IgG4-SC and PSC. In addition, we found that the number of infiltrated Foxp3-positive cells was positively correlated with the number of IgG4-positive cells in intra-IgG4-SC patients, but not in those with other liver diseases (PSC, AIH, and PBC). Data obtained in previous studies showed that the level of $Foxp3^+$ Tregs decreased in the liver of PBC patients as the histological stage of the disease advanced [35–37]. Of our 26 cases of PSC, 23 liver specimens were obtained during liver transplantation surgery, indicating that the histological stages in our cases were advanced. Further studies are necessary in order to be able to draw a reliable conclusion on the relationship between $Foxp3^+$ Tregs and PBC because there is a possibility that staging of PSC may affect the severity of infiltration of Tregs similar to that observed in PBC.

We previously reported that circulating Th1 type $CD4^+$ T cells, but not Th2 type $CD4^+$ T cells [40], and $CD4^+CD25^{high}$ Tregs were increased in the peripheral blood of AIP patients [17]. Recent studies of immune tolerance and allergy show that high-dose antigen exposures can cause both immune deviations of the Th2 response in favor of a Th0/Th1 and the generation of IL-10- and TGF-β-producing Tregs. During high-dose antigen exposures, the activation and/or maintenance of the usual Th2 T cell response is inhibited. Additionally, IL-10 induces preferential switching of the B cell response in favor of producing IgG4 antibodies, and possibly IgA antibodies, under the influence of TGF-β [41]. $CD4^+CD25^+$ Tregs also produce IL-10 to educate antigen presenting cells [42]. Therefore, increased Treg levels may correlate with the production of IL-10 in the involved organs, which in turn may influence the switching of B cells to IgG4-producing plasmacytes and the production of serum IgG4. We have previously reported that serum levels of IL-10 and TGF-β in AIP patients were not different from those in healthy and

other disease controls (alcoholic and idiopathic chronic pancreatitis patients) [17]. In other experiments, there was no difference in the serum levels of TGF-β among patients with PSC [43], PBC [44], and healthy controls but serum TGF-β levels in AIH patients were higher than those in healthy controls [45]. Zen et al. [11] reported that Tregs producing IL-10 and TGF-β infiltrated the liver of patients with IgG4-SC and that Foxp3-positive cells were lower in PSC patients than in IgG4-SC patients. However, different from IgG4-SC, our data suggest that such a mechanism is unlikely in AIH or PBC. It still remains unclear why there is difference in the relationship between IgG4-positive cells and Tregs in patients with IgG4-SC, PSC, AIH, and PBC. There are at least two possibilities explaining these differences: (1) it may be due to an originally different subpopulation of Tregs [46–49]; (2) it may be due to different activity of Tregs, such as acting Tregs and resting Tregs [50]. In contrast to murine $Foxp3^+$ Tregs, human $Foxp3^+$ cells may not be functionally homogenous [46, 47]. In general, high amounts of IL-10-producing Tregs, which also produce TGF-β, are well known as type 1 regulatory (Tr1) cells. There is some evidence (based on CD25 expression on $CD4^+$Tr1 cells) in adult humans that constitutive $CD4^+CD25^+$ Tregs and inducible IL-10- and TGF-β-secreting Tr1 cells represent overlapping populations [41]. Furthermore, some $Foxp3^+$ cells are phenotypically naive (e.g., $CD45RA^+$), being present in cord blood as well as in the peripheral blood of adults, and suppressive in vitro [48], whereas other $Foxp3^+$ cells phenotypically resemble memory T cells (e.g., $CD45RA^-$) and possibly originate from peripheral memory $Foxp3^-CD4^+$ T cells [49], in which case they may use different suppressive mechanisms by secreting different immunosuppressive cytokines, such as IL-10 and TGF-β [51]. Taken together, our data may support a hypothesis that decreased naive Tregs may be involved in the pathogenesis of IgG4-SC, resulting in the activation of Th1 type immune responses, while a high-dose antigen (carbonic anhydarase II or lactoferrin, etc.) may induce $CD4^+CD25^{high}$ Tregs from the peripheral blood [52]. This mechanism correlates with the production of IL-10 switching B cells to IgG4-producing plasmacytes in the chronic active phase, resulting in the suppression of both Th2 and Th2 type immune cells [53].

In conclusion, the IgG4/G1 ratio may be another useful marker for the differential diagnosis between intra-IgG4-SC and PSC. We have also demonstrated that the infiltration of $Foxp3^+$ Tregs in the liver was significantly increased in the livers of patients with intra-IgG4-SC, AIH, and PBC relative to those of patients with PSC and that there is a possibility that the function of infiltrated $Foxp3^+$ Tregs is different in intra-IgG4-SC and other liver diseases (PSC, AIH, and PBC). Further studies are needed to clarify the real function of $Foxp3^+$ Tregs infiltrating into the liver of patients with intra-IgG4-SC and other autoimmune liver diseases as well as whether IL-10 or TGF-β is upregulated in the local microenvironment or not.

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References

1. Sarles H, Sarles JC, Muratore R, Guien C. Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatic disease? Am J Dig Dis. 1961;6:688–98.
2. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci. 1995;40:1561–8.
3. Horiuchi A, Kawa S, Akamatsu T, Aoki Y, Mukawa K, Furuya N, et al. Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of Japanese literature. Am J Gastroenterol. 1998;93:260–3.
4. Uchida K, Okazaki K, Konishi Y Ohana M, Takakuwa H, Hajiro K, et al. Clinical analysis of autoimmune-related pancreatitis. Am J Gastroenterol. 2000;95:2788–94.
5. Okazaki K, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I, et al. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. J Gastroenterol. 2006;41:626–31.
6. Okazaki K, Uchida K, Matsushita M, Takaoka M. Autoimmune pancreatitis. Intern Med. 2005;44:1215–23.
7. Kamisawa T, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Extrapancreatic lesions in autoimmune pancreatitis. J Clin Gastroenterol. 2005;39:904–7.
8. Nakazawa T, Ohara H, Sano H, Ando T, Aoki S, Kobayashi S, et al. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. Pancreas. 2005;30:20–5.
9. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med. 2001;344:732–8.
10. Kamisawa T, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. J Gastroenterol. 2006;41:613–25.
11. Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology. 2007;45:1538–46.
12. Nakazawa T, Ohara H, Sano H, Aoki S, Kobayashi S, Okamoto T, et al. Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. Gastrointest Endosc. 2004;60:937–44.
13. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. $CD4^+$ CD25high regulatory cells in human peripheral blood. J Immunol. 2001;167:1245–53.
14. Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. J Exp Med. 2001;193:1303–10.

15. Wing K, Ekmark A, Karlsson H, Rudin A, Suri-Payer E. Characterization of human $CD25^+$ $CD4^+$ T cells in thymus, cord and adult blood. Immunology. 2002;106:190–9.
16. Makita S, Kanai T, Oshima S, Uraushihara K, Totsuka T, Sawada T, et al. $CD4^+$ CD25bright T cells in human intestinal lamina propria as regulatory cells. J Immunol. 2004;173:3119–30.
17. Miyoshi H, Uchida K, Taniguchi T, Yazumi S, Matsushita M, Takaoka M, et al. Circulating Naive and $CD4^+$ CD25high regulatory T cells in patients with autoimmune pancreatitis. Pancreas. 2007;36:133–40.
18. Otsuki M, Chung JB, Okazaki K, Kim MH, Kamisawa T, Kawa S, et al. Asian diagnostic criteria for autoimmune pancreatitis: consensus of the Japan-Korea Symposium on Autoimmune Pancreatitis. J Gastroenterol. 2008;43:403–8.
19. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology. 2008;134:706–15.
20. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999;31:929–38.
21. Vergani D, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol. 2004;41:677–83.
22. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med. 2005;353:1261–73.
23. LaRusso NF, Weisnor RH, Ludwig J, MacCarty RL. Current concept: primary sclerosing cholangitis. N Engl J Med. 1984;310:899–903.
24. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol. 2006;16:335–40.
25. Masaki Y, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, 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. 2009; 68:1310–5.
26. Hayashi K, Nakazawa T, Ohara H, Ando T, Takada H, Tanaka H, et al. Autoimmune sclerosing cholangiopancreatitis with little pancreatic involvements by imaging findings. Hepatogastroenterology. 2007;54:2150–5.
27. Hamano H, Kawa S, Uehara T, Ochi Y, Takayama M, Komatsu K, et al. Immunoglobulin G4–related lymphoplasmacytic sclerosing cholangitis that mimics infiltrating hilar cholangiocarcinoma: part of a spectrum of autoimmune pancreatitis? Gastrointest Endosc. 2005;62:152–7.
28. Nishino T, Oyama H, Hashimoto E, Toki F, Oi I, Kobayashi M, et al. Clinicopathological differentiation between sclerosing cholangitis with autoimmune pancreatitis and primary sclrosing cholangitis. J Gastroenterol. 2007;42:550–9.
29. Ohara H, Nakazawa T, Ando T, Joh T. Systemic extrapancreatic lesions associated with autoimmune pancreatitis. J Gastroenterol. 2007;42:15–21.
30. Uehara T, Hamano H, Kawa S, Sano K, Honda T, Ota H. Distinct clinicopathological entity autoimmune pancreatitis-associated sclerosing cholangitis. Pathol Int. 2005;55:405–11.
31. Zen Y, Harada K, Sasaki M, Sato Y, Tsuneyama K, Haratake J, 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. 2004;28:1193–203.
32. Umemura T, Zen Y, Hamano H, Kawa S, Nakanuma Y, Kiyosawa K. Immunoglobin G4-hepatopathy association of immunoglobin G4-bearing plasma cells in liver with autoimmune pancreatitis. Hepatology. 2007;46:463–71.
33. Umemura T, Zen Y, Hamano H, Ichijo T, Kawa S, Nakanuma Y, et al. IgG4 associated autoimmune hepatitis: a differential diagnosis for classical autoimmune hepatitis. Gut. 2007;56:1471–2.
34. Chung H, Watanabe T, Kudo M, Maenishi O, Wakatsuki Y, Chiba T. Identification and characterization of IgG4-associated autoimmune hepatitis. Liver Int. 2010;30:222–31.
35. Lan RY, Cheng C, Lian ZX, Tsuneyama K, Yang GX, Moritoki Y, et al. Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. Hepatology. 2006;43:729–37.
36. Sasaki M, Ikeda H, Sawada S, Sato Y, Nakanuma Y. Naturally-occurring regulatory T cells are increased in inflamed portal tracts with cholangiopathy in primary biliary cirrhosis. J Clin Pathol. 2007;60:1102–7.
37. Sakaki M, Hiroishi K, Baba T, Ito T, Hirayama Y, Saito K, et al. Intrahepatic status of regulatory T cells in autoimmune liver diseases and chronic viral hepatitis. Hepatol Res. 2008;38:354–61.
38. Longhi MS, Hussain MJ, Mitry RR, Arora SK, Vergani GM, Vergani D, et al. Functional study of $CD4^+$ $CD25^+$ regulatory T cells in health and autoimmune hepatitis. J Immunol. 2006;176:4484–91.
39. Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. J Hepatol. 2004;41:31–7.
40. Okazaki K, Uchida K, Ohana M, Nakase H, Uose S, Inai M, et al. Autoimmune-related pancreatitis is associated with autoantibodies and Th1/Th2 cellular immune response. Gastroenterology. 2000;118:573–81.
41. Robinson DS, Larche M, Durhan SR. Tregs and allergic disease. J Clin Invest. 2004;114:1389–97.
42. Alpan O, Bachelder E, Isil E, Arnheiter H, Matzinger P. 'Educated' dendritic cells act as messengers from memory to naive T helper cells. Nat Immunol. 2004;5:615–22.
43. Watt JK, Hawkins K, Zhang M, Lipschitz J, Sandha G, Gong Y, et al. Transforming growth factor-beta (TGF-beta) protein levels are not elevated in the blood or bile of patients with primary sclerosing cholangitis: a pilot study. Dig Dis Sci. 2004;49:5–8.
44. Rong G, Zhou Y, Xiong Y, Zhou L, Geng H, Jiang T, et al. Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population. Clin Exp Immunol. 2009;156:217–25.
45. Sakaguchi K, Kitano M, Nishimura M, Senoh T, Ohta T, Terao M, et al. Serum level of transforming growth factor-beta1 (TGF-beta1) and the expression of TGF-beta receptor type II in peripheral blood mononuclear cells in patients with autoimmune hepatitis. Hepatogastroenterology. 2004;51:1780–3.
46. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol. 2007;19:345–54.
47. Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human $CD4^+$ FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. Blood. 2007; 110:2983–90.
48. Valmori D, Merlo A, Souleimanian NE, Hesdorffer CS, Ayyoub M. A peripheral circulating compartment of natural naive CD4 Tregs. J Clin Invest. 2005;115:1953–62.
49. Vukmanovic-Stejic M, Zhang Y, Cook JE, Fletcher JM, McQuaid A, Masters JE, et al. Human $CD4^+$ CD25hi$Foxp3^+$ regulatory T cells are derived by rapid turnover of memory populations in vivo. J Clin Invest. 2006;116:2423–33.

50. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional Delineation and Differentiation Dynamics of Human CD4(+) T Cells Expressing the FoxP3 Transcription Factor. Immunity. 2009;30:1–13.
51. Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, et al. Two functional subsets of FOXP3^{+} regulatory T cells in human thymus and periphery. Immunity. 2008;28:870–80.
52. Uchida K, Okazaki K, Nishi T, Uose S, Nakase H, Ohana M, et al. Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydarase II and lactoferrin. Lab Invest. 2002;82:411–24.
53. Okazaki K, Uchida K, Fukui T. Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis. J Gastroenterol. 2008;43:409–18.

Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease

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Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease

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ABSTRACT

We report a case of immunoglobulin G4 (IgG4)-related sclerosing disease involving the pancreas, liver and salivary glands. Massive infiltration of IgG4-expressing plasma cells was seen in the liver and submandibular lymph nodes. Interestingly, accumulation of IgG4-expressing plasma cells was also seen in the colon and terminal ileum. Peripheral blood mononuclear cells (PBMCs) isolated from this patient exhibited enhanced production of IgG4 and interleukin-10 upon stimulation with Toll-like receptor (TLR) ligands as compared with those from a healthy control. In contrast, production of tumour necrosis factor α and interferon γ by PBMCs from this patient was markedly reduced. Since colonic mucosa is always exposed to TLR ligands derived from commensal organisms, the results of immunological studies suggest that enhanced T helper type 2 responses to intestinal microflora may underlie the immunopathogenesis in this patient with IgG4-related sclerosing disease.

INTRODUCTION

Autoimmune pancreatitis (AIP) is an inflammatory disorder which is characterised by increased serum levels of immunoglobulin G4 (IgG4) or by an IgG4-positive plasmacytic infiltrate into the inflamed tissue.[1] Another important feature of AIP is a wide variety of extrapancreatic manifestations such as sialadenitis, cholangitis, retroperitoneal fibrosis and inflammatory pseudotumour of the liver and lung.[2] Since these extrapancreatic and pancreatic lesions share common histopathological findings (ie, abundant infiltration by $IgG4^+$ plasmacytes), Kamisawa *et al* proposed a new clinicopathological entity: 'IgG4-related sclerosing disease'.[2] However, little is understood regarding the role played by this IgG subtype in the inflammatory process. In this regard, IgG4 itself does not seem to be responsible for the development of tissue damage since this IgG subtype does not cause cell-mediated lysis due to poor binding activity to complement.[3] In addition, anti-inflammatory activity of IgG4 has been shown.[4] Consistent with these biological functions of IgG4, clinical manifestations of immune complex disease such as arthritis and glomerulonephritis are rarely seen in patients with IgG4-related sclerosing disease.[5] These facts suggest that abnormal immunological environments leading to enhanced IgG4 responses, rather than IgG4 antibody itself, underlie the pathogenesis of this disease.

Distribution of IgG4-expressing plasmacytes in the gastrointestinal tract of patients with AIP has been observed.[6 7] However, it is unknown whether this distribution of $IgG4^+$ cells is directly induced by immune reactions occurring in the gastrointestinal tract or is indirectly induced by systemic IgG4 responses. Given the fact that mucosa of the gastrointestinal tract is always exposed to antigens derived from intestinal microflora, it is tempting to speculate that immune responses against microbial antigens create abnormal environments leading to enhanced IgG4 responses in the gut. Indeed, we experienced a case of IgG4-related sclerosing disease in which accumulation of IgG4-expressing plasmacytes was visualised as colonic inflammatory pseudopolyps.[8] Here we report a case with IgG4-related sclerosing disease whose ileal and colonic mucosa bore a marked infiltration of IgG4-expressing plasma cells. Interestingly, peripheral blood mononuclear cells (PBMCs) isolated from this case show enhanced T helper type 2 (Th2) and IgG4 responses upon stimulation with Toll-like receptor (TLR) ligands. These results indicate possible involvement of excessive Th2 responses against intestinal microflora in some cases with IgG4-related sclerosing disease.

CASE REPORT

A 70-year-old asymptomatic man was admitted for further investigation of swelling of the pancreas and submandibular lymph nodes. He had a history of systemic lymphadenopathy of unknown aetiology at the age of 45. Laboratory tests revealed elevation of serum levels of amylase (229 IU/l; normal range <129 IU/l) and IgG (2144 mg/dl; normal range <1840 mg/dl). Abdominal CT using contrast reagent showed focal swelling of the pancreatic head without an enhancement effect. Endoscopic retrograde cholangiopancreatography revealed irregular narrowing of the main pancreatic duct and the stricture of the lower bile duct. These radiographic findings were consistent with those of AIP.[1] A hypoechoic tumour was detected in the lateral segment of the liver on abdominal ultrasonography. Since a marked elevation of serum IgG4 level was detected (918 mg/dl; normal range <135 mg/dl), this patient was strongly suspected to have IgG4-related scrclosing disease involving the pancreas, bile duct and liver. Biopsy of the liver tumour revealed massive infiltration of plasmacytes expressing IgG and IgG4 around the bile duct (figure 1). More than 50% of IgG-expressing cells

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were positive for IgG4 staining, which suggests that this liver tumour was a pseudotumour due to IgG4-associated cholangitis. Similar histological findings were obtained in the immunohistochemical analyses using biopsy specimens from submandibular lymph nodes (figure 1). Based on these results, this patient was finally diagnosed as having IgG4-related sclerosing disease.

Colonoscopy was performed to exclude the involvement of the gastrointestinal tract before starting prednisolone treatment. Although no inflammatory mucosa was seen from the terminal ileum to the rectum on colonoscopic examination, biopsy specimens taken from the intact mucosa of the terminal ileum and colon revealed a marked infiltration of plasmacytes expressing IgG into the submucosa without destruction of crypt architecture or fibrosis (figure 1). Interestingly, >50% of IgG-expressing cells were positive for IgG4 staining. Accumulation of IgG4-expressing plasma cells in the colonic mucosa led us to hypothesise that abnormal immunological responses to gut microbial antigens might underlie the development of enhanced IgG4 responses. PBMCs from this case and healthy controls were stimulated with TLR ligands to see immune responses against antigens derived from intestinal microflora.[9] Ethical permission for this study was granted by the review board of Kyoto University. As shown in figure 2, production of IgG4 as well as interleukin-10 (IL-10) was enhanced upon stimulation with TLR4 (lipopolysaccharide (LPS)) and TLR5 (flagellin) ligands. Production of IgG4 was also enhanced by stimulation of a TLR2 ligand (peptidoglycan (PGN)). In contrast, production of Th1 cytokines (interferon γ (IFNγ) and tumour necrosis factor α (TNFα)) in response to TLR ligands by the patient's PBMCs was impaired as compared with that by control PBMCs. No difference was seen in the production of IL-8 or IL-17 upon stimulation with TLR ligands or TNFα. These data suggest that activation of TLRs generates both IgG4 and Th2 responses in PBMCs from this case since IFNγ and IL-10 are prototypical Th1 and Th2 cytokines, respectively.[9] We determined the type of immune cells producing these cytokines by cell depletion study

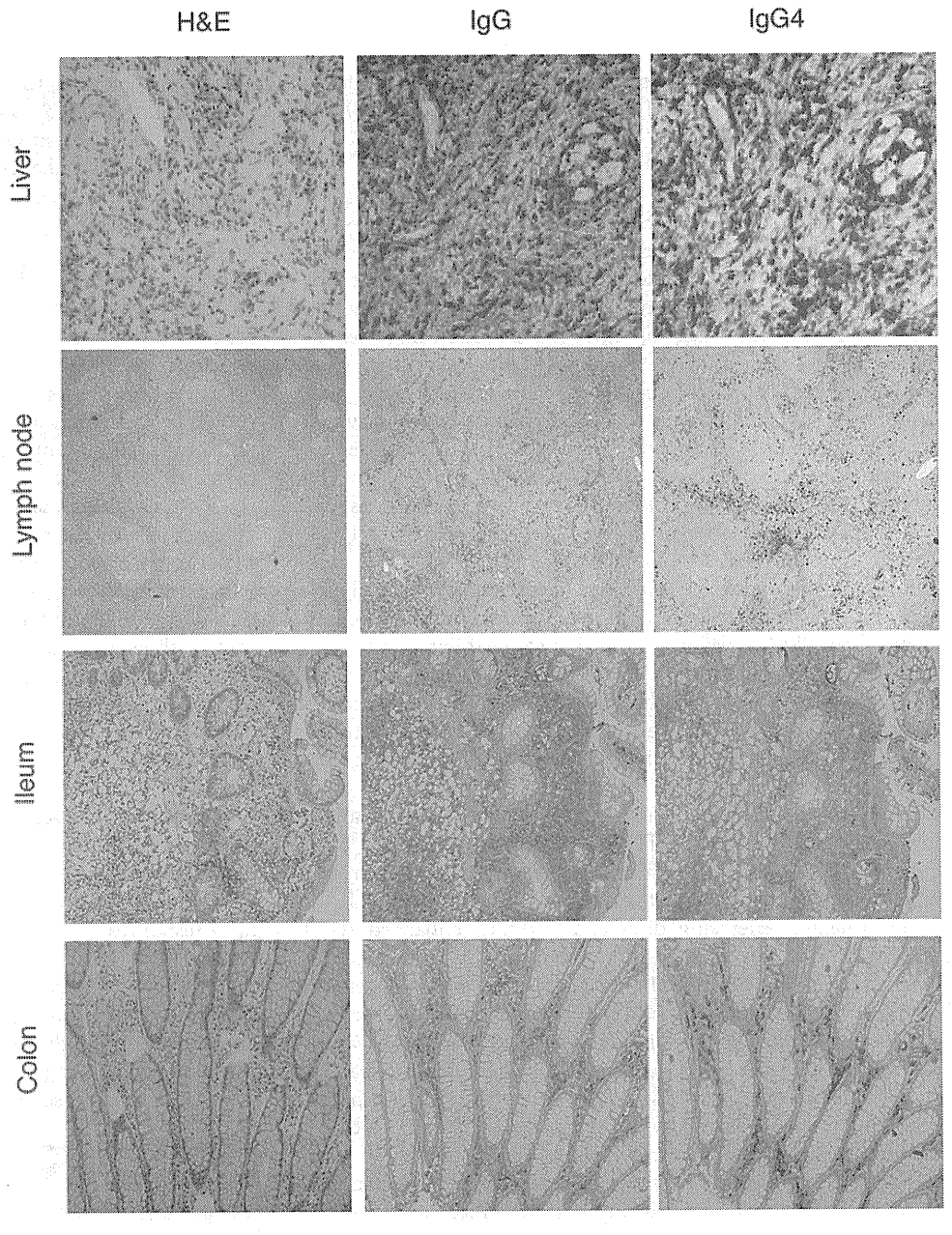

Figure 1 Immunohistochemical staining of immunoglobulin G4 (IgG4) and IgG. Biopsy specimens obtained from the liver, submandibular lymph nodes, terminal ileum and colon were stained with anti-IgG4 or anti-IgG antibody for visualisation of plasma cells expressing IgG4 or IgG.

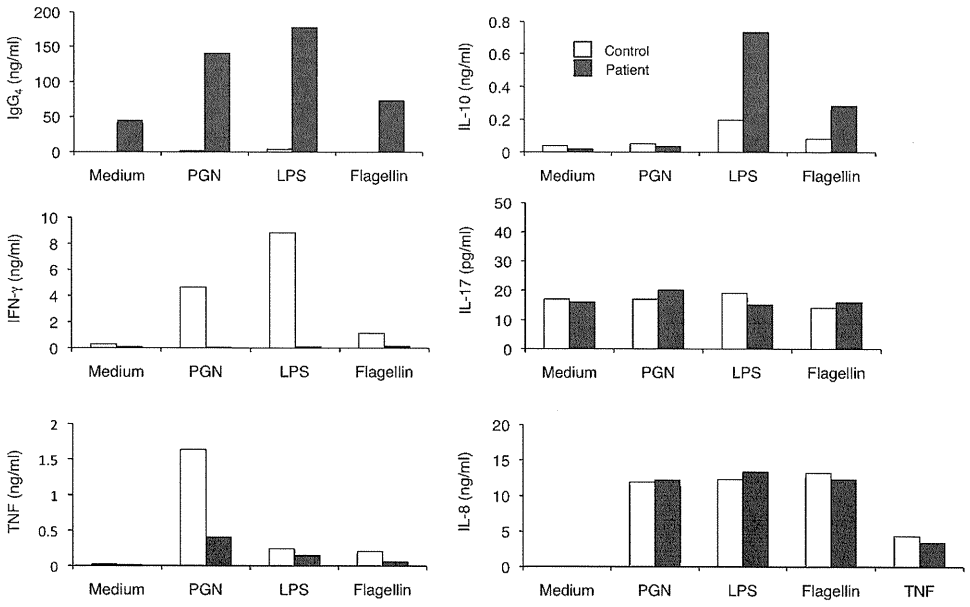

Figure 2 Enhanced T helper type 2 (Th2) responses to Toll-like receptor (TLR) ligands by peripheral blood mononuclear cells (PBMCs) isolated from the patient. PBMCs (2×10^6/ml) isolated from the patient and healthy controls were stimulated with peptidoglycan (PGN, 10 μg/ml), lipopolysaccharide (LPS, 1 μg/ml), flagellin (1 μg/ml) or tumour necrosis factor (TNF, 20 ng/ml). PBMCs were cultured for 48 h for interleukin-8 (IL-8) and TNF assay, and for 14 days for IgG4, interferon γ (IFNγ), IL-10 and IL-17 assay. Results shown are means of triplicate wells.

using control samples. We found that $CD3^+$ T cells produced IFNγ and IL-10 whereas $CD14^+$ monocytes produced IL-10 and TNFα (data not shown).

DISCUSSION

An interesting observation in this case with IgG4-related sclerosing disease was a marked infiltration of IgG4-expressing plasmacytes into the colonic mucosa which appeared to be normal on endoscopic examination. It remains unclear whether we can regard this case as IgG4-related sclerosing disease involving the colonic mucosa since no pathological findings were present in colonic biopsy specimens other than marked infiltration of $IgG4^+$ cells. Thus, unlike our previous case in which infiltration of IgG4-expressing plasmacytes was visualised as colonic polypoidal lesions,[8] we have to be cautious in the interpretation of infiltration of IgG4-expressing plasma cells into endoscopically normal colonic mucosa in the setting of IgG4-related sclerosing disease.

Immune responses leading to accumulation of IgG4-expressing plasmacytes in the gastrointestinal tract are poorly understood. PBMCs isolated from this case exhibited enhanced production of IgG4 and Th2 cytokines upon stimulation with TLR ligands, suggesting that enhanced immune reactions against microbial antigens cause infiltration of lymphocytes as in the case of inflammatory bowel disease (IBD).[10] In fact, this idea is supported by clinical evidence that a significant population of patients with AIP have a diagnosis of IBD.[11] Importantly, IgG4 responses induced by TLR activation are associated with enhanced IL-10 production. In this regard, two different groups report involvement of regulatory T cells (Tregs) producing IL-10 in IgG4-related sclerosing disease.[12 13] Thus, enhanced IL-10 production seen in this case may be partially mediated by circulating Tregs. Given the fact that IL-10 is an important cytokine for IgG4 class switching,[14] we assume that excessive Th2 responses triggered by TLR ligands together with activation of Tregs create abnormal immunological environments leading to enhanced IgG4 responses. This idea partially explains immunological mechanisms of enhanced Th2 responses in patients with IgG4-related sclerosing disease.[12]

Although storiform fibrosis is a characteristic pathological finding of IgG4-related sclerosing disease,[2] molecular mechanisms of fibrosis in this disorder are poorly understood. Th2 cytokines mediated by activation of TLRs on macrophages have been identified as positive regulators of tissue fibrosis in the liver and lung.[15] Thus, enhanced Th2 responses to TLR ligands might be involved in the development of storiform fibrosis in IgG4-related sclerosing disease. However, analysis of expression of both Th2 cytokines and TLRs using fibrotic tissue samples is necessary to address this issue.

What is the mechanism by which enhanced Th2 responses against intestinal microflora cause IgG4-related sclerosing disease without the development of colitis? In this regard, immune reactions causing tissue injury and those causing IgG4 responses should be considered separately as shown by the fact that IgG4 antibody itself has anti-inflammatory properties.[4] Indeed, tissue destruction was not seen in the lower gastrointestinal tract of this case despite a marked infiltration of IgG4-expressing plasmacytes into the submucosa. Several mechanisms for preventing hyper-responsiveness to microbial antigens operate in the gut. For example, the intestine is the preferential site where naïve T cells differentiate into Tregs.[10] Thus, one possible explanation is that pathogenic immune reactions causing tissue injury are suppressed in the gut by regulatory mechanisms, whereas such reactions cause tissue injury in other sterile organs such as the pancreas and bile duct due to the lack of regulatory mechanisms. Based on this, it is tempting to speculate that the gastrointestinal tract is an induction site for systemic IgG4 responses and functions as a reservoir for IgG4-expressing plasmacytes even if disease-associated pathogenic changes are absent. Alternatively, distribution of IgG4-expressing plasmacytes into the colonic mucosa may be an epiphenomenon associated with systemic IgG4 responses.

In conclusion, the results of immunological studies using PBMCs from this case suggest involvement of excessive Th2 responses to intestinal microflora in the development of IgG4-related sclerosing disease. Confirmation of this idea awaits further studies using a large number of patients with IgG4-related sclerosing disease.

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Competing interests None.

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Patient consent Obtained.

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REFERENCES

1. **Finkelberg DL,** Sahani D, Deshpande V, *et al*. Autoimmune pancreatitis. *N Engl J Med* 2006;**355**:2670–6.
2. **Kamisawa T,** Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 2006;**41**:613–25.
3. **Kawa S,** Kitahara K, Hamano H, *et al*. A novel immunoglobulin–immunoglobulin interaction in autoimmunity. *PLoS One* 2008;**3**:e1637.
4. **van der Neut Kolfschoten M,** Schuurman J, Losen M, *et al*. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 2007;**317**:1554–7.
5. **Okazaki K,** Chiba T. Autoimmune related pancreatitis. *Gut* 2002;**51**:1–4.
6. **Kamisawa T,** Egawa N, Nakajima H, *et al*. Gastrointestinal findings in patients with autoimmune pancreatitis. *Endoscopy* 2005;**37**:1127–30.
7. **Deheragoda MG,** Church NI, Rodriguez-Justo M, *et al*. The use of immunogiobulin g4 immunostaining in diagnosing pancreatic and extrapancreatic involvement in autoimmune pancreatitis. *Clin Gastroenterol Hepatol* 2007;**5**:1229–34.
8. **Ueno K,** Watanabe T, Kawata Y, *et al*. IgG4-related autoimmune pancreatitis involving the colonic mucosa. *Eur J Gastroenterol Hepatol* 2008;**20**:1118–21.
9. **Watanabe T,** Asano N, Murray PJ, *et al*. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. *J Clin Invest* 2008;**118**:545–59.
10. **Strober W,** Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007;**117**:514–21.
11. **Ravi K,** Chari ST, Vege SS, *et al*. Inflammatory bowel disease in the setting of autoimmune pancreatitis. *Inflamm Bowel Dis* 2009;**15**:1326–30.
12. **Zen Y,** Fujii T, Harada K, *et al*. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007;**45**:1538–46.
13. **Miyoshi H,** Uchida K, Taniguchi T, *et al*. Circulating naive and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis. *Pancreas* 2008;**36**:133–40.
14. **Jeannin P,** Lecoanet S, Delneste Y, *et al*. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998;**160**:3555–61.
15. **Wynn TA.** Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;**117**:524–9.

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Advance Publication

REVIEW

Pituitary and Stalk Lesions (Infundibulo-hypophysitis) Associated with Immunoglobulin G4-related Systemic Disease: an Emerging Clinical Entity

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Abstract. Inflammatory lesions of the pituitary gland are rarely encountered. Recently, the concept of immunoglobulin G4 (IgG4)-related systemic disease was proposed in Japan, and more than 20 cases have been reported as possibly associated with infundibulo-hypophysitis since 2000. We herein review such case reports in the published literature and in the abstracts of scientific meetings. Almost all cases involved middle-aged to elderly men presenting with various degrees of hypopituitarism and diabetes insipidus and demonstrating a thickened pituitary stalk and/or pituitary mass. These structures shrank remarkably in response to glucocorticoid therapy, even in a lower dose range similar to that prescribed as a replacement for adrenocortical insufficiency. Some of the anterior pituitary insufficiencies were also resolved by glucocorticoid administration. The presence of IgG4-related systemic disease and an elevated serum IgG4 level before glucocorticoid therapy were the main clues to a correct diagnosis of IgG4-related infundibulo-hypophysitis. Autoimmunity is suggested but not yet established to play a role in the pathogenesis for IgG4-related systemic disease. The fact that hypertrophic pachymeningitis and para-sinusitis accompanied some cases suggested that both sellar and parasellar structures are involved in the chronic inflammation. We therefore classify this disorder not as a variant form of primary autoimmune hypophysitis but as a secondary form of infundibulo-hypophysitis associated with IgG4-related systemic disease.

Key words: Secondary hypophysitis, Immunoglobulin G4, Multifocal fibrosclerosis, Hypopituitarism, Diabetes insipidus

INFLAMMATORY LESIONS of the pituitary and pituitary stalk are rarely encountered. However, due to the availability of magnetic resonance (MR) imaging, so-called lymphocytic hypophysitis has been reported more frequently in Japan than in Western countries [1]. Infundibulo-hypophysitis may be categorized into three groups according to the involved tissues: adenohypophysitis, infundibulo-neurohypophysitis and panhypophysitis [2]. An autoimmune mechanism is thought to be involved in adenohypophysitis and infundibulo-hypophysitis. However, panhypophysitis may be heterogeneous; that is, it may be primary or secondary as a direct result of systemic infectious or inflammatory processes or as a result of local processes such as a ruptured Rathke cleft cyst, craniopharyngioma, or germinoma. Chronic inflammation of parasellar structures such as seen in hypertrophic pachymeningitis or Tolosa-Hunt syndrome may spread into the pituitary gland. Rare cases associated with multifocal fibrosclerosis have been reported [3, 4].

Recently, the new concept of immunoglobulin G4 (IgG4)-related systemic disease was proposed from the close observation of autoimmune pancreatitis (AIP) and lymphoproliferative diseases [5, 6]. Involvement of the pituitary gland may be recognized as a possible extra-pancreatic manifestation of AIP. More than 20 cases have been reported since 2000. In the present article, we review these case reports and summarize the clinical features of IgG4-related infundibulo-hypophysitis. We also discuss

the relationship to autoimmune hypophysitis and the possible pathogenesis of IgG-4 related disease.

1. IgG4-related systemic disease

IgG4-related sclerosing disease is a systemic disease characterized by extensive infiltration of IgG4-positive plasma cells and T-lymphocyte into various organs [5]. Clinical manifestations are apparent in the pancreas, bile duct, gallbladder, salivary gland, retroperitoneum, kidney, lung, and prostate, in which organ tissue fibrosis with occlusive phlebitis is pathologically induced. Most IgG4-related sclerosing diseases have been found to be associated with AIP, but IgG4-related diseases without pancreatic involvement have been reported. Some inflammatory pseudotumors may be involved in this disease. The disease occurs predominantly in elder men, is frequently associated with lymph node swelling, and responds well to glucocorticoid therapy. Serum IgG4 levels and immunostaining with anti-IgG4 antibody are useful for making a diagnosis.

Multifocal fibrosclerosis is an uncommon fibroproliferative systemic disorder with multiple manifestations, including sclerosing cholangitis, salivary gland fibrosis, retroperitoneal fibrosis, Riedel's thyroiditis, and fibrotic orbital pseudotumor [7]. As the histopathological findings of these disorders are similar - i.e., fibrotic changes with lymphoplasmacytic infiltration and occasional phlebitis - it is suggested that they are all interrelated and probably different manifestations of a common disorder of fibroblastic proliferation. The histopathology of the extrapancreatic lesions associated with AIP strongly suggests that multifocal fibrosclerosis is an IgG4-related sclerosing disease [8].

2. Pituitary and stalk lesions (infundibulo-hypophysitis) associated with IgG4-related systemic disease

Chronic inflammatory diseases of the pituitary gland, inflammatory pseudotumor [9] or plasma cell granuloma [10] have been described, and several cases of pituitary lesion associated with retroperitoneal fibrosis have been reported [3, 4]. More recently, a new disease entity consisting of hypophysitis associated with IgG4-related systemic disease has been described [11-14]. We surveyed the case reports involving this entity both in the published literature and in the abstracts of scientific meetings since 2000. Inclusion criteria for the survey were the presence of pituitary and stalk lesions associated with at least one IgG4-related systemic disease or multifocal fibrosclerosis, and/or the biopsy-proven inflammatory pseudotumor of the pituitary mass infiltrated with IgG4-positive plasma cells.

There were 22 such cases (Table 1: [11-34]). Twenty-one cases were male and only one was female. The age distribution was as followed; 2 patients were in their 40s, 4 in their 50s, 8 in their 60s, and 8 in their 70s. The median age was 64 years. Whether the observed extreme male predominance is the characteristics for the IgG4-related hypophysitis or not remains to be determined, since the male: female ratio of AIP was reported as 2.77:1 [35].

1) Clinical manifestations

Symptoms related to the hypothalamic-hypophyseal system were general malaise (11 cases), headache (6 cases), visual disturbances including impaired eye movement (6 cases), fever (5 cases), polyuria (6 cases), appetite loss (4 cases), weight loss (4 cases), and decreased libido (3 cases). General malaise, loss of appetite and weight loss was closely related with ACTH deficiency.

2) Pituitary function

Various degrees of anterior pituitary hormone deficiency were observed in 19 cases, and central diabetes insipidus was observed in 12 cases. Eleven cases had both hypopituitarism and diabetes insipidus. Masked diabetes insipidus was diagnosed in 3 cases. Diabetes insipidus may have preceded the development of hypopituitarism in 4 cases.

Isolated hypogonadism, isolated central hypothyroidism, and isolated ACTH deficiency were observed in 2 patients, one patient and one patient, respectively. Another 15 cases had combined anterior pituitary hormone deficiencies. Decreased secretion of LH/FSH (15 cases), ACTH (14 cases), TSH (12 cases), GH (8 cases) were documented. It is unclear whether specific combinations of pituitary hormone deficiencies may exist, since provocative tests to examine the pituitary hormone reserve were not used in every patient. Three cases with hyperprolactinemia were reported. Altered hypothalamic regulation of pituitary hormone secretion was demonstrated in one case [22].