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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 (TNF) and interferon (IFN) 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 Th2 responses 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+ plasmocytes), 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 showed 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. A hypoechogenic 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 sclerosing 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...
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. 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. 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.
using control samples. We found that CD8+ 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, 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 plasma cells 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). In fact, this idea is supported by clinical evidence that a significant population of patients with AIP have a diagnosis of IBD. Importantl, 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 and TNFα (data not shown).

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.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Kyoto University review board.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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