Table 2. Methylation of the IGFBP-3 promoter in biliary tract cancer tissues

	Hypermethylated	Hypomethylated	Fisher's exact test
Gallbladder cancer tissues	13 (43%)	17 (57%)	
p53(+)	2	11	$P = 0.009$
p53(−)	11	6	
Extrahepatic bile duct cancer tissues	11 (37%)	19 (63%)	
p53(+)	2	12	$P = 0.021$
p53(−)	9	7	
Carcinoma tissues of the ampulla of Vater	9 (45%)	11 (55%)	
p53(+)	1	7	$P = 0.025$
p53(−)	8	4	

Effect on survival. The effects of BMS-536924 in combination with chemotherapy were assessed. All of the tested drugs, 5-FU, gemcitabine and cisplatin, induced caspase-3 activity in TGBC-1TKB (Fig. 3C). BMS-536924 enhanced 5-FU-induced apoptosis synergistically and both gemcitabine-induced and cisplatin-induced apoptosis additively. In TGBC-2TKB, BMS-536924 upregulated drug-induced apoptosis synergistically for all three chemotherapies (Fig. 3D). These results suggest that BMS-536924 enhances the effects of chemotherapy.

BMS-536924 suppressed tumors in mice. To assess the effect of this drug on cancer *in vivo*, TGBC-1TKB cells were inoculated into nude mice and allowed to form evident tumors. Oral administration of 70 mg/kg BMS-536924 significantly inhibited tumor growth in mice (Fig. 4A).

To evaluate the effect of BMS-536924 on apoptosis induction in tumors, TUNEL assays were performed (Fig. 4B). BMS-536924 upregulated apoptosis in xenografts tumors. The treatment did not have adverse effects on the body weight of mice or the glucose levels at the time of death (Fig. 4C), suggesting tolerable toxicity.

Effect of dominant negative insulin-like growth factor-I receptor on biliary tract carcinomas in mice. To assess the effect of IGF-IR/482st on BTC *in vivo*, TGBC-1TKB cells were inoculated in nude mice and allowed to form evident tumors. Intra-tumoral injection of adenovirus-IGF-IR/482st for five successive days markedly suppressed tumor growth without influencing body weight or glucose concentration at death (Table 3). These results suggest that IGF-IR might be a candidate molecular target for human BTC.

Discussion

In the current study, IGF-positivity in carcinoma cells was associated with tumor size in all types of BTC. IGF-II-positivity tended to correlate with high T-stage and advanced overall stage. IGF-IR-positivity was seen immunohistochemically in over 60% of patients, and was associated with advanced tumor stage. Moreover, IGF-II and IGF-IR expression were correlated to one another in all types of BTC and IGF-I ligand and receptor expression were associated in GBC, suggesting possible aberrant activation of IGF-IR by paracrine/autocrine loops. We found an association of p53-positivity and IGF-IR in GBC, in agreement with published data that mutated p53 upregulates IGF-IR expression. Thus, the IGF-IR axis might contribute to the aggressive phenotype of tumor cells, resulting in the progression of BTC.

Hypermethylation of the IGFBP-3 promoter was detected in approximately 40% of the BTC studied. Thus, the activities of IGF might be upregulated, at least in part, by epigenetic silencing of IGFBP-3 in these cancers. IGFBP-3 methylation was detected more frequently in p53-negative tumors than in

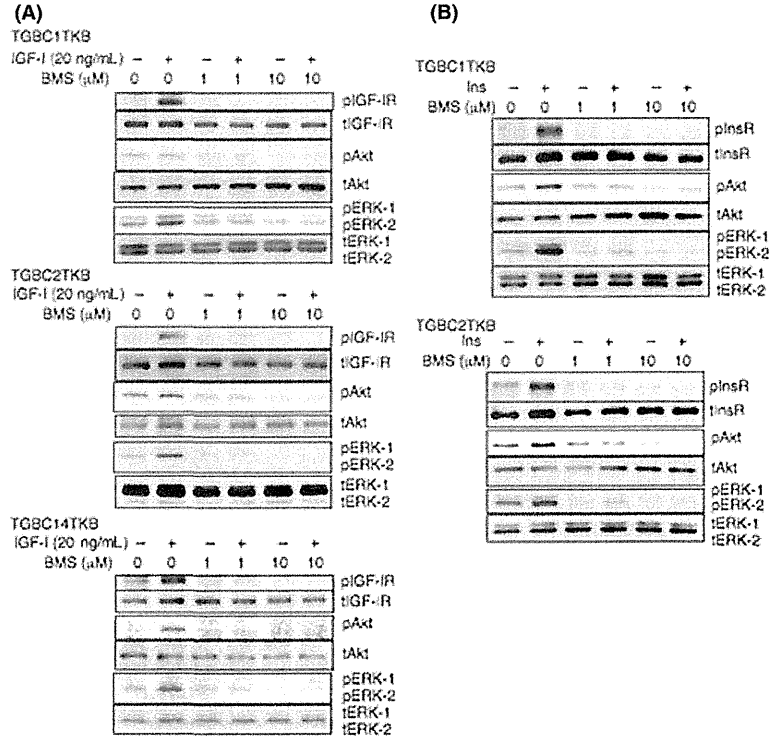

Fig. 2. BMS-536924 blocked insulin-like growth factor (IGF)-I/insulin signals of three biliary tract carcinomas cell lines by Western blotting. (A) BMS-536924 reduced IGF-I induced IGF-IR autophosphorylation of all cell lines. BMS-536924 blocked both Akt and ERK signals. (B) BMS-536924 also blocked insulin-induced InsR autophosporylation and the downstream signals. Ins, insulin; pIGF-IR, phosphorylated IGF-IR; tIGF-IR, total IGF-IR; pAkt, phosphorylated Akt-1; tAkt, total Akt-1; pERK, phosphorylated ERK-1/-2; tERK, total ERK-1/-2; pInsR, phosphorylated InsR; tInsR, total InsR.

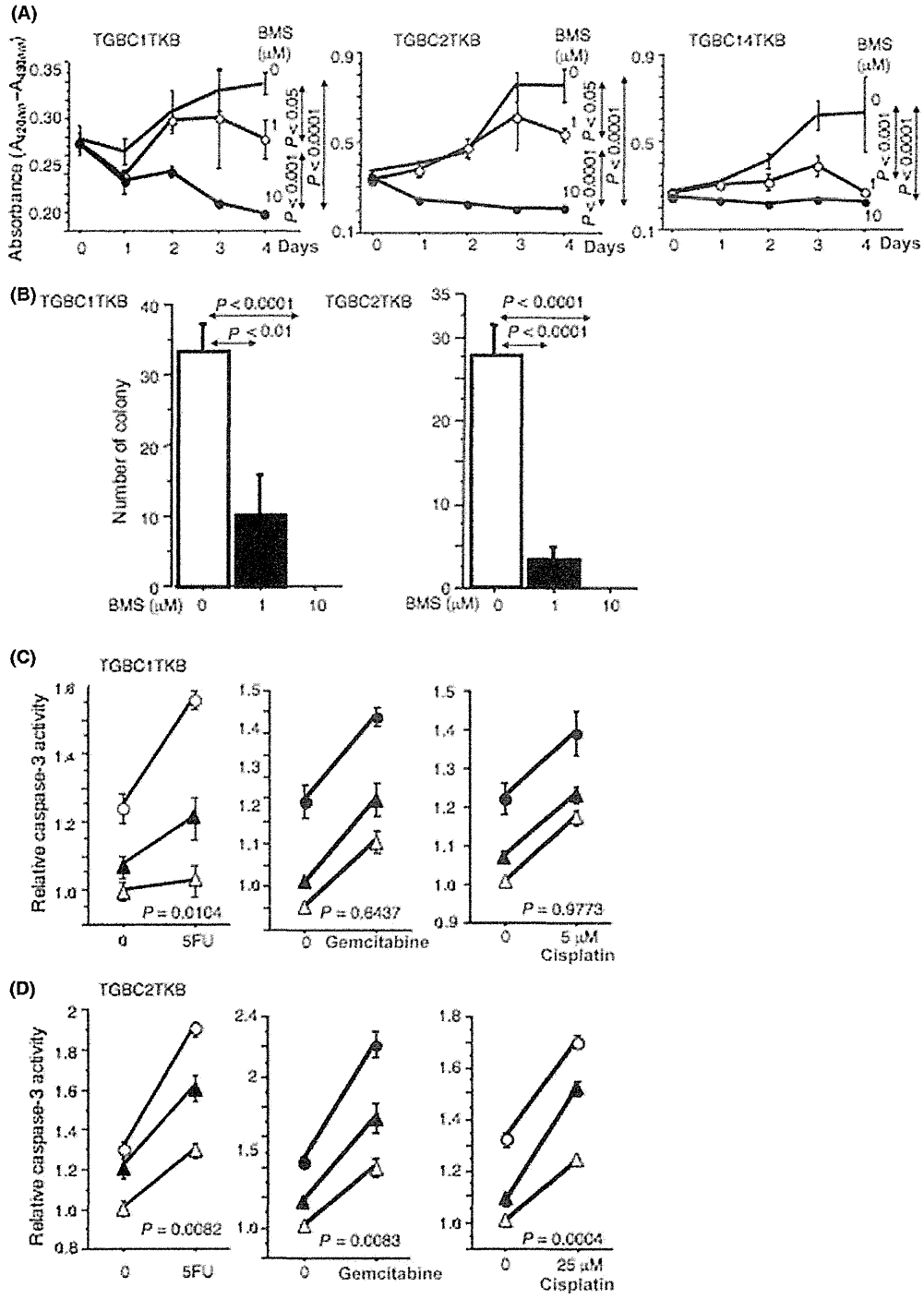

Fig. 3. BMS-536924 reduces *in vitro* growth and colony formation and enhances the effect of chemotherapy of BTC. (A) BMS-536924 reduced growth of three cell lines on plastic with dose dependency, detected by WST-1 assay. Without circle, control; open circle, 1 μM BMS-536924; closed circle, 10 μM BMS-536924. (B) Colony formation assay shows that BMS-536924 reduces *in vitro* tumorigenicity, in a dose-dependent manner. (C, D) In TGBC-1TKB (C) and TGBC-2TKB (D), caspase-3 assay showed that BMS-536924 enhanced chemotherapy-induced apoptosis (analyzed by two-factor factorial ANOVA). △, control; ▲, 1 μM BMS-536924; ○, 5 μM BMS-536924; ●, 10 μM BMS-536924.

p53-positive tumors, suggesting that IGFBP-3 methylation might be more important in p53 wild-type tumors than in p53 mutated tumors, which might have downregulated IGFBP-3 through other mechanisms.

Matrilysin is revealed to play a key role in the development of BTC.[26] Interestingly, IGF-IR and matrilysin were related to each other in all types of BTC. IGFBP activity can be modulated by IGFBP proteases, and there are at least three classes of such

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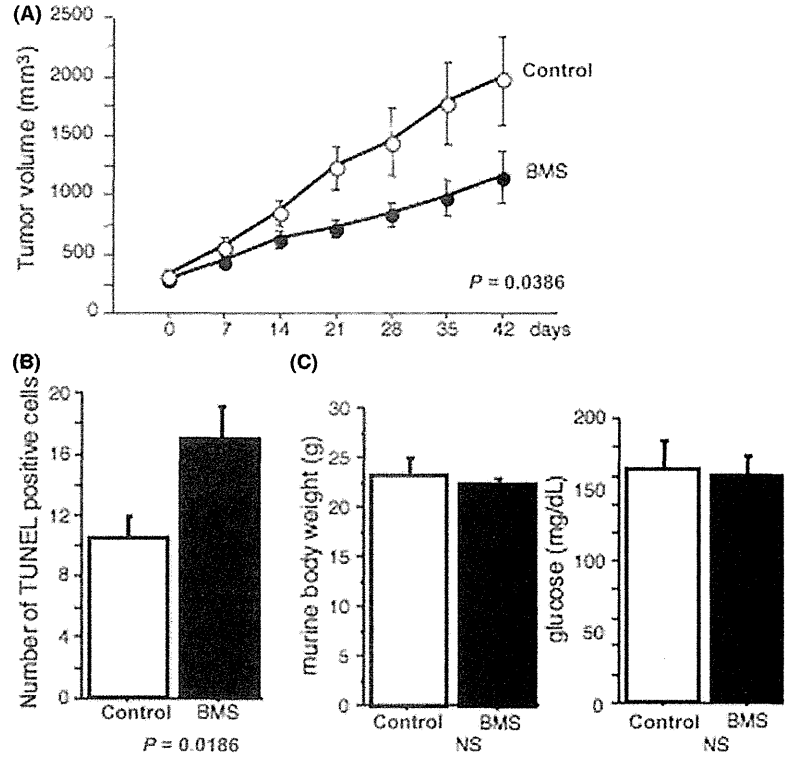

Fig. 4. The effect of BMS-536924 on TGBC-1TKB established subcutaneous tumors in nude mice. (A) 1×10^6 1TKB cells were injected subcutaneously on day −24. After tumors were palpable (day 0), animals were treated orally once daily for 2 weeks, either with BMS-536924 (closed circle) or with control (open circle) ($n = 15$ in each group). (B) TUNEL assay showed that BMS-536924 upregulated apoptosis. (C) There is no difference in either murine weight or serum glucose concentration at death. NS, no significant difference.

Table 3. Effect of dominant negative insulin-like growth factor-I reactor on TGBC1TKB xenografts on nude mice

	Control (mean ± SE)	IGF-IR/dn (mean ± SE)	
Tumor volume (mm^3)	2158.9 ± 369.9	765.8 ± 435.2	$P = 0.0349$
Murine body weight (g)	23.3 ± 0.6	21.7 ± 0.7	NS
Glucose (mg/dL)	164.7 ± 9.9	150.0 ± 23.8	NS

NS, no significant difference.

proteases: cathepsins, kallikreins and MMP.[39] MMP-7 cleaves all IGFBP and thus activates IGF-IR signaling.[39,40] The results of the present study combined with previous research showing an IGF-IR/matrilysin positive feedback loop in gastrointestinal carcinoma,[13] indicate that both molecules might contribute to the progression of BTC.

Here, we used a new IGF-IR-TKI, BMS-536924, for the accurate dissection of the responsible signaling pathways. Even in low concentrations, this agent suppressed colony formation efficiency and enhanced chemotherapy-induced apoptosis *in vitro*. In an *in vitro* study, another IGF-IR-TKI, NVP-AEW541, is also reported to suppress the growth of BTC cell lines;[17] however, its effects were slightly different from our results. Its activity was lower in GBC. NVP-AEW541 dephosphorylated both IGF-IR and Akt, but not ERK. Combined with gemcitabine, NVP-AEW541 exerted synergistic effects, while the combination with 5-FU was only additive.[17] Moreover, we revealed that not only TKI but also IGF-IR/dn effectively suppressed xenograft growth in mice. These results indicate that IGF-IR blockade is a promising treatment for BTC.

A major hurdle to the targeting of IGF-IR is the close homology to InsR.[41] Therefore, it is believed that any strategy designed to block IGF-IR signaling has to have specificity for IGF-IR without significant influence on InsR signaling. In contrast, an important role for InsR in regulating IGF action, as either a hybrid or holoreceptor, has been reported.[42] Moreover, increased insulin sensitivity in breast cancer has been observed by targeting IGF-IR.[43] Agents targeting all of the receptors responsible for IGF signaling might be necessary to disrupt the malignant phenotype regulated by this growth factor receptor family. BMS-536924, a TKI potent against both IGF-IR and InsR,[32] might not only be an advantage but a prerequisite in treating cancers. In this study, although BMS-536924 blocked both IGF-IR and InsR signals, and showed marked antitumor effects both *in vitro* and *in vivo*, it did not affect either body weight or blood glucose concentration.

Therefore, there are two opposing strategies for blockade IGF-IR signaling. One is to avoid adverse effects by shutting down only IGF-IR signaling without influencing InsR signaling. Such selective IGF-IR blockade can be achieved by IGF-IR-mAb or IGF-IR/dn, for prevention of recurrence or maintenance of remission. The other approach is to achieve maximum antitumor effects by blocking both receptors simultaneously, using BMS-536924. This latter approach might be significantly more effective, but potentially also more toxic than the former.

Meanwhile, several mechanisms for resistance to BMS-536924 have been reported. Although IGF and IGF-IR are highly expressed in BMS-536924-sensitive sarcoma cell lines, IGFBP-3 and IGFBP-6 are highly expressed in primary BMS-536924-resistant cell lines.[44] IGFBP are elevated 7–15-fold in cells with acquired resistance to BMS-536924 compared with parental sensitive cells. Overexpression of epidermal growth factor receptor (EGFR) and its ligands in the resistant cells might represent another mechanism for resistence.[44]

Many breast cancer tumors that achieve an initial response to trastuzumab ultimately acquire resistance to it. One mechanism of resistance is overexpression of IGF-IR[45] and another is the

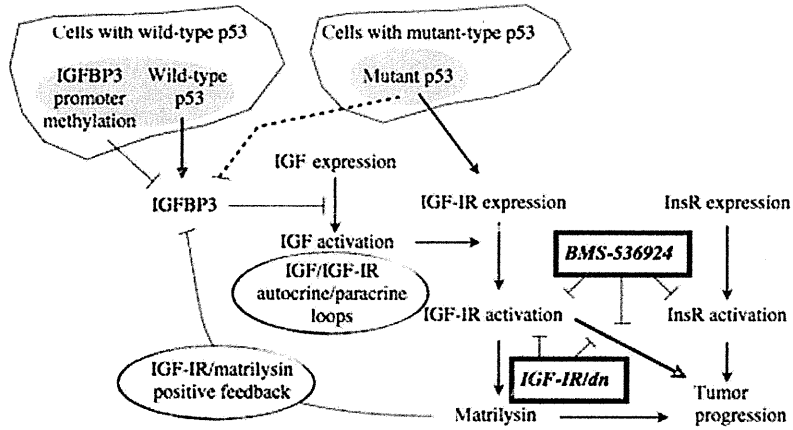

Fig. 5. Insulin-like growth factor (IGF)/IGF-I receptor (IGF-IR) axis in the progression of biliary tract carcinomas. Aberrant activation of IGF-IR is suggested by overexpressions of IGF ligands and receptor in tumor cells, simultaneously (IGF/IGF-IR autocrine or paracrine loops). Moreover, mutant p53 or IGF binding protein (IGFBP)-3 promoter hypermethylation reduced expressions of IGFBP-3, and IGF-IR signals upregulated matrilysin expression, which lysed IGFBP-3 (IGF-IR/matrilysin positive-feedback), which lead much free-form of IGF ligands. Strategies of targeting IGF-IR, BMS-536924 and dominant negative IGF-IR could inhibit tumor progression of biliary tract carcinomas.

formation of IGF-IR/Her2 heterodimers.[46] These data suggested that IGF-IR blockade might be specifically effective for trastuzumab resistant tumors. As four cholangiocarcinoma cell lines are reported to express EGFR, HGFR and IGF-IR,[18] there are several potentially crosstalking signals between the IGF-IR and other receptors. Dual targeting TKI or combination strategies of IGF-IR inhibitors with other targeted therapies might achieve improved patient outcomes.[47]

In this study, we demonstrated that IGF-IR might be a marker of advanced disease and that the IGF/IGF-IR axis might contribute to a particularly aggressive phenotype in BTC (Fig. 5). IGF-IR blockade with BMS-536924 or IGF-IR/dn suppresses tumorigenicity and tumor survival both *in vitro* and in animal models. In addition, BMS-536924 has the advantage of being orally bioavailable. This study thus validates IGF-IR as a therapeutic target in human biliary tract malignancies and suggests that BMS-536924 might be a promising anticancer therapeutic for this disease.

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Disclosure Statement

The authors have no conflict of interest.

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ORIGINAL ARTICLE

Value of serum IgG4 in the diagnosis of IgG4-related disease and in differentiation from rheumatic diseases and other diseases

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Abstract IgG4-related disease (IgG4-RD) is a novel disease entity that includes Mikulicz's disease, autoimmune pancreatitis (AIP), and many other conditions. It is characterized by elevated serum IgG4 levels and abundant IgG4-bearing plasmacyte infiltration of involved organs. We postulated that high levels of serum IgG4 would comprise a useful diagnostic tool, but little information is available about IgG4 in conditions other than IgG4-RD, including rheumatic diseases. Several reports have described cutoff values for serum IgG4 when diagnosing IgG4-RD, but these studies mostly used 135 mg/dL in AIP to differentiate from pancreatic cancer instead of rheumatic and other common diseases. There is no evidence for a cutoff serum IgG4 level of 135 mg/dL for rheumatic diseases and common diseases that are often complicated with rheumatic diseases. The aim of this work was to re-evaluate the usual cutoff serum IgG4 value in AIP (135 mg/dL) that is used to diagnose whole IgG4-RD in the setting of a rheumatic clinic by measuring serum IgG4 levels in IgG4-RD and various disorders. We therefore constructed ROC curves of serum IgG4 levels in 418 patients who attended Sapporo Medical University Hospital due to IgG4-RD and various rheumatic and common disorders. The optimal cut-off value of serum IgG4 for a diagnosis of IgG4-RD was 144 mg/dL, and the sensitivity and specificity were 95.10 and 90.76%, respectively. Levels of serum IgG4 were elevated in IgG4-RD, Churg–Strauss syndrome, multicentric Castleman's disease, eosinophilic disorders, and in some patients with rheumatoid arthritis, systemic sclerosis, chronic hepatitis, and liver cirrhosis. The usual cut-off value of 135 mg/dL in AIP is useful for diagnosing whole IgG4-RD, but high levels of serum IgG4 are sometimes observed in not only IgG4-RD but also other rheumatic and common diseases.

Keywords Autoimmune pancreatitis · Churg–Strauss syndrome · IgG4 · Mikulicz's disease · Rheumatoid arthritis

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Introduction

IgG4-related disease (IgG4-RD) is considered a systemic, chronic, and inflammatory disorder that is characterized by elevated levels of IgG4, abundant infiltration of plasmacytes with IgG4, and fibrosis in involved organs. Allergic

reactions may play a role in the pathogenesis. Mikulicz's disease (MD), autoimmune pancreatitis (AIP), and other conditions are among those associated with IgG4-RD. Serum levels of IgG4 are very useful for diagnosing IgG4-RD; however, though several reports have described a cutoff value, most of them were limited to AIP, and the comparator was often pancreatic cancer [1–5]. Gastroenterologists in Japan have often used a cutoff value of 135 mg/dL [6] to diagnose AIP. However, rheumatologists dealing with IgG4-RD do not know the optimal IgG4 cutoff values for diagnosing various diseases. Except for biliary and pancreatic diseases, no information is available about serum IgG4 levels in other disorders. Accordingly, the purpose of the analysis desribed in this paper was to establish the cutoff value for rheumatic diseases. We measured levels of serum IgG4 in various rheumatic and common diseases that are often complicated with rheumatic diseases, and evaluated whether the usual cutoff value in AIP (135 mg/dL) is useful for diagnosing whole IgG4-RD.

Methods

Samples

We analyzed data from 402 patients who presented at Sapporo Medical University Hospital, Teine Keijinkai Hospital, and JR Sapporo Hospital (126 men, 276 women) between October 2010 and March 2011, and from 16 patients with multicentric Castleman's disease (MCD) obtained from Nishimoto et al. (Wakayama Medical University). The patients with IgG4-RD comprised MD ($n = 66$), Küttner's tumor (chronic sclerosing sialadenitis) ($n = 17$), IgG4-related dacryoadenitis ($n = 11$), and AIP ($n = 8$).

We compared IgG4 values with those of patients with systemic lupus erythematosus ($n = 18$), antiphospholipid antibody syndrome ($n = 5$), rheumatoid arthritis (RA; $n = 29$), systemic sclerosis (SSc; $n = 40$), polymyositis and dermatomyositis ($n = 6$), mixed connective tissue disease ($n = 5$), Sjögren's syndrome ($n = 84$), Behçet's disease ($n = 9$), microscopic polyangiitis ($n = 5$), and Churg–Strauss syndrome (CSS; n = 5). In addition, we also analyzed IgG4 values in patients with the following non-rheumatic diseases: bronchial asthma ($n = 7$), idiopathic pulmonary fibrosis ($n = 6$), chronic hepatitis ($n = 21$), liver cirrhosis ($n = 22$), MCD ($n = 16$), urticaria and atopic dermatitis ($n = 2$ each) and eosinophilic fasciitis, eosinophilic pneumonia, and food-dependent exercise-induced anaphylaxis ($n = 1$ each). We also included 21 healthy individuals and patients with multiple myeloma ($n = 2$), lymphoma ($n = 3$), gastric cancer ($n = 3$), colorectal cancer ($n = 1$), and prostate cancer ($n = 2$).

Both AIP and IgG4-RD were diagnosed if the Japan [6] and the Mayo Clinic HISORt criteria [7] were satisfied, and if supported by physical, pathological, and radiological findings. Rheumatic diseases were diagnosed according to the ACR (American College of Rheumatology), EULAR (The European League Against Rheumatism), and international criteria [8–17]. Respirologists and hepatologists diagnosed bronchial asthma, idiopathic pulmonary fibrosis, chronic hepatitis, and liver cirrhosis by computed tomography, pulmonary function tests, and biopsy. Hematological and pathological specialists diagnosed MCD.

Measurement of serum IgG and IgG4

All samples were collected before the treatments. Serum IgG and IgG4 levels were measured with a Behring nephelometer (Dade Behring, Deerfield, IL, USA) using following antibodies; BS-NIA IgG1-4 (The Binding Site, Birmingham, UK), and serum IgG4/IgG ratios were calculated and values were averaged for each disorder. We then constructed ROC curves to identify the optimal serum IgG4 cutoff value for diagnosing IgG4-RD. The frequency of patients with elevated serum IgG4 levels and their clinical characteristics were analyzed.

Written consent to use the information from these cases was obtained from the patients in accordance with the Declaration of Helsinki. This study proceeded under the approval of our institutional IRB (SMU 22-57).

Results

Levels of serum IgG, IgG4, and the IgG4/IgG ratio in IgG4-RD and other diseases

Table 1 shows the average serum IgG concentration in each disease. Hypergammaglobulinemia was evident in all of the rheumatic diseases that we analyzed, and the trend was similar in samples from idiopathic pulmonary fibrosis, chronic hepatitis, cirrhosis, and MCD. The serum IgG value was higher in IgG4-RD, as reported. The average value of serum IgG4 in the patients with IgG4-RD was higher, as predicted. Levels of serum IgG4 were elevated only in CSS among the rheumatic diseases, and in MCD (Fig. 1; Table 1), eosinophilic fasciitis, and eosinophilic pneumonia among the non-rheumatic disorders. The serum IgG4/IgG ratio was >7% in CSS, MCD, and IgG4-RD (Fig. 2), and it was also higher in eosinophilic fasciitis and eosinophilic pneumonia (data not shown). In contrast, these values were not elevated in healthy controls and in patients with malignancies.

Table 1 Serum IgG and IgG4 levels and IgG4/IgG ratios

	IgG (±SD) (mg/dL)	IgG4 (±SD) (mg/dL)	IgG4/IgG (±SD) (%)
SLE (n = 18)	1842 ± 418	44 ± 36	2.7 ± 2.3
APS (n = 5)	1839 ± 245	84 ± 82	4.5 ± 4.3
RA (n = 29)	1770 ± 373	93 ± 68	5.2 ± 3.3
SSc (n = 40)	1398 ± 257	42 ± 63	3.1 ± 4.7
PMDM (n = 6)	1478 ± 330	48 ± 61	3.5 ± 4.6
MCTD (n = 5)	2005 ± 707	21 ± 20	1.2 ± 1.3
SS (n = 84)	1705 ± 542	38 ± 65	2.2 ± 2.4
BD (n = 9)	1257 ± 196	26 ± 23	2.3 ± 2.4
MPA (n = 5)	1950 ± 616	44 ± 54	2.4 ± 3.2
CSS (n = 5)	2013 ± 450	494 ± 328	23.4 ± 15.2
BA (n = 7)	1687 ± 433	67 ± 49	4.3 ± 2.8
IPF (n = 6)	1823 ± 244	50 ± 35	2.7 ± 1.7
CH (n = 21)	1830 ± 440	57 ± 48	3.1 ± 2.4
LC (n = 22)	2027 ± 666	72 ± 73	3.2 ± 2.4
MCD (n = 16)	2583 ± 1706	213 ± 234	7.3 ± 4.9
HC (n = 21)	1393 ± 262	43 ± 31	2.9 ± 1.8
MD (n = 66)	2479 ± 1478	814 ± 682	25.3 ± 9.9
KT (n = 17)	2188 ± 754	698 ± 625	22.1 ± 10.2
DA (n = 11)	1747 ± 700	391 ± 407	16.4 ± 9.7
AIP (n = 8)	1975 ± 832	398 ± 259	17.8 ± 8.5
G4RD (n = 102)	2312 ± 1287	716 ± 639	23.2 ± 10.2

SLE Systemic lupus erythematosus, *APS* anti-phospholipid syndrome, *RA* rheumatoid arthritis, *SSc* systemic sclerosis, *PMDM* polymyositis/dermatomyositis, *MCTD* mixed connective tissue disease, *SS* Sjögren's syndrome, *BD* Behçet's disease, *MPA* microscopic polyangiitis, *CSS* Churg–Strauss syndrome, *BA* bronchial asthma, *IPF* idiopathic pulmonary fibrosis, *CH* chronic hepatitis, *LC* liver cirrhosis, *MCD* multicentric Castleman's disease, *HC* healthy control, *MD* Mikulicz's disease, *KT* Küttner's tumor, *DA* IgG4-related dacryoadenitis, *AIP* autoimmune pancreatitis, *G4RD* IgG4-related disease

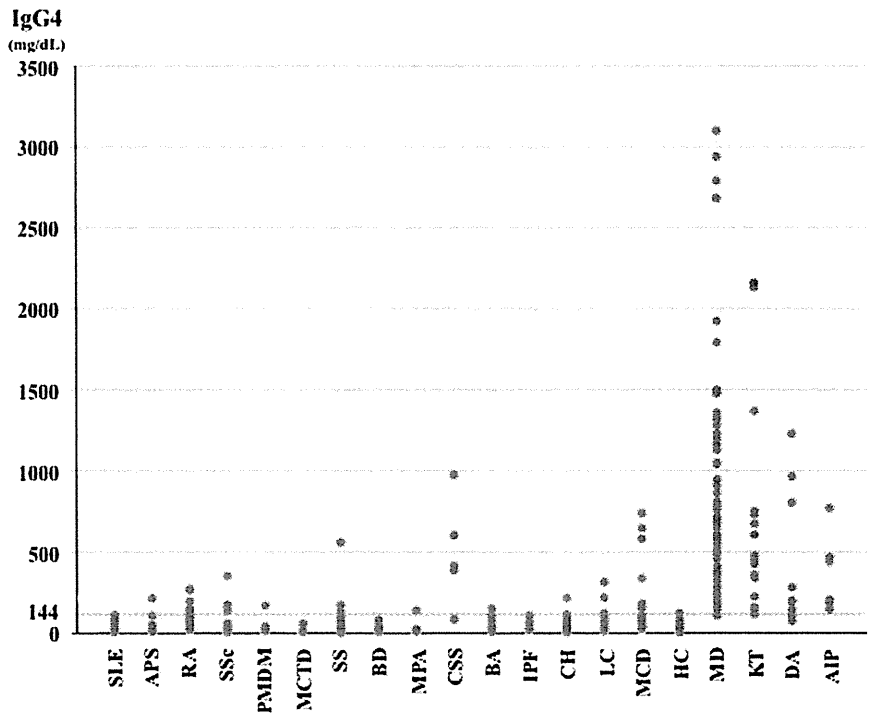

Fig. 1 Levels of serum IgG4 in each disease. Some patients presented with elevated levels of serum IgG4 in RA, SSc, CSS, hepatic disorders, and MCD. The serological tests showed this phenomenon in almost all patients with IgG4-RD

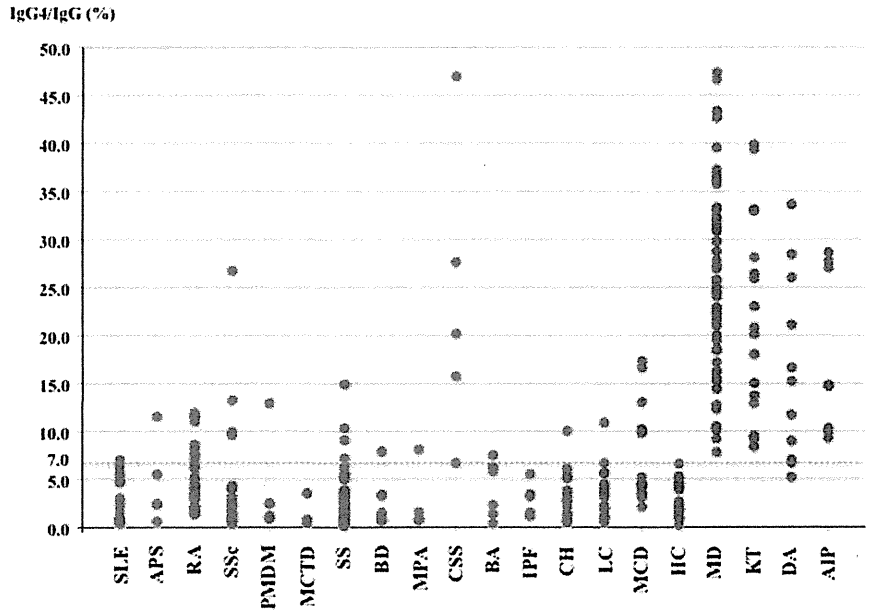

Fig. 2 Serum IgG4/IgG ratio in each disease. Some patients presented with elevated levels of serum IgG4 in RA, SSc, SS, CSS, and MCD. The ratio was found to be higher in patients with IgG4-RD

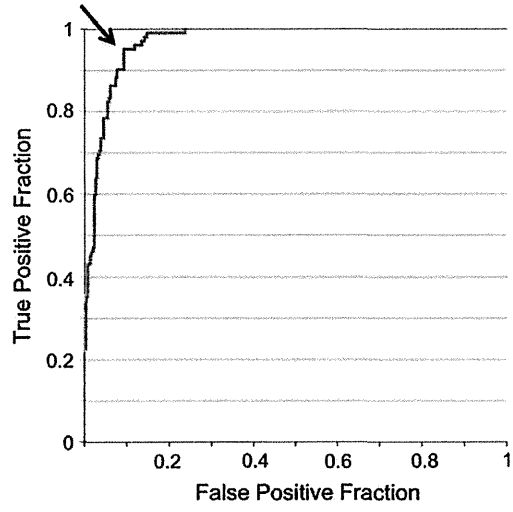

Fig. 3 ROC curves for diagnosing IgG4-related diseases. Optimal cutoff, 144 mg/dL; sensitivity and specificity, 95.10 and 90.76%, respectively. Area under the curve (AUC) was 0.970

ROC curves and optimal cutoff value of serum IgG4 for diagnosing IgG4-RD

We determined from ROC curves that the optimal cutoff value of serum IgG4 for diagnosing IgG4-RD was 144 mg/dL, and that the sensitivity and the specificity were 95.10 and 90.76%, respectively. The area under the curve (AUC) was 0.970 (Fig. 3).

95.1% of the patients with IgG4-RD had >144 mg/dL of IgG4. We found that the average values of serum IgG4 among patients with IgG4-RD were higher in cases of MD and Küttner's tumor than in cases of dacryoadenitis and AIP. In contrast, the ratio of healthy individuals with elevated serum IgG4 was not high, whereas serum IgG4 was clearly higher in four patients (80%) with CSS, in five patients (17.2%) with RA, and in three patients (7.5%) with SSc. Among the non-rheumatic diseases, serum IgG4 levels were elevated in bronchial asthma (one patient, 14.3%), chronic hepatitis (one patient, 4.8%), and liver cirrhosis (two patients, 9.1%). In addition, seven patients (43.8%) with MCD also had elevated serum IgG4 levels (Table 2).

Characteristics of the patients who presented with elevated levels of serum IgG4

Aside from IgG4-RD, the disorders that appeared to lead to elevated levels of serum IgG4 were RA, SSc, CSS, eosinophilic fasciitis, and eosinophilic pneumonia. It was clear that elevated concentrations of serum IgG4 were often present in eosinophilic disorders. On the other hand, we focused on RA and SSc, and found that there were not very many patients with atopic factors. Eosinophils in RA with elevated IgG4 were 126.8 ± 155.6 (SD)/μL, and levels of serum IgE were 47.2 ± 30.3 IU/mL; neither were increased. Among the patients with rheumatic disorders who had elevated serum IgG4, disease activity at onset in RA was very severe [disease activity score (DAS)28 [18], 5.10 ± 1.15 (SD)], and all had anti-citrullinated peptide antibodies. Sixty percent of them are currently receiving a tumor necrosis factor (TNF) blocker (infliximab or etanercept). Others progressed to organizing pneumonia during the course, and were treated with a high dose of glucocorticoid. Eosinophils in SSc with elevated IgG4

Table 2 Frequency of patients with elevated serum IgG4 levels

	Patients of elevated IgG4 (%)
SLE ($n = 18$)	0.0
APS ($n = 5$)	20.0
RA ($n = 29$)	17.2
SSc ($n = 40$)	7.5
PMDM ($n = 6$)	16.7
MCTD ($n = 5$)	0.0
SS ($n = 84$)	2.4
BD ($n = 9$)	0.0
MPA ($n = 5$)	20.0
CSS ($n = 5$)	80.0
BA ($n = 7$)	14.3
IPF ($n = 6$)	0.0
CH ($n = 21$)	4.8
LC ($n = 22$)	9.1
MCD ($n = 16$)	43.8
HC ($n = 21$)	0.0
MD ($n = 66$)	98.5
KT ($n = 17$)	94.1
DA ($n = 11$)	72.7
AIP ($n = 8$)	100.0
G4RD ($n = 102$)	95.1

were 32.9 ± 57.0/μL, and serum IgE levels were 204.3 ± 163.3 IU/mL. Both of these values were within the normal range. The modified Rodnan total skin score (TSS) [19] at first visit was 14.7 ± 9.0 points, and it increased to 15.3 ± 12.1 points after a year despite the prescription of glucocorticoids. Two of the three patients with SSc and elevated IgG4 had anti-RNA polymerase III antibodies. One of them had pulmonary fibrosis, but there was no cardiac or renal dysfunction.

Discussion

The systemic, chronic, and inflammatory disorder IgG4-RD is characterized by elevated levels of IgG4, abundant infiltration of plasmacytes with IgG4, and fibrosis of involved organs, and it comprises mainly AIP [20] and MD [21]. It is important to be able to differentiate IgG4-RD from cancer because IgG4-RD presents with mass-forming lesions. It is also important to distinguish IgG4-RD from rheumatic diseases because IgG4-RD can present with hypergammaglobulinemia, hypocomplementemia, elevated circulating immune complexes, and positive rheumatoid factor. Now that the concept of IgG4-RD has been established, a precise definition of elevated serum IgG4 levels is required.

Among the four subclasses of IgG, the half-life of IgG4 in serum is 23 days, and it can pass through the placenta. Complement binding is unusual. Because the Fc portion of IgG4 binds to mast cells, it is believed to be involved in allergic reactions. The production of IgG4 does not depend on the release of chemical mediators. Some investigators have suggested that IgG4 competes with IgE and prevents the release of chemical mediators. IgG4 can pass through the placenta. The serum IgG4 concentration in neonates immediately after birth decreases for 3 months due to diminished production. The concentrations of serum IgG4 then increase to 60% of the adult value at the age of 1 year, and then reach the adult value 5 years later. The IgG1 and IgG3 subclasses increase rapidly, whereas IgG4 increases gradually throughout infancy, which results in temporarily lower serum IgG4/IgG ratios [22–24]. Several reports have described mean levels of serum IgG4 in healthy adults [22, 24–33] after the first description was published in 1970 [25]. It is known that the serum IgG4 concentration is lowest in the IgG subclasses. The average values of serum IgG4 in these reports range from 30 to 60 mg/dL, and the value of 43 mg/dL in the present study agrees with these findings.

However, serum IgG4 concentrations in various disorders have not been analyzed in detail. The cutoff values of serum IgG4 that can differentiate AIP from pancreatic cancer range from 130 to 141 mg/dL [1–5] (135 mg/dL in Japan [6]), since a relationship between AIP and IgG4 has been established [20]. These values are higher than those of healthy volunteers. These can only be useful to gastroenterologists and pancreatologists, because they are established values in biliary and pancreatic disorders. Patients with systemic and chronic inflammatory IgG4-RD present in a variety of clinical settings, so it is important to know the serum IgG4 levels that are associated with other diseases. We published primitive data on rheumatic diseases in 2004 [34], when we identified elevated IgG4 levels only in MD. However, we later found that levels are elevated in CSS [35] and in some patients with MCD [36]. Therefore, it became obvious that rheumatologists should understand serum IgG4 levels in various rheumatic disorders and common diseases: a notion that prompted this study. Thus, we analyzed serum IgG4 in rheumatic and common diseases establish the usefulness of measuring serum IgG4 in rheumatology, and re-evaluated the cutoff value used in AIP (135 mg/dL).

We found that the average values of serum IgG4 among IgG4-RD were higher in MD and Küttner's tumor than in dacryoadenitis and AIP. There was a significant difference between MD and dacryoadenitis ($p < 0.05$). In other words, serum IgG4 levels tended to be significantly elevated in patients with IgG4-RD who had salivary lesions. At this time, it is difficult to determine which organ

involvement led to the elevation of serum IgG4 because there were cases with many complications, but there was a lack of cases with complications to analyze. Among the rheumatic disorders, IgG4 levels were elevated only in CSS. We discovered elevated serum IgG4 in several individuals with RA and SSc. There was a case report of arthropathy with IgG4-RD [37], but the RA samples for this study met the international classification criteria. We did not have to consider the possibility that our cases with RA were also complicated with IgG4-RD. Most of our RA patients had high disease activity, and biological agents were prescribed during the course. There are some reports of the relationship between RA and IgG4. IgG1 and IgG4 are the predominant subclasses among anti-citrullinated peptide antibodies (ACPA) in RA. The meaning of this phenomenon is still unknown, but it is suggested that the predominance of IgG4 indirectly obstructs the elimination of autoreactive B cells through the inhibitory Fc receptor (FcγRIIB) [38]. The values of IgG4-ACPA in RA were significantly decreased after treatment with TNF blockade [39]. Thus, it is possible that the decline in serum IgG4 in RA is due to disease remission [40]. We continued to analyze the serial changes in IgG4 after therapy in the RA cases. The meaning of the elevated levels of serum IgG4 accompanied by anti-RNA polymerase III antibodies in two of the three patients with SSc remains obscure. Rapid and progressive skin sclerosis is a feature of SSc with this antibody [41]. Okazaki [42] explained that the elevated IgG4 and fibrosis are regulated by interleukin (IL)-10 or transforming growth factor (TGF)-β in IgG4-RD. It is suggested that IL-10 and TGF-β from regulatory T cells led to the switch in class to IgG4 [43, 44]. It has been pointed out that regulatory T cell expansion in peripheral blood in cases with SSc [45] and serum levels of IL-10 correlate with skin fibrosis, such as the total skin thickness score [46]. It has also been reported that elevated levels of TGF-β reflect the severity of chronic hepatitis and liver cirrhosis [47, 48], and that IL-10 gene polymorphisms are associated with the progression of liver fibrosis [49] as well as SSc. Progressive fibrosis in SSc might be associated with IgG4 through these cytokines. On the other hand, serum IgG4 levels were elevated in patients with severe asthma. Bronchial asthma is an early symptom of CSS. Whether asthma with elevated IgG4 leads to CSS remains unknown. Concentrations of serum IgG4 were also elevated in eosinophilic disorders, although we analyzed only a few patients. To correct the cytokine balance that deviate toward Th1-type usual inflammation or Th2-type allergic inflammation, inhibitory cytokines are produced through regulatory T cells, which leads to elevated levels of serum IgG4. An anti-inflammatory process may be seen. It is possible that IgG4 plays a role as an inhibitory humoral factor.

The present findings indicated that high serum IgG4 concentrations are not specific to IgG4-RD. Indeed, we do not diagnose IgG4-RD based only on serum IgG4 values, but high values are problematic because an appropriate cutoff value for serum IgG4 has not been established. The optimal cutoff value in the present study was 144 mg/dL when compared with rheumatic, allergic, and common diseases. This value was determined at >90% sensitivity and specificity, and together with the AUC it was useful for diagnosis. When we analyzed the sensitivity and specificity at 135 mg/dL using same population, the sensitivity and the specificity were 96.08 and 89.87%, respectively. There were no significant differences between the sensitivity and the specificity values obtained with a cutoff value of 144 mg/dL and those obtained with a cutoff of 135 mg/dL. Therefore, in the daily clinic, the use of the usual cutoff value of 135 mg/dL in AIP does not cause any problems, and it is also useful for diagnosing any IgG4-RD.

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Conflict of interest None.

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ORIGINAL ARTICLE

Risk of malignancies in IgG4-related disease

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Abstract IgG4-related disease (IgG4-RD) is considered a systemic, chronic, and inflammatory disorder that is characterized by the enlargement of involved organs, elevated levels of IgG4, and abundant infiltration of plasmacytes with IgG4 and fibrosis in involved organs. It is necessary to differentiate IgG4-RD from malignant tumors. Recently we have looked at case reports of IgG4-RD with malignancy that was discovered at systemic screening. In this study, we analyzed the relationship between IgG4-RD and malignancies. The study subjects were 106 patients with IgG4-RD who had been referred to our hospital since April 1997. We analyzed the clinical characteristics of IgG4-RD patients who had cancer that was observed upon the initial diagnosis of IgG4-RD or that occurred during an average follow-up period of 3.1 years. Using data from national cancer registries that monitor cancer incidence in Japan, we evaluated the standardized incidence ratio (SIR) for malignancies in IgG4-RD. Malignancies were observed in 11 of the IgG4-RD patients (10.4%). The malignancies were all different and included lung cancer, colon cancer, and lymphoma. With the exception of the age at which the IgG4-RD diagnosis was made, there were no common features in patients with cancer and those without. The SIR for these malignancies in IgG4-RD was 383.0, which was higher than that for the general population. We should be cognizant of the possible existence of malignancies in patients with IgG4-RD at the time of diagnosis and during follow-up care.

Keywords Cancer · IgG4 · Lymphoma

Introduction

IgG4-related disease (IgG4-RD) is characterized by enlargement of the affected organs along with elevated levels of serum IgG4, and abundant infiltration of IgG4-bearing plasma cells and fibrosis. IgG4-RD is a new concept of systemic and chronic inflammation [1], and usually includes Mikulicz's disease (MD) and autoimmune pancreatitis (AIP) [2, 3]. IgG4-RD is an important disease for rheumatologists, as it often presents as positive for rheumatoid factor, and patients have hypocomplementemia and elevated levels of circulating immune complexes [4].

Previously, we have often diagnosed IgG4-RD simply by the elevation of serum IgG4, but it has since been shown that differential diagnosis is important so as to exclude the possibility of malignant tumors. IgG4-RD with pancreatic cancer has often been reported in patients with AIP [5, 6]. We extracted IgG4-RD cases complicated by malignancies at diagnosis or during the follow-up of patients who were registered in the SMART database (Sapporo Medical University and Related institutes database for investigation and best Treatments of IgG4-RD), and analyzed their clinical characteristics. We examined whether the cancer incidence in these patients was higher than that in the general population.

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Patients, materials, and methods

The subjects were 106 patients with IgG4-RD, who had visited Sapporo Medical University Hospital beginning in April 1997 and were registered in the SMART database. The patients comprised 50 males and 56 females, with a mean age of 59.02 years at the onset of IgG4-RD, and 60.85 years at the time of diagnosis. Of the 106 patients, 67 were diagnosed with MD, 17 with Küttner's tumor (chronic sclerosing sialadenitis), 12 with IgG4-related dacryoadenitis, and 10 with AIP.

These patients satisfied the tentative criteria for IgG4-RD that consisted of: (1) enlargement of the affected organs; (2) elevated levels of serum IgG4 (>1.35 g/L); and (3) abundant infiltration of IgG4-positive plasmacytes and fibrosis. We separated the patients with diagnoses of IgG4-RD complicated by malignancies and those who developed complications during follow-up (average 3.1 years, maximum 14.3 years) and analyzed their clinical features. We calculated the standardized incidence ratio (SIR) for malignant neoplasms in IgG4-RD using the aforementioned database employed in the monitoring of cancer incidence in Japan [7].

Results

Two patients with malignant neoplasms

Case 1

A 30-year-old woman noticed a mass in her left breast in the winter of 1999. A nipple-areola-sparing mastectomy was performed and revealed a pathologic, inflammatory pseudotumor. Later that same year, the patient was admitted to our hospital; a biopsy taken from her right eyelid disclosed mucosa-associated lymphoid tissue (MALT) lymphoma of the lacrimal gland. Southern blotting IgH gene rearrangement was detected. There were additional complications due to mediastinal lymphoma lesions, and radiation therapy and chemotherapy were administered. The patient was then in remission until 2008, when the enlargement of bilateral submandibular glands was observed. The serological data showed no antinuclear antibody or anti-SS-A antibody. She did not suffer from sicca symptoms. The Saxon's test result was 3.55 g/2 min and the Schirmer's test results were 12 mm/5 min for the right eye, and 14 mm/5 min for the left eye. We noticed an elevation of serum IgG4 (13.50 g/L) and performed a submandibular gland biopsy, which revealed prominent infiltration of IgG4-bearing plasmacytes and fibrosis. There was no IgH rearrangement in the submandibular gland. We retrospectively analyzed the breast and lacrimal gland specimens taken in 1999; both showed remarkably high IgG4-positive plasma cell infiltration. The patient was diagnosed with MALT lymphoma, which subsequently led to MD.

Case 2

A 77-year-old woman became aware of bilateral submandibular enlargements in June 2009. Otolaryngologists suspected Sjögren's syndrome (SS), and the patient was admitted to our hospital. There was no evidence of sicca syndrome. Serological analysis revealed negativity for anti-SS-A antibody, but did show elevated levels of serum IgG4 (4.48 g/L). The submandibular gland biopsy revealed prominent infiltration of plasmacytes with IgG4. IgH rearrangement was not found. We diagnosed Küttner's tumor (chronic sclerosing sialadenitis). Positron emission tomography (PET) revealed no other accumulation of FDG except in the submandibular glands. Administration of glucocorticoid led to remission; however, 1 year after the glucocorticoid was begun, a small ulcer appeared at the right edge of her tongue. The patient underwent oral surgery, and a biopsy of the ulcer disclosed squamous cell carcinoma.

The SMART database registry, which included our 106 patients, showed 11 patients (10.4%) with IgG4-RD with either a concurrent diagnosis of malignancy, or the development of malignancies during follow-up. These patients consisted of three with MD (n = 69, 4.5%), five with Küttner's tumor (chronic sclerosing sialadenitis) (n = 17, 29.4%), one with IgG4-related dacryoadenitis (n = 12, 8.3%), and two with AIP (n = 10, 20.0%). Breast, colorectal, lung, ovarian, lingual, renal, prostate cancer, and hematological malignancies such as malignant lymphoma were apparent (Table 1).

Table 1 Profile of patients with IgG4-related disease complicated by malignancy

IgG4-related disease (n = x)	No. of Pts with Ca.	Malignancy and frequency
Mikulicz's disease (n = 69)	3	4.5% breast, ovary, MALT
Küttner's tumor (n = 17)	5	29.4% colon (2), lung, lingual, NHL
IgG4-related dacryoadenitis (n = 12)	1	8.3% lung
Autoimmune pancreatitis (n = 10)	2	20.0% renal, prostate
Total (n = 106)	11	10.40%

No. number, *Pts* patients, *Ca.* cancer, *MALT* mucosa-associated lymphoid tissue lymphoma, *NHL* non-Hodgkin's lymphoma

Temporal relationship between diagnosis of IgG4-RD and malignancies

A simultaneous diagnosis of IgG4-RD and malignancies was confirmed in 2 of the 11 patients. It was a tendency that the discovery of malignancies was often observed in the first 2 years of follow-up. There were four patients in whom IgG4-RD had developed first (of whom two showed malignant lymphoma) and five patients in whom malignancies had developed first.

Clinical characteristics of the patients with malignancies

Comparing the malignancy and non-malignancy groups of IgG4-RD patients, the average ages at IgG4-RD onset were 67.64 and 58.02 years, respectively, and the mean ages at IgG4-RD diagnosis were 70.27 and 59.76 years, respectively. In the malignancy group, age at onset and age at diagnosis were both significantly higher than these ages in the group without malignancies (both $P < 0.05$). The sex ratio was 6:5 in favor of males in the malignancy group, and 44:51 in favor of females in the non-malignancy group. There was no significant difference in sex ratios between the groups. Serological data showed that IgG and IgG4 levels were 22.11 and 5.82 g/L, respectively, in the malignancy group, and 23.61 and 7.55 g/L, respectively, in the non-malignancy group. There was a tendency towards higher serum IgG and IgG4 concentrations in the non-malignancy group, but this was not statistically significant. As for levels of complement and circulating immune complexes, the malignancy group presented with levels of 42.4 U/mL and 4.4 μg/mL, respectively, and the non-malignancy group presented with levels of 39.8 U/mL and 7.3 μg/mL, respectively. The levels of rheumatoid factor were 16.6 U/mL in the malignancy group and 35.2 U/mL in the non-malignancy group. There were no significant differences in these data between the two groups.

Standardized incidence ratio for cancers in IgG4-related disease

We evaluated the SIR for cancers in IgG4-RD, using the database of national cancer registries in the monitoring of cancer incidence in Japan (2005) [7]. We found from this registry that the SIR for malignancies in male patients with IgG4-RD was 331.1, and that in female patients with IgG4-RD was 471.6. The total was 383.0, which was very high (Table 2).

Discussion

IgG4-RD is a new disease concept defined as a systemic, chronic, and inflammatory disorder. Differentiation from malignant tumors in this disease is clinically very important, as it is characterized by enlargement of the involved

Table 2 Standardized incidence ratio for malignancies in IgG4-related disease

Standard population					IgG4-related disease				
Population aged more than 20 years in Japan, 2005					SMART, 2011				
Sex	Age (years)	Population	No. of Pts with Ca.	Incidence of Ca.[a]	No. of Pts with IgG4-RD	Expected No./1 year	Expected No./3.1 years	Observed No. (average 3.1 years)	Standardized incidence ratio[b]
Male	20–39	17,289,425	5,902	34.136	2	0.000683	0.002117	0	
	40–59	17,393,579	62,474	359.179	21	0.075427	0.233824	2	
	60–79	12,995,595	238,028	1,831.605	26	0.476217	1.476273	3	
	80–	2,033,533	65,458	3,218.930	1	0.032189	0.099786	1	
Male					50	0.584517	1.812003	6	331.13
Female	20–39	16,831,860	15,303	90.917	6	0.005455	0.016911	1	
	40–59	17,464,541	68,770	393.769	22	0.086629	0.268550	0	
	60–79	14,881,942	125,854	845.683	26	0.219877	0.681619	3	
	80–	4,305,564	64,687	1,502.405	2	0.030048	0.093149	1	
Female					56	0.342010	1.060231	5	471.60
Total		103,196,039	646,476	8,276.624	106	0.926527	2.872234	11	382.98

No. number, *Pts* patients, *Ca.* cancer, *IgG4-RD* IgG4-related disease, *SMART* Sapporo Medical University and Related institutes database for investigation and best Treatments of IgG4-RD

[a] Per 100,000 people

[b] The value for the standard population per year is regarded as 100

organs. Several IgG4-RD patients with pancreatic cancer have been reported over the past few years [5, 6, 8, 9].

It is known that PET is very useful for systemic evaluation in IgG4-RD [10, 11]. Whenever we make a diagnosis of IgG4-RD, we consult PET images. In some instances, we are presented with cases involving abnormal accumulation of FDG at lesion sites outside the involved organs; this is known as organic dysfunction of IgG4-RD. Upon further examination, we often find that the lesion is cancer. In such instances, PET is very useful for detecting cancer [12].

The present study demonstrated that malignancies occurred in 10.4% of IgG4-RD patients, approximately 3.5 times higher than the incidence of cancer in the general population. These results suggest that when diagnosing IgG4-RD, it is necessary not only to discriminate between the enlarged organs and cancers but also to consider the possibility of cancer complications in other parts of the body.

Malignancies as complications to IgG4-RD are categorized as lymphoma and non-lymphoid tumors. Lymphoma includes MALT lymphoma and non-Hodgkin lymphoma. Lymphoma can present as a background of chronic inflammation.

In SS, antigenic activation of B cells, together with oncogenic events, including p53 inactivation and bcl-2 activation, may play important roles in B-cell monoclonal proliferation and malignant transformation [13]. Zhang et al. reported that 2.2% of patients with primary SS developed malignancies during 4.4 years' follow-up [14]. The SIR for malignancies in SS was reported to be 1.5–3.3 [14, 15]. The SIR for lymphoma was higher, at 8.7–48.1 [14–16], but it was lower than that in IgG4-RD. The SIR for malignant lymphoma in SS showed an 18.8-fold risk [17], and that in IgG4-RD showed a 16.0-fold risk [18].

In our Case 1, it was difficult to interpret the temporal relationship between the onset of IgG4-RD and lymphoma, as the patient was retrospectively diagnosed with IgG4-RD; however, we assumed that the IgG4-RD was present before diagnosing lymphoma from the findings of the breast and lacrimal gland specimens. The SMART database revealed malignancies in two of the four patients in whom IgG4-RD progressed into lymphoma. Thus, it is suggested that lymphoma could occur in IgG4-RD as well as occurring in SS.

Various carcinomas were observed in the non-lymphoid tumors in our study. Cancers might be commonly associated in IgG4-RD patients. Case 2 was an example of the progression from IgG4-RD to cancer. Recently, it has been demonstrated that helper 2 and regulatory T cells play a role in the pathogenesis of IgG4-RD [19]. It is thus possible that regulatory T cells suppress not only the usual inflammation but also tumor immunity [20].

Considering the relationship between cancers and IgG4, it is known that in pancreatic cancer, but not AIP, there is an infiltration of IgG4-bearing plasmacytes in the normal pancreatic tissues surrounding cancers. Some patients with pancreatic cancer show elevated levels of serum IgG4 [21]. These phenomena do not apply only to pancreatic cancer. We found that pathological and serological phenomena associated with IgG4 in hepatocellular carcinoma resulted from chronic hepatitis and cirrhosis, scirrhous-type gastric cancer, and colorectal carcinoma (data not shown). Thus, we must avoid misdiagnosing a case that presents only with malignant neoplasms as IgG4-RD. We should recognize that IgG4 plays roles other than those in the pathogenesis of IgG4-RD and can be involved in tumor immunity. In the present analysis, we did not check whether the patients with malignancies had any relevant hereditary predisposition or environmental exposure factors, such as smoking for lung cancers, or human papillomavirus infection for oral cancers. We will have to analyze the relationships between IgG4-RD and malignancies by taking these factors into account.

In conclusion, malignancies are possible complications in patients with IgG4-RD. In this study, the complication rate of malignant neoplasms in patients with IgG4-RD was 10.4% and the SIR for malignancies in IgG4-RD was 383.0, which is substantially higher than that for the general population. As rheumatologists, we should consider the possibility that malignancies can be complications at the diagnosis or subsequent follow-up of IgG4-RD patients.

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Conflict of interest None.

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LETTER

Adult-onset Still's disease in pregnancy

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We report a case of adult-onset Still's disease (AOSD) in a pregnant patient that was successfully treated using intravenous methylprednisolone, cyclosporine, and plasma exchange, allowing safe delivery. Pregnancy appears to be one risk factor for AOSD, and we summarize the relationship between AOSD and pregnancy.

A 28-year-old Japanese woman, in the 21st week of her first pregnancy, had suffered from low-grade fever and sore throat for 2 weeks. She was referred to us for a high spiking fever, cervical lymph node enlargement, non-pruritic diffuse rash with typical Köbner's sign, and painful polyarthritis with palpable synovitis affecting the wrists, knees, and ankle joints bilaterally. She was referred to our department and admitted in September 2010. Chest radiography revealed bilateral pleural effusion, which was suggestive of pleuritis. Laboratory studies showed high levels of C-reactive protein (115 mg/L) and ferritin (24,883 ng/mL, normal <120 ng/mL), anemia (hemoglobin 10.5 g/dL), and an elevated white blood cell count (25,100/μL with 96.5% peripheral neutrocytes). Liver dysfunction was identified (aspartate transaminase 123 IU/L, normal <39 IU/L; alanine aminotransferase 94 IU/L, normal <40 IU/L). Negative results were obtained for serum rheumatoid factor and antinuclear antibody. Blood cultures and exhaustive serological investigation for bacterial and viral etiologies of AOSD-like syndromes repeatedly yielded negative results. Her serum interleukin (IL)-18 level was significantly elevated (128,000 pg/mL), but IL-12 was not detectable. A diagnosis of AOSD was therefore made, according to the criteria described by Yamaguchi et al. [1]. Her symptoms worsened despite the initiation of prednisolone at 60 mg/day (treatment conducted in consultation with the obstetrician). As hemophagocytic syndrome developed as a complication after a few days, she received two courses of intravenous methylprednisolone pulse therapy, and plasma exchange, performed 15 times. Because the short-term effect of the pulse therapy was insufficient, we consulted her obstetrician again, and together with the obstetrician, we judged that maternal survival was the priority. We obtained informed consent for the use of cyclosporine in pregnancy from the patient and her family. Cyclosporine and liposomal dexamethasone palmitate were added after the pulse therapy, leading to improvement in the patient's condition. During the clinical course, the patient suffered from severe oral ulcers induced by cytomegalovirus. Ganciclovir was prescribed because of concerns that congenital cytomegalovirus infection could develop in the fetus. At gestational week 33, she underwent urgent Cesarean section because the fetus showed intrauterine growth restriction, and she gave birth to a 1.3-kg healthy girl with no apparent abnormalities. After 1 month, with no clinical or biological signs of AOSD recurrence, she was discharged with her baby.

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Inflammatory arthritis, such as rheumatoid arthritis, sometimes shows spontaneous improvement. It is suggested that the changes in sex hormones and cytokines during pregnancy lead to the spontaneous remission of autoimmune rheumatic diseases such as systemic lupus erythematosus, but the details are not understood. In AOSD, as occurred in our patient, there are cases in which onset or exacerbation presents in relation to pregnancy.

Our patient's characteristics met all the criteria proposed by Yamaguchi et al. [1] for definite AOSD. To date, 25 pregnancies that could be analyzed in detail have been reported in 19 patients with AOSD [2–15] (Table 1). Occurrence in pregnancy was identified in 9 patients, and most suffered from the second trimester until puerperium. No clinical or hematological differences were seen between typical AOSD and AOSD occurring in pregnancy. The precise role of pregnancy in the onset and course of AOSD is unclear, because improvement, flare, and no effect on AOSD have all been described during both pregnancy and postpartum, even in the same multiparous patient.

Dominant Th1 cytokine production is detected in the peripheral blood and pathological tissues of patients with active AOSD [16], and serum IL-18 levels are known to be related to disease activity in AOSD [17]. The level of serum IL-18 is reported to be less than 100 pg/mL in healthy controls, and a mean level of 9,200 pg/mL has been reported in the active phase of AOSD [18]. Serum

Table 1 Reported AOSD in pregnant patients

Case no.			Age (years)	OT	FER	WBC	Sym	Main treatment	Outcome
1	Bywaters [2]	1971	34				F, A, R	Gold	Not satisfactory
2	Stein et al. [3]	1980	28	8 weeks					
3	Green et al. [4]	1982	23	21 weeks		22000	F, A, R	PSL60 mg/day	Neonatal death
4	Yebra Bango et al. [5]	1985	19	FT			F, A, R	PSL	Poor
5									
A	Katz et al. [6]	1990	32	PP2 weeks			A, R	NSAID	Satisfactory
B			35	PP2 months			F, R	PSL40 mg/day	Not satisfactory
6	Leff [7]	1990	23	PP			F, A, R	PSL20 mg/day	Not satisfactory
7									
A	de Miguel et al. [8]	1992	38	PP3 months			F, A, R	PSL30 mg/day/AZA	Satisfactory
B			40	PA				MTX	Fetal loss
8	Parry et al. [9]	1992	27						
9									
A	Le Loët et al. [10]	1993	27	5 months			F, A, R	PSL60 mg/day	Satisfactory
B			30						
10	Le Loët et al. [10]	1993	24	5 months			F, A, R	PSL20 mg/day	Satisfactory
11	Le Loët et al. [10]	1993	24	PP			R	PSL10 mg/day	IUGR
12	Le Loët et al. [10]	1993	25	6 months					
13	Falkenbach et al. [11]	1994	25	8 weeks			F, A	PSL/IVCY	Termination
14	Mahmud and Hughes [12]	1999	33	30 weeks					
15	Liozon et al. [13]	1999	28	10 weeks	2960	12100	F, A, R	IVIG/IVMP	Preeclampsia
16									
A	Pan et al. [14]	2003	21	20 weeks	33900	6500	F, R	PSL60 mg/day/HCQ/MTX	IUGR
B			38	22 weeks	2710	12800	F, A, R	PSL60 mg/day	Satisfactory
17	Mok et al. [15]	2004	30	17 weeks	3985	15600	A, R	PSL5 mg/day/HCQ	IUGR
18									
A	Mok et al. [15]	2004	24		1990				Fetal loss
B			24						IGT
C			27	21 weeks		12200	F, A, R	PSL60 mg/day	Not satisfactory
19	Mok et al. [15]	2004	22	PP			A	NSAID	IUGR
20	Our patient	2011	28	21 weeks	24883	25100	F, A, R	IVMP/DEX/PE/CsA	IUGR

OT onset time, *PP* postpartum, *FT* first trimester, *PA* post-abortion, *FER* ferritin (ng/mL), *WBC* white blood cell count (/μl), *Sym* symptoms, *F* fever, *A* arthralgia, *R* skin rash, *PSL* prednisolone, *NSAID* non-steroidal anti-inflammatory drug, *AZA* azathioprine, *MTX* methotrexate, *IVCY* intravenous cyclophosphamide, *IVIG* intravenous immunoglobulin, *IVMP* intravenous methylprednisolone, *HCQ* hydroxychloroquine, *DEX* liposomal dexamethasone, *PE* plasma exchange, *CsA* cyclosporine A, *IUGR* intrauterine growth retardation, *IGT* impaired glucose tolerance

IL-18 concentrations in pregnant women gradually become elevated with the approach of delivery (500–3,000 pg/mL), and decrease postpartum. IL-18 has been suggested to act as a defensive cytokine protecting the mother from infectious complications of pregnancy such as premature rupture of the membranes, but also as a cytokine responding to foreign antigens originating from the conceptus [19].

IL-18 usually requires IL-12 to induce a Th1-type immune response, and IL-18 without IL-12 induces a Th2-type immune reaction. However, the cytokine balance changes during the course of pregnancy. A relationship between pregnancy and the Th2-immune response has been identified. Our patient showed a significantly elevated level of serum IL-18, but negligible production of IL-12 at gestational week 21. These results could not explain an extreme Th1-immune response by IL-12, and suggested the existence of other mechanisms inducing a Th1-immunoreaction in AOSD during pregnancy. We could not understand the origin of the elevated IL-18 concentration in our patient, but it seemed that the pregnancy itself increased the risk of developing AOSD, because pregnancy alone induced the elevation of serum IL-18. The mechanism of IL-18 production in pregnancy is also unknown, so it is necessary to conduct studies in this area.

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Conflict of interest None.

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