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# Ⅳ．研究成果の刊行物・別冊

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ORIGINAL ARTICLE

# Total lesion glycolysis as an IgG4-related disease activity marker

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**Abstract**

*Objectives.* 2-[18F]-fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography (FDG-PET/CT) was reported to be useful for monitoring immunoglobulin G4-related disease (IgG4-RD); however, a quantitative FDG-PET/CT analysis such as total lesion glycolysis (TLG) has not yet been conducted. This study aimed to investigate whether TLG would correlate with serum markers in IgG4-RD, and the utility of TLG for disease monitoring.

*Methods.* This retrospective study included 17 patients (12 men; median age, 62 years) who were followed up at Kyoto University Hospital and underwent FDG-PET/CT from April 2009 to November 2013. TLG was calculated for the involved lesions. Correlations between serum markers [IgG4, soluble IL-2 receptor (sIL-2R), lactate dehydrogenase (LDH), and C-reactive protein (CRP)] and TLG concomitant with FDG-PET/CT scans were investigated. Serial changes in TLG were assessed in patients who underwent follow-up FDG-PET/CT ($n = 6$).

*Results.* The calculated median (IQL) TLG value was 154.8 (63.7–324.4). A significant correlation was found between the sIL-2R level and TLG ($P = 0.001$, rs = 0.763). In contrast, no correlations were found between the IgG4, LDH, or CRP levels and TLG. Increased or decreased TLG corresponded with clinical disease improvement or worsening.

*Conclusions.* TLG correlated significantly with the serum sIL-2R level and may be useful for disease monitoring in IgG4-RD.

**Keywords**

FDG-PET, IgG4-related disease, Soluble IL-2 receptor, Total lesion glycolysis

**History**

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## Introduction

Immunoglobulin G4-related disease (IgG4-RD) is a multiorgan disease that is histologically characterized by the marked infiltration of IgG4-positive plasma cells into affected organs and elevated serum IgG4 levels [1,2]. Commonly affected sites include the pancreas (autoimmune pancreatitis), salivary glands (Mikulicz disease), retroperitoneal space (retroperitoneal fibrosis), lymph nodes, and lungs [3]. To date, there are no established clinical indices for evaluating IgG4-RD activity [4].

2-[18F]-fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography (FDG-PET/CT) is a commonly used diagnostic tool for malignant diseases. FDG-PET/CT has been reported to be useful for lesion detection and disease activity evaluations in IgG4-RD cases [4,5]. A major advantage of FDG-PET/CT is that it provides quantitative data regarding lesion metabolic activity on a whole-body scale [5]. Total lesion glycolysis (TLG) is a recently proposed FDG uptake parameter; this parameter is calculated as the mean standardized uptake value (SUVmean) × the metabolic tumor volume (MTV). TLG reflects the total FDG uptake in the lesion, and a summation of TLG value from each lesion can indicate global metabolic disease activity in patients with multiorgan disorder [6]. However, there is no paper that investigated TLG in IgG4-RD; therefore, it is unknown in IgG4-RD patients whether TLG correlates serum markers of inflammations and whether serial change of TLG coincides with clinical course.

In the present study, to evaluate the utility of TLG for assessing IgG4-RD activity, we calculated the TLG values of IgG4-RD patients and determined whether these values correlated with serum levels of inflammatory biomarkers. Additionally, we compared changes in TLG with clinical courses of patients who underwent serial FDG-PET/CT studies.

## Materials and methods

### Study subjects

This retrospective study included 17 patients (12 men and 5 women) who were diagnosed with definite IgG4-RD at Kyoto University Hospital and underwent FDG-PET/CT before the initiation of immunosuppressive therapy during the period from April 2009 to November 2013. Seven of these cases were recurrent for which corticosteroid or immunosuppressive therapy had not been administered within 6 months prior to the FDG-PET/CT scan. The diagnoses were based on the criteria proposed by Umehara et al. [1]. Only the definite cases were included which fulfilled all of the following criteria: characteristic lesion

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distribution, an elevated serum IgG4 level (> 135 mg/dL), and histopathological findings compatible with IgG4-RD. The median (interquartile) age at the first test was 62 (range, 58 − 73) years. Patients initially underwent FDG-PET/CT because of suspected malignant disease (e.g., malignant lymphoma) and were ultimately diagnosed with IgG4-RD. Patients were excluded from study if FDG-PET/CT had been performed at an institute other than Kyoto University Hospital, or if corticosteroid or another immunosuppressive therapy had been administered at the time of the initial FDG-PET/CT scan. Patients with active malignant diseases were excluded; however, three cases with histories of malignancies within 5 years prior to FDG-PET/CT (thyroid cancer, colon cancer, and double lung and breast cancer) were included because in each case, radical treatment had been performed and malignant recurrence had been clinically excluded.

Twelve patients were symptomatic, and salivary gland swelling and hydronephrosis-related symptoms such as abdominal pain were comparatively common, whereas five cases did not complain of any symptoms. Comorbidities included diabetes mellitus in three cases, bronchial asthma in two and Grave's disease in two; in all cases the comorbid diseases were well controlled. The number of involved organs varied with a maximum of eight affected organs. Thirteen patients received corticosteroid therapy at a median (interquartile) dose of 35 (31.3 − 36) mg/day upon conversion to prednisolone. Patient characteristics are summarized in Table 1.

The Kyoto University Hospital Institutional Review Board approved this study (E201).

### FDG-PET/CT imaging

PET/CT scan was performed on a combined PET/CT scanner (Discovery ST Elite; GE Healthcare, Little Chalfont, UK). Patients fasted for at least 4 h before the study and after the plasma glucose level was confirmed to be < 150 mg/dL, each patient received an intravenous administration of a standard dose of 3.7MBq/kg of FDG. Approximately 60 min after the injection, a low-dose CT scan and successive PET scan covering the levels from the upper thigh to the skull was performed while the patient maintained shallow breathing. In this PET/CT scan system, CT and PET images were coregistered and the CT data were used for attenuation correction. These images were reconstructed using the VUE Point Plus 3-dimensional iterative reconstruction algorithm.

Table 1. Patient characteristics.

| | All patients, N = 17 |
|---|---|
| Sex, male/female | 12/5 |
| Age, years | 66 (57–73) |
| Symptoms | |
| Symptomatic* | 12 |
| Hydronephrosis-related symptoms | 6 |
| Salivary gland swelling | 2 |
| Exophthalmos | 3 |
| Dry mouth or dry eye | 2 |
| Asymptomatic | 5 |
| Comorbidities | |
| Hypertension | 5 |
| Diabetes | 3 |
| Asthma | 2 |
| Grave's disease | 2 |
| Angina pectoris | 2 |
| Number of involved organs | |
| 1 | 4 |
| 2 | 4 |
| 3 | 3 |
| 4 | 4 |
| 5 ≦ | 2 |
| Serum IgG, mg/dl | 2179 (1820–2761) |
| Serum IgE, mg/dl | 740 (400–1100) |
| Corticosteroid therapy | 13 |
| Initial corticosteroid dose, mg/day | 35 (32.3–36) |

Values are presented as medians (interquartile ranges) or numbers.
*MTV* metabolic tumor volume, *TLG* total lesion glycolysis, *sIL-2R* soluble interleukin- 2 receptor, *IgG4* immunoglobulin G4.
*One patient complained of abdominal pain due to hydronephrosis as well as dry mouth and was counted separately.

### Image interpretation

All images were reviewed for consensus by two observers (one nuclear medicine physician and one pulmonary physician). FDG accumulation was assessed on a workstation (Advantage Workstation, GE Healthcare) by calculating the standardized uptake value (SUV) in the regions of interest. SUV was calculated using the following formula: SUV = Cdc/(Di/W), where Cdc is the decay-corrected tracer tissue concentration (in Bq/g); Di is the injected dose (in Bq); and W is the patient's body weight (in g). Abnormal FDG uptake was identified based on a visual comparison of FDG uptake between the background organ and target site. All sites with abnormal FDG uptake were considered approximate foci of IgG4-RD lesions, although pathological analysis was not available for all sites. Next, we manually drew a 3D cuboid contour surrounding the abnormal uptake site. Within this contour, the voxels with SUV equal to or greater than the cut-off value were automatically extracted and defined as the optimal lesion border (Figure 1). In the present study, the cut-off value was fixed at 2.5 in accordance with previous reports [7–10]. The lesion volume was calculated as the metabolic tumor volume (MTV). The average SUV in the lesion was defined as the SUVmean, and TLG was calculated as the product of SUVmean and MTV [6]. After calculating the TLG of all affected lesions, we summed the TLG of each lesion to generate a final TLG score for each patient.

Furthermore, as FDG is excreted into the urine, kidneys or urinary ducts show a physiologically high uptake of FDG; therefore, we considered that the physiological uptake of FDG in urine was inevitably included when evaluating kidney lesions. To account for this, two observers carefully excluded the urinary tract or a healthy area of kidney by the visual assessment based on a CT scan. Moreover, to minimize the influence of physiological urine uptake, we subtracted the FDG uptake of urine from that of each lesion. SUVmax of the lesions other than those of the kidney did not exceed 10.0 in the present study; therefore, we defined the areas with SUV > 10 as urine uptake. Finally, we calculated the TLG of

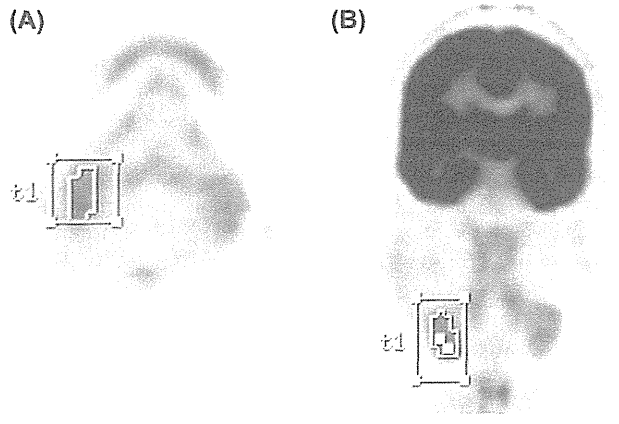


Figure 1. TLG measurement in a 47-year-old male with a salivary gland lesion. Sample coronal (A) and axial (B) PET images with quantitative parameters from a workstation are shown. Within the three-dimensional cuboid contour surrounding the right submandibular gland, voxels equal to or greater than the cut-off value (2.5) were automatically extracted. In this lesion, the maximum SUVmax, MTV, and TLG were 3.2, 6.5, and 18.1, respectively.

the area with an SUV > 10.0 within the contour and subtracted this from the TLG of the area with an SUV > 2.5. In addition, to completely exclude the influence of physiological urine excretion, we also performed sub-analysis in patients without renal lesions.

In this study, a maximum value of SUV within the region (SUVmax) of all affected organs was also assessed. When SUVmax was below 2.5, it was recorded as < 2.5. Owing to the methodological limitation, physiological excretion in the urine may influence SUVmax more strongly than TLG. Therefore, patients with kidney lesions were excluded when SUVmax was used in this analysis.

### Laboratory data

Laboratory data were obtained from the medical records. The serum C-reactive protein (CRP), lactate dehydrogenase (LDH), soluble IL-2 receptor (sIL-2R), and IgG4 concentrations were included if the evaluations had been conducted within 2 months before FDG-PET/CT. Laboratory evaluations after FDG-PET/CT were allowed for those who did not receive treatment. Next, correlations between initial TLG or SUVmax values and serum markers were investigated in all patients.

All serum CRP and LDH tests were performed within 1 month before or after FDG-PET/CT. For sIL-2R and IgG4, 13 and 11 tests were performed within 1 month before or after FDG-PET/CT, respectively. Three and two cases lacked sIL-2R and IgG4 data, respectively.

### Follow-up study

Among patients who underwent a follow-up FDG-PET/CT study, serial changes in TLG between initial and follow-up studies were evaluated and increasing or decreasing trends were assessed. Next, an assessment of each patient's course of global disease activity course was made from the 2 physicians' consensus. The course of disease activity was assessed based on image findings other than FDG-PET/CT, patients' symptoms, and physical finding [11]. The temporal change regarding each parameter was classified according to a three-grade system (improved, no change, or worsened), and the global disease course was determined by considering each temporal change. In brief, when classification of each parameter matched, the global disease course was judged according to that specific classification. When a conflicting case existed (such as a case when some parameters both improved and worsened), it was agreed that a decision would be made according to the most significant finding judged by each observer; however, no such case occurred within this study. We investigated whether the trend in serial TLG change and SUVmax change coincided with the global activity course.

### Statistical analysis

Statistical analyses were performed using modified R software programs (The R Foundation for Statistical Computing, Perugia, Italy) as described previously [12,13]. Continuous data were presented as medians with interquartile ranges (IQR). The Spearman rank correlation coefficient test was used to analyze correlations between TLG and serum markers. A P-value of < 0.05 was considered statistically significant.

## Results

### Organs with abnormal FDG uptake

Organs with abnormal FDG uptake are listed in Table 2. Most affected organs exhibited positive FDG uptake, while there was no area with an SUV > 2.5 in any normal organ except for the kidney and intraorbital space (likely due to the physiological uptake of urine or external ocular muscles, respectively). Among these organs, the hilar and mediastinal lymph nodes were the most commonly affected, followed by the salivary glands. In contrast, three suspected lesions did not show abnormal FDG uptake. Two of these were typical of IgG4-RD lung involvement (one was a ground-glass opacity with interlobular septum thickening and reticular shadow and the other was a peribronchovascular infiltrative shadow with ill-defined nodules), and the third was a space-occupying pancreatic lesion with a 5-mm diameter that was identified by abdominal echography. Pathological examinations were not conducted for these three lesions.

Table 2. Organs with abnormal FDG uptake.

| Organs with abnormal FDG uptake | n* |
|---|---|
| Lacrimal gland | 1 |
| Intraorbital space | 3 |
| Salivary gland | 9 |
| Pharyngolarynx | 1 |
| Supraclavicular lymph nodes | 3 |
| Hilar/mediastinal lymph nodes | 13 |
| Lung | 2 |
| Pancreas | 1 |
| Kidney | 3 |
| Thoracic aorta | 1 |
| Abdominal aorta | 1 |
| Retroperitoneal fibrosis | 5 |
| Prostate | 5 |

*We counted multiple lesions within in a single organ as an one-organ lesion.

### Quantitative analysis of FDG-PET/CT and serum markers

Quantitative analyses of the FDG-PET/CT and serum biomarker data are summarized in Table 3. The median MTV was 51.4 mL (20.2–92.5 mL) and TLG was 154.8 (63.7–324.4). The serum CRP (median: 0.2 mg/dL, IQR: 0.1–0.5 mg/dL) and LDH (median: 170 IU/L, IQR: 157–192 IU/L) levels were normal or slightly elevated in a majority of the cases. In contrast, elevated sIL2-R (median: 871.5 U/mL, IQR: 652.5 − 1337.5 U/mL) and IgG4 (median: 736 mg/dL, IQR: 539–1150 mg/dL) levels were observed.

### Correlations between TLG and serum markers

Among the serum markers investigated in the present study, sIL-2R levels were found to significantly, positively correlate with TLG ($P = 0.001$, rs = 0.763) and SUVmax ($P = 0.018$, rs = 0.723). CRP ($P = 0.913$, rs = 0.029), LDH ($P = 0.444$, rs = − 0.199), and IgG4 ($P = 0.192$, rs = 0.357) levels did not significantly correlate with either TLG or SUVmax (Figure 2, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/14397595.2014.990674). Even when the two cases with the highest TLG were excluded from the analysis, the correlation between sIL-2R and TLG was still significant ($P = 0.030$, rs = 0.623).

Table 3. Summary of quantitative data.

| Parameters | |
|---|---|
| MTV (ml) | 51.4 (20.2–92.5) |
| TLG | 154.8 (63.7–324.4) |
| Biomarkers | |
| CRP (mg/dl) | 0.2 (0.1–0.5) |
| LDH (IU/l) | 170 (157–192) |
| sIL-2R (U/ml) | 871.5 (652.5–1337.5) |
| IgG4 (mg/dl) | 736 (539–1150) |

Values are presented as medians (interquartile ranges).
*MTV* metabolic tumor volume, *TLG* total lesion glycolysis, *sIL-2R* soluble interleukin-2 receptor, *IgG4* immunoglobulin G4.

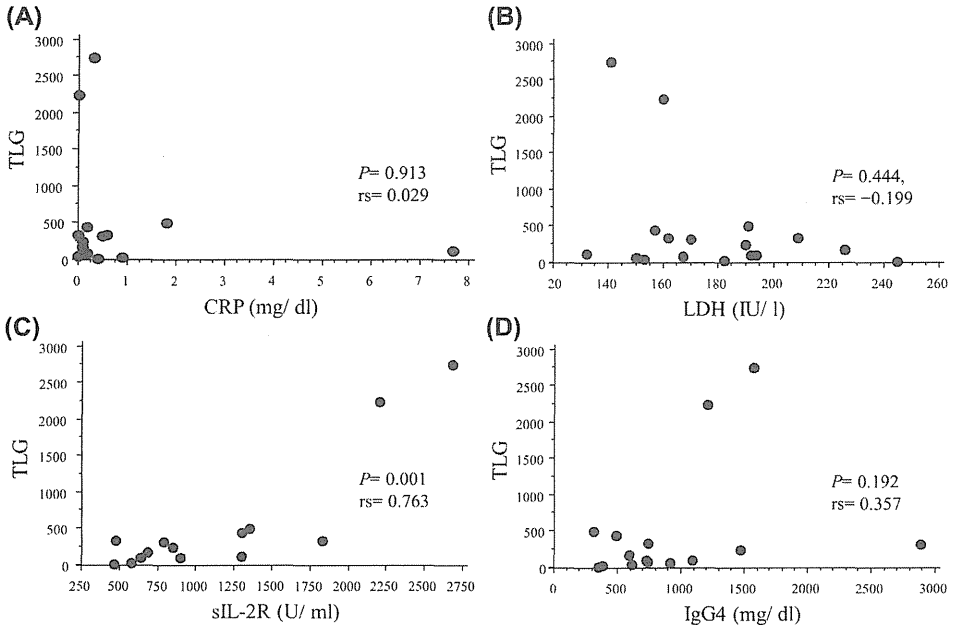


Figure 2. Correlations between TLG and serum biomarker levels. A: CRP, B: LDH, C: sIL-2R, D: IgG4. The serum sIL-2R level correlated significantly with TLG ($P = 0.001$, rs = 0.763), whereas no significant correlations were observed with the other markers. Each graph includes the rs and P values as determined using the Spearman rank correlation coefficient test.

We also performed the same analysis in patients without kidney lesions ($n = 15$). In this analysis, sIL-2R levels also correlated significantly with TLG ($P = 0.020$, rs = 0.717), whereas those of the other markers did not (Supplementary Figure 2 to be found online at http://informahealthcare.com/doi/abs/10.3109/14397595.2014.990674).

### Serial changes in TLG or SUVmax coincident with the clinical course

Follow-up studies were performed in six patients in whom serial changes in TLG FDG-PET/CT were assessed (Table 4 and Supplementary Table 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/14397595.2014.990674). Among these, three cases had received corticosteroid therapy and the others were followed up without therapy. Although intervals between the initial and follow-up studies were not uniform, reductions in TLG were observed in all cases that had received steroid therapy. In contrast, two of the three patients who did not receive steroid therapy showed increases in TLG. When comparing the trends in serial TLG changes with the global disease activity courses, all cases with decreased TLG were considered to have achieved improved global disease activity, whereas all cases with increased TLG were considered to have worsened. On the other hand, in two out of the five patients assessed, the course of SUVmax was not associated with the clinical course.

## Discussion

In this study, we demonstrated that serum sIL-2R levels correlated significantly and positively with TLG. However, no other serum marker levels, including IgG4, correlated significantly with TLG. Serial changes in TLG paralleled clinical disease improvement or worsening. These results indicated the utility of TLG as a marker with which to monitor IgG4-RD activity. To the best of our knowledge, this was the first report to investigate the significance of TLG in IgG4-RD.

In IgG4-RD, although the symptoms are usually mild [14] and resolution is sometimes obtained naturally, as seen in the present study, adequately timed and intense treatment is needed to prevent the irreversible progression of fibrosis [15]. Therefore, evaluations of disease activity are important. However, IgG4-RD affects various organs; therefore, it is difficult to establish an integrated measurement that can evaluate severities of lesions in different organs. To date, several scoring systems have been pro-

Table 4. Serial change of TLG in clinical course.

| | Treatment (+) | | | Treatment (−) | | |
|---|---|---|---|---|---|---|
| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 |
| Age | 66 | 73 | 77 | 73 | 54 | 53 |
| Gender | Male | Male | Female | Male | Male | Female |
| Affected Organs | Kidney<br>Pancreas | RF<br>SG<br>HMLN | OC<br>Lung<br>HMLN | OC | RF<br>HMLN | Lung<br>Pancreas |
| Test interval (month) | 27 | 1 | 12 | 22 | 18 | 22 |
| Initial dose of corticosteroid* | 40 | 35 | 30 | None | None | None |
| Initial TLG | 86.5 | 154.8 | 324.4 | 11.9 | 108.7 | 0.0 |
| Initial SUVmax | NA | 5.9 | 8.9 | 4.6 | 10.0 | <2.5 |
| Follow-up TLG | 66.9 | 8.0 | 157.6 | 10.6 | 591.5 | 16.8 |
| Follow-up SUVmax | NA | 3.0 | 5.2 | 4.9 | 5.0 | 3.2 |
| Course of global activity | Improved | Improved | Improved | Improved | Worsened | Worsened |

*TLG* total lesion glycolysis, *RF* retroperitoneal fibrosis, *SG* salivary glands, *HMLN* hilar and mediastinal lymph nodes, *OC* ocular cavity

Treatment (+) or (−) indicates whether corticosteroid therapy was initiated or not.

*Recorded when corticosteroid therapy was initiated during the period between initial and follow-up FDG-PET/CT. The corticosteroid dose was adjusted for prednisolone.

posed as tools for evaluating the global disease activity [11,16], but these systems have not been sufficiently established. Moreover, serum IgG4 level was included in the scoring, but in the present study, most of the cases did not undergo follow-up serum IgG4 measurements; therefore, the scoring systems were considered inapplicable. Accordingly, a physician's global assessment was used as a tool for evaluating changes in the disease activity [11,17].

FDG uptake is increased at sites of inflammation [18]. Elevated FDG uptake usually reflects more abundant glucose consumption, thereby indicating accelerated proliferation and increased metabolism [18–20]. Therefore, the degree of FDG uptake is considered to provide information about disease activity [21]. Actually, in some inflammatory diseases such as rheumatoid arthritis or Takayasu aortitis, FDG-PET was reported to have a diagnostic value and function effectively in evaluating treatment response [22–24].

Ebbo et al. performed a qualitative analysis of the FDG-PET/CT scans of IgG4-RD patients. The authors showed increased FDG uptake at the sites of affected lesions and reduced uptake after clinical remission was achieved [4]. Based on these results, they concluded that the FDG-PET/CT findings correlated with the disease activity. However, to date there have been no reports about the quantitative evaluation of FDG-PET/CT for IgG4-RD; therefore, we investigated the clinical significance of TLG in IgG4-RD.

SUVmax is a common and widely used quantitative analysis parameter of PET. This parameter is easily obtained, reproducible, and reader-independent, but it can be influenced by statistical noise. In addition, IgG4-RD patients are likely to have multiple organ involvement and thus multiple lesions. Because TLG is calculated as a summation of SUV of involved areas, we adopted and analyzed TLG as a quantitative value in this investigation.

Regarding the serum biomarkers, we demonstrated in the present study that serum sIL-2R levels strongly correlated with TLG. Although the precise biological role of sIL-2R remains unclear, the serum level of this receptor is considered as an indicator of lymphocyte activation [25] and elevated sIL-2R levels have been reported in several inflammatory diseases such as sarcoidosis [26] and systemic lupus erythematosus [25]. Moreover, elevated serum sIL-2R levels can predict sarcoidosis relapse after therapy [27]. Furthermore, in IgG4-RD, IL-2 is considered to have an important role in lymphocyte activation [28]. Matsubayashi et al. reported elevated serum sIL-2R levels in autoimmune pancreatitis patients and significant reductions in the level of this marker after corticosteroid therapy [29]. Autoimmune pancreatitis is now considered as a pancreatic manifestation of IgG4-RD [1]; therefore, sIL-2R might act as a biomarker of disease activity in IgG4-RD patients. The correlation between TLG and sIL-2R might thus indicate the utility of TLG as a biomarker in IgG4-RD.

In the previous studies, IgG4 is the most intensively investigated biomarker, and its serial changes in its levels were regarded as indicative of treatment responses or reactivation [30]. However, in the present study, IgG4 did not correlate significantly with TLG. The pathophysiological role of IgG4 remains unclear; however, IgG4 secretion is currently believed to occur in response to IL-10 stimulation, which acts to suppress immune responses [31]. Therefore, IgG4 may not directly reflect the intensity of inflammation. This indirect relationship might result in lack of correlation with TLG, although this idea is speculative.

In the analysis of serial FDG-PET/CT studies, all patients who exhibited decreases in TLG showed clinical improvement. On the contrary, two cases with increased TLG exhibited clinical deterioration. This result agreed with the above-described qualitative study [4], and suggested the utility of TLG as a parameter with which to monitor disease activity. On the other hand, in two cases the course of SUVmax was not associated with clinical course (Table 4). In these cases (Case 4 and 5), ocular movement and urinary tract obstruction improved or worsened in parallel with the change in TLG. We assume that the lesion volume might directly affect the function of these organs. In addition, after the treatment, case 2 showed a marked reduction in volume of the affected areas in parallel with TLG (from 154.8 to 8.0), whereas SUVmax showed just a moderate decrease (from 5.9 to 3.0); thus, TLG might exhibit a better association with global activity than SUVmax.

A major disadvantage of FDG-PET/CT is its high cost. Therefore, frequent and regular use of FDG-PET/CT is not possible in daily practice. Given the utility of sIL-2R and IgG4 in disease monitoring, measurement of these biomarkers may be advantageous in terms of cost and convenience compared with FDG-PET/CT. However, levels of both of these biomarkers might be elevated non-specifically because of other diseases or conditions [32,33]. Moreover, it is impossible to obtain site-specific information of activity when multiple lesions exist. In contrast, FDG-PET/CT can evaluate each organ separately [4]; therefore, it is comparatively easier to selectively investigate IgG4-RD lesion activities. For these reasons, serum biomarker evaluation is beneficial in daily practice, whereas more detailed FDG-PET/CT and TLG evaluation is useful as needed. On the other hand, it is still unclear whether comparing a TLG score in different organs has any clinical significance, because even lesions with a low TLG score could significantly aggravate patients (for example, intraorbital space lesions). Given this, further research is needed to better understand this problem.

This study has several limitations. First, this is a retrospective study with existing data deficits including the follow-up data of serum markers. Second, a pathological evaluation was not performed for all estimated lesions; therefore, sites of non-specific inflammation might have been included as IgG4-RD lesions. Third, the number of patients who participated in follow-up FDG-PET/CT studies was small, and intervals between the initial and follow-up FDG-PET/CT studies were not uniform; therefore, it was difficult to perform a statistical analysis regarding changes in TLG. Moreover, given the lack of information about the minimally clinically important difference in TLG, it was difficult to interpret the significance of a small change in TLG as seen in case 4. Fourth, SUV might be influenced by several factors such as the serum glucose level or the interval between the FDG injection and PET emission scan; therefore, the TLG calculations in the present study might exhibit a certain degree of fluctuation. In addition, physiological FDG uptake (such as urine) inevitably influenced TLG values, whereas SUV may show a low level even in the presence of definite lesions (such as interstitial pneumonia) and such lesions were omitted from analysis when SUV was below 2.5. Therefore, in cases where these lesions were present, an accurate evaluation was challenging. Despite these limitations, the present study showed a correlation between TLG and the sIL-2R level, and the serial change of TLG was associated with the clinical course. These findings indicated the utility of TLG for disease monitoring in IgG4-RD. In future studies, validations of these markers using clinically significant outcomes or parameters will be required.

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## Conflict of interest

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## Supplementary material available online

Supplementary Figures 1, 2 and Table 1.

REVIEW

*Gut and Liver, Vol. 8, No. 5, September 2014, pp. 462-470*

# Recent Advances in the Concept and Pathogenesis of IgG4-Related Disease in the Hepato-Bilio-Pancreatic System

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Recent studies have proposed nomenclatures of type 1 autoimmune pancreatitis (AIP) (IgG4-related pancreatitis), IgG4-related sclerosing cholangitis (IgG4-SC), IgG4-related cholecystitis, and IgG4-related hepatopathy as IgG4-related disease (IgG4-RD) in the hepato-bilio-pancreatic system. In IgG4-related hepatopathy, a novel concept of IgG4-related autoimmune hepatitis (AIH) with the same histopathological features as AIH has been proposed. Among organs involved in IgG4-RD, associations with pancreatic and biliary lesions are most frequently observed, supporting the novel concept of "biliary diseases with pancreatic counterparts." Targets of type 1 AIP and IgG4-SC may be periductal glands around the bile and pancreatic ducts. Based on genetic backgrounds, innate and acquired immunity, Th2-dominant immune status, regulatory T (Treg) or B cells, and complement activation via a classical pathway may be involved in the development of IgG4-RD. Although the role of IgG4 remains unclear in IgG4-RD, IgG4-production is upregulated by interleukin 10 from Treg cells and by B cell activating factor from monocytes/basophils with stimulation of toll-like receptors/nucleotide-binding oligomerization domain-like receptors. Based on these findings, we have proposed a hypothesis for the development of IgG4-RD in the hepato-bilio-pancreatic system. Further studies are necessary to clarify the pathogenic mechanism of IgG4-RD. **(Gut Liver 2014;8:462-470)**

**Key Words:** IgG4-related disease; Autoimmune pancreatitis; IgG4-related sclerosing cholangitis; IgG4-related hepatopathy

## INTRODUCTION

In 1961, Sarles *et al.*[1] observed a case of particular pancreatitis with hypergammaglobulinaemia, which is supposed to be a prototype of autoimmune pancreatitis (AIP) (Table 1). In 1995, Yoshida *et al.*[2] proposed a novel concept of AIP, which has been accepted as type 1 AIP (IgG4-related pancreatitis), the pancreatic manifestation of IgG4-related disease (IgG4-RD).[3] IgG4-RD is recognized worldwide as a novel clinical entity following the epoch-making evidence of increased serum levels of IgG4 in the history of AIP.[4] The histopathological findings are characterized by the periductal localization of predominantly CD4 positive T cells, IgG4-positive plasma cells, storiform fibrosis with acinar cell atrophy, and obliterative fibrosis,[5,6] which is also called lymphoplasmacytic sclerosing pancreatitis (LPSP).[7] On the other hand, mainly in the Western countries, histological analyses using resected pancreatic samples in patients with chronic non-alcoholic pancreatitis demonstrated a different histological pattern of pancreatitis from LPSP, so called idiopathic duct-centric pancreatitis (IDCP) or AIP with granulocytic epithelial lesion. In 2003, Kamisawa *et al.*[8] first suggested that AIP showing LPSP is a systemic sclerosing disease based on the concept of multifocal fibrosclerosis proposed by Comings *et al.*,[9] because the pancreas and other involved organs have fibrosis with abundant infiltration of IgG4-positive plasma cells. On the other hand, patients with IDCP, rarely observed in Japan, are not associated with either serum IgG4 elevation or with other organ involvement typically seen in LPSP. AIP is subclassified according to the International Consensus of Diagnostic Criteria for AIP as either type 1 (LPSP) or type 2 (IDCP).[10] Type 2 AIP, unlike type 1 AIP, is thought to be a specific pancreatic disease with occasional coexistence with ulcerative colitis.[10,11]

On the other hand, in 1892, Mikulicz[12] first observed a patient with symmetrical swelling of the lachrymal, parotid and submandibular glands, with massive infiltration of mononuclear cells. The condition was called Mikulicz's disease; however, it has since been classified as an atypical type of Sjögren's syn-

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**Table 1.** Transition of the Concept of IgG4-Related Disease

| Author (Year) | Evidences/Contents |
|---|---|
| Mikulicz (1892)[12] | Mikulicz's disease *(Z Chir Fesrschr)* |
| Sarles *et al.* (1961)[1] | Hypergammaglobulinemia in CP *(Am J Dig Dis)* |
| Comings *et al.* (1967)[9] | Familial multifocal fibrosclerosis *(Ann Intern Med)* |
| Küttner (1972)[13] | Küttner tumor *(Beitr Klin Chir)* |
| Kawaguchi *et al.* (1991)[7] | Lymphoplasmacytic sclerosing pancreatitis *(Hum Pathol)* |
| Yoshida *et al.* (1995)[2] | Autoimmune pancreatitis *(Dig Dis Sci)* |
| Hamano *et al.* (2001)[4] | High IgG4 levels in sclerosing pancreatitis *(N Eng J Med)* |
| Kamisawa *et al.* (2003)[8] | IgG4-related sclerosing disease *(J Gastroenterol)* |
| Kamisawa *et al.* (2006)[14] | IgG4-related sclerosing disease *(J Gastroenterol)* |
| Yamamoto *et al.* (2006)[15] | IgG4-related plasmacytic disease *(Mod Rheumatol)* |
| Masaki *et al.* (2009)[16] | IgG4-multiorgan lymphoproliferative syndrome (MOLPS) *(Ann Rheum Dis)* |
| Shimosegawa *et al.* (2011)[11] | International Consensus Diagnostic Criteria for AIP *(Pancreas)* |
| Umehara *et al.*[3,17] | Concept and comprehensive diagnostic criteria for IgG4-related disease *(Mod Rheumatol)* |
| Deshpande *et al.* (2012)[18] | International Pathological Consensus for IgG4-RD *(Mod Pathol)* |
| Stone *et al.* (2012)[19] | Nomenclatures of individual organ manifestation of IgG4-RD *(Arthritis Rheum)* |

CP, chronic pancreatitis; AIP, autoimmune pancreatitis.

**Table 2.** The Three Major Histopathological Features Associated with IgG4-Related Disease and the Minimal Criteria in a New Organ/Site in the International Pathological Consensus[18]

| |
|---|
| The three major histopathological features associated with IgG4-RD |
| 1. Dense lymphoplasmacytic infiltrate |
| 2. Fibrosis, arranged at least focally in a storiform pattern |
| 3. Obliterative phlebitis |
| Other histopathological features associated with IgG4-RD are: |
| 1. Phlebitis without obliteration of the lumen |
| 2. Increased numbers of eosinophils |
| Minimal criteria for IgG4-RD in a new organ/site |
| 1. Characteristic histopathological findings with an elevated IgG4t plasma cells and IgG4-to-IgG ratio |
| 2. High serum IgG4 concentrations |
| 3. Effective response to glucocorticoid therapy |
| 4. Reports of other organ involvement that is consistent with IgG4-RD |

IgG4-RD, IgG4-related disease.

drome, which also presents with bilateral, painless, and symmetrical swelling of the lachrymal, parotid, and submandibular glands. Küttner[13] reported a tumor-like enlargement of the submandibular gland that was sometimes a result of stones in the Wharton duct. These patients, lacking anti-SS-A/Ro or anti-SS-B/La antibodies, often show other systemic organ involvement with elevated serum levels of IgG4, infiltration of IgG4-positive plasma cells into the glands, and recovery of secretion with steroid treatment similar to AIP.[4-6] Referring to the original concept of multifocal fibrosclerosis, recent studies led us to develop a novel concept of a systemic disease such as IgG4-related systemic sclerosing disease,[14] systemic IgG4-related plasmacytic syndrome,[15] or IgG4-positive multiorgan lymphoproliferative syndrome,[16] all of which may refer to the same conditions. Based on these findings, although it is unclear whether the pathogenetic mechanisms in individual organs are same or not,[3,17] the comprehensive term "IgG4-related disease IgG4-RD," which was internationally endorsed with the proposal of nomenclatures for individual organ lesions as well as pathological consensus, and diagnostic criteria have been proposed from the Japanese investigators.[17] In this review, we discussed the current concepts of hepato-bilio-pancreatic lesions and recent advances in our understanding of the pathogenesis of IgG4-RD.

## CURRENT CONCEPTS OF IgG4-RD IN THE HEPATO-BILIO-PANCREATIC SYSTEM

The patients with IgG4-RD show diffuse or focal organ enlargement and mass-forming or nodular/thickened lesions in various organs, either synchronously or metachronously. This is due to the prominent infiltration of lymphocytes and plasmacytes with fibrosis.[3,5,14] The causes are still unclear; however, some abnormal immunological mechanisms are involved. The organs known to be affected include the pancreas, biliary duct, lacrimal/salivary glands, retroperitoneum, central nervous system, thyroid gland, lungs, liver, gastrointestinal tracts, kidneys, prostate gland, and lymph nodes.[5,14-19] Clinical symptoms vary depending on the organ in which the lesions are located, but many cases are treated effectively by steroid therapy. All of

them show similar pathological findings with abundant infiltration of IgG4-positive cells and fibrosis, and international minimum histological consensus was proposed (Table 2). Although the infiltration of IgG4-positive cells and increased serum levels of IgG4 are characteristic in IgG4-RD, the severity of fibrosis seems to be different among the individual involved organs.[18] Storiform fibrosis and obliterative phlebitis are characteristic in pancreatic and biliary tract lesions, but rarely observed in the salivary or lymphnodes.[18] Although most patients have multiorgan lesions synchronously or metachronously, about 10% to 20% of the patients have solitary organ involvement.[20] Therefore, it is unclear whether the pathogenetic mechanism is same among individual organs or not. Type 1 AIP (IgG4-related pancreatitis), IgG4-related sclerosing cholangitis (IgG4-SC), IgG4-related cholecystitis, and IgG4-related hepatopathy are recommended as the nomenclatures of IgG4-RD in the hepato-biliopancreatic system.[19]

### 1. Type 1 AIP (IgG4-related pancreatitis)

AIP is a distinct form of pancreatitis clinically characterized by frequent presentation with obstructive jaundice with or without a pancreatic mass, histologically by a lymphoplasmacytic infiltrate and fibrosis and therapeutically by a dramatic response to steroids.[5,21] Recent studies have suggested that "AIP" manifests two distinct subtypes, type 1 and type 2 AIP (Table 3).[10,11] Type 1 AIP (IgG4-related pancreatitis) is more prevalent in Japan and Korea, whereas type 2 AIP, with granulocytic epithelial lesion, is more commonly observed in Europe and the United States.

In type 1 AIP, the pancreatic histopathology shows the following characteristic features of LPSP: 1) abundant infiltration of plasma cells ($IgG4^+$ cells; >10/hpf, 40%>IgG4/IgG cells) and lymphocytes, 2) peculiar storiform or swirling fibrosis, and 3) perivenular infiltration with lymphocytes and plasma cells often leading to obliterative phlebitis. Clinically, it is characterized by swelling of the pancreas, elevated serum IgG4 levels and extrapancreatic lesions (e.g., sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis) associated with infiltration of abundant $IgG4^+$ plasma cells. Patients with type 1 AIP often have obstructive jaundice in elderly males, and the pancreatic and extrapancreatic manifestations respond to steroid therapy.[21] Therefore, it is a pancreatic manifestation of a systemic disorder, IgG4-RD.[19,21]

### 2. IgG4-SC

About 60% to 80% of patients with type 1 AIP are associated with IgG4-SC,[5,20-22] in which cholangiographic features are similar to those of primary sclerosing cholangitis (PSC), pancreatic cancer, and cholangiocarcinoma. The steroid responses and the prognoses of IgG4-SC differ from patients with PSC, which suggests different pathological conditions.[5,20-22] Four types of the characteristic cholangiographic features of IgG4-

**Table 3.** Subtypes of Autoimmune Pancreatitis

| Subtype of AIP | Type 1 | Type 2 |
|---|---|---|
| Other nomenclatures | AIP without GEL | AIP with GEL |
| | IgG4-related | IgG4-unrelated |
| | LPSP | IDCP |
| Prevalence | Asia>USA, EU | EU>USA>Asia |
| Age | High aged | Younger |
| Gender | Male>>Female | Male=Female (NS) |
| Symptoms | | |
| Obstructive jaundice | Often | Often |
| Abdominal pain | Rare | Common |
| Pancreas swelling | Common | Common |
| Serology | High serum IgG, IgG4, autoAbs (+) | Normal IgG, normal IgG4, autoAbs (-) |
| OOI | Sclerosing cholangitis | Unrelated with OOI |
| | Sclerosing sialadenitis | |
| | Reteroperitoneal fibrosis | |
| | Others | |
| Ulcerative colitis | Rare | Often |
| Steroid | Responsive | Responsive |
| Relapse | High rate | Rare |

AIP, autoimmune pancreatitis; GEL, granulocytic epithelial lesion; LPSP, lymphoplasmacytic sclerosing pancreatitis; IDCP, idiopathic duct-centric chronic pancreatitis; NS, not significant; OOI, other organ involvement.

SC have been proposed based on the regions of stricture (Fig. 1).[22] IgG4-SC with only stenosis of the distal common bile duct (type 1) is difficult to differentiate from pancreatic cancer. This stricture might be due to both the thickening of bile duct and the effect of inflammation and/or edema of pancreas without wall thickness. IgG4-SC with diffuse stenosis throughout the intrahepatic/proximal bile ducts (type 2) is similar to PSC. IgG4-SC with stenosis in the hilar hepatic bile duct (type 3 and 4) is difficult to differentiate from hepatic hilar colangiocarcinoma.[22] In addition to stenosis of bile ducts, circular and symmetric thickening of the bile duct wall, smooth outer and inner margin, and homogenous internal echo demonstrated by abdominal ultrasonography, abdominal computed tomography, abdominal magnetic resonance imaging, endoscopic ultrasonography, and intraductal ultrasonography are most characteristic images.[22] These characteristic features are recognized not only in the stenotic areas or occasionally in the gallbladder but also in areas without stenosis that appear normal in cholangiogram. Most cases of IgG4-SC (80% to 90%) are associated with AIP.[20-22] It is particularly difficult to accurately diagnose IgG4-SC without AIP. In contrast to PSC, inflammatory bowel disease is rarely observed in the patients with IgG4-SC.[20-22]

Histopathologically, similar to LPSP in type 1 AIP, massive infiltration of IgG4-positive plasma cells, storiform fibrosis and/or obliterative phlebitis in the bile duct wall are characteristic and called as lymphoplasmacytic sclerosing cholangitis.[19,22] Such fibroinflammatory involvement is mainly observed in the submucosa of the bile duct wall, whereas the epithelium of the bile duct is intact.[23] Endoscopic transpapillary bile duct biopsy or cytological examinations are useful for differential diagnosis of cholangiocarcinoma, although it is difficult to take enough biopsy samples for characteristic histopathological findings of IgG4-SC.[22] Liver biopsy is sometimes useful in the diagnosis of IgG4-SC in cases of intrahepatic bile duct involvement.[22]

### 3. IgG4-related hepatopathy

Liver dysfunction is frequently observed in AIP patients and most of them show various pathological changes with infiltration of IgG4-bearing plasma cells in the liver; portal inflammation with or without interface hepatitis, large bile duct obstructive features, portal sclerosis, lobular hepatitis, and canalicular cholestasis.[24] As a very few of IgG4-RD patients without AIP or IgG4-SC show the same histological features as autoimmune hepatitis (AIH), a novel concept of IgG4-related AIH has been proposed.[25,26] To establish the concept of IgG4-related AIH, further studies are required.

## RECENT ADVANCES IN THE PATHOGENIC MECHANISMS OF IgG4-RD IN THE HEPATO-BILIO-PANCREATIC SYSTEM

### 1. Immunogenic backgrounds

Although immunogenic backgrounds of IgG4-RD are not well understood, Japanese patients with AIP, most of whom are

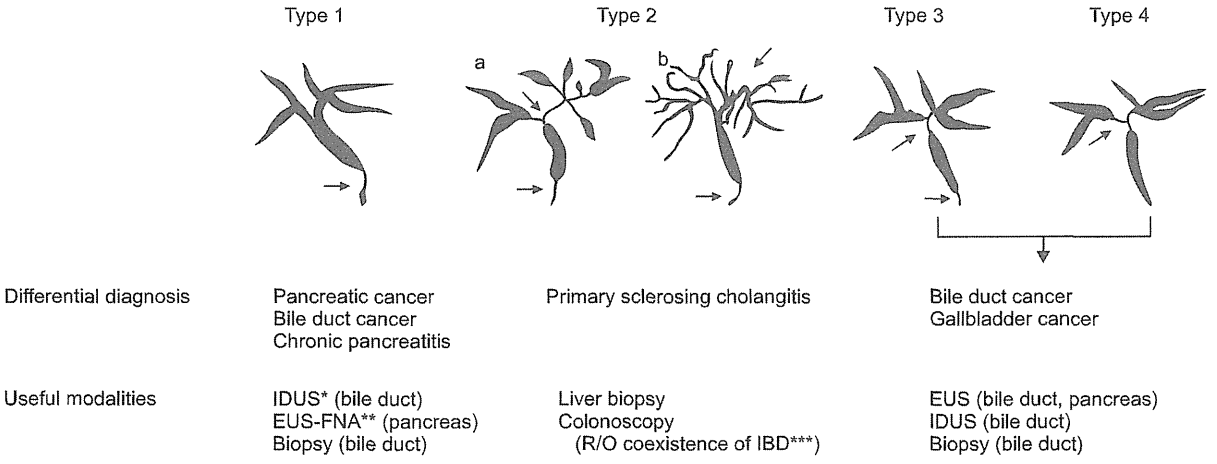


**Fig. 1.** Classification of cholangiography in IgG4-related sclerosing cholangitis (IgG4-SC). The characteristic features of IgG4-SC can be classified into four types, based on the regions of stricture as revealed by cholangiography and differential diagnosis. Type 1 IgG4-SC shows stenosis only in the lower part of the common bile duct, which should be differentiated from chronic pancreatitis, pancreatic cancer, or cholangiocarcinoma. Type 2 IgG4-SC, in which stenosis is diffusely distributed throughout the intrahepatic and extrahepatic bile ducts, should be differentiated from primary sclerosing cholangitis. Type 2 is further subdivided into two types. Type 2a has a narrowing of the intrahepatic bile ducts with prestenotic dilation, and Type 2b has a narrowing of the intrahepatic bile ducts without prestenotic dilation and reduced bile duct branches, caused by marked lymphocytic and plasmacyte infiltration into the peripheral bile ducts. Type 3 IgG4-SC is characterized by stenosis in both the hilar hepatic lesions and the lower portion of the common bile duct. Type 4 IgG4-SC shows strictures of the bile duct only in the hilar hepatic lesions. Cholangiographic findings of types 3 and 4 need to be discriminated from those of cholangiocarcinoma. From Ohara H, *et al.* J Hepatobiliary Pancreat Sci 2012;19:536-542, with permission from Springer.[27]
IDUS, intraductal ultrasonography; EUS, endoscopic ultrasonography; EUS-FNA, EUS-guided fine-needle aspiration; IBD, inflammatory bowel disease.

IgG4-related, may be associated with class II antigen haplotype of the major histocompatibility complex (HLA-DRB1*0405-DQB1*0401),[27] polymorphism of nuclear factor-κB and Fc-receptor-like 3 genes expressed on B cells.[28] An inhibitory molecule, cytotoxic T lymphocyte antigen-4 (CTLA-4; CD152) expressed on the activated memory T cells or $CD4^+CD25^+$ regulatory T cells (Tregs), was independently reported as a susceptibility factor.[29,30] Based on immunogenic backgrounds, abnormal conditions of immune responses may be involved in the development of type 1 AIP, although the precise pathogenic mechanisms remain unclear.[5]

### 2. Innate immunity

Recently, abnormal innate immunity has been demonstrated in some patients with IgG4-RD.[5,21] Activation of NOD-2 and TLR ligands on monocytes or basophils from patients with IgG4-related AIP enhances IgG4 responses via B cell activating factor (BAFF) and interleukin (IL)-13, although specific pathogens still remain unclear.[31,32] In animal models, activation of TLR3 by polyinosinic:polycytidylic acid or TLR4 by lipopolysaccharide can induce immune-mediated cholangitis, pancreatitis and sialadenitis similar to human IgG4-RD.[33]

### 3. Possible roles of IgG4 in IgG4-RD

Although the association of IgE-mediated allergy and IgG4 antibodies is well known, IgG4 characteristics are still poorly understood. IgG4 is involved in an immune process referring to as 'Fab-arm exchange,' which is a swapping of a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule; this usually results in asymmetric antibodies with two different antigen-combining sites.[34] While these modified antibodies are hetero-bivalent, they behave as monovalent antibodies. Another aspect of IgG4 is that it mimics IgG rheumatoid factor activity by interacting with IgG, namely Fc-mediated aggregation.[35] IgG4 seems to be associated with a pathogenic effect in a few situations. In pemphigus, recognition of skin autoantigens (desmogleins) by IgG4 is at the origin of the disease process.[36] A most recent study of structural determinants of human IgG4-Fc by crystallography suggested that Fc-Fc interactions are compatible with intact IgG4 molecules and may provide a model for the formation of aggregates of IgG4 that can cause disease pathology in the absence of antigen.[37]

Another recent data on regulation of IgG4 showed that IgG4-RD may reflect an excessive production of anti-inflammatory cytokines such as IL-10 that triggers an overwhelming expansion of IgG4-producing plasma cells.[38-42] Increased peripheral inducible-memory Tregs are positively correlated with serum levels of IgG4.[39] In addition, prominent infiltration of Tregs upregulated IL-10 in livers of the patients with IgG4-SC.[40] These findings suggest that IgG4 do not act as a pathogenic factor, but as an anti-inflammartory factor in IgG4-RD. Further studies are necessary to clarify the precise role of IgG4 in IgG4-RD.

### 4. The complement system

Patients in active stages of AIP occasionally show decreased complement (C3, C4) with elevated circulating immune complex as well as serum levels of IgG4 and the IgG4 subclass of immune complexes.[43] However, a previous study showed that the classical pathway of complement activation through IgG1 may be involved in the development of AIP rather than mannose-binding lectin or alternative pathways through IgG4.[43]

### 5. Autoantibodies and candidate of target antigens

Although some patients with IgG4-RD have nonspecific antibodies such as an antinuclear antibody, there is scarce association of IgG4-RD. From the view of IgG4 function, the big mystery is whether IgG4-RD is an autoimmune or an allergic disease. Although disease specific targets are unknown, the occasional coexistence of multiorgan involvements leads us to consider that there may be common target antigens. Among candidate antigens previously reported, lactoferrin (LF),[44,45] carbonic anhydrase (CA)-II,[44-47] CA-IV,[48] and pancreatic secretory trypsin inhibitor (PSTI)[49] are expressed in the pancreas, salivary glands, biliary duct, lungs, renal tubules, and so forth. Immunization with CA-II or LF induced systemic lesions such as pancreatitis, sialadenitis, cholangitis, interstitial nephritis in the mice models similar to human IgG4-RD.[50] Amylase α-2A,[51] HSP-10,[52] and *Helicobacter pylori*[53-56] are also candidates of disease-associated antigens. Among the involved organs in IgG4-RD, recent studies suggest an extremely high association of pancreatic and biliary lesions.[5,20,21] As both peribiliary glands in the biliary tract and pancreatic duct glands associated with pancreatic ducts in human are intermingled with small amounts of pancreatic exocrine acini,[57] and the biliary tree-derived stem cells may be involved in a pancreatic organogenesis in mice.[58] Nakanuma *et al.*[59] have proposed a new concept of the "biliary diseases with pancreatic counterparts," in which targets of type 1 AIP and IgG4-SC may be periductal glands around the bile and pancreatic ducts. Further studies of the biliary tract's pathophysiology based on its similarity to pancreatic counterparts are warranted.

### 6. Role of B cells

In addition to steroid and immune-modulators, the B cell depletion by rituximab, which reduces only IgG4, but not IgG1, IgG2, or IgG3, is useful in the therapeutic strategy in IgG4-RD.[60,61] A recent study showed expansion of $IgG4^+$ B cell receptor clones in blood and tissue of patients with active IgG4-cholangiopathy, and disappearance by corticosteroid treatment.[62] A recent study showed that the increased $CD19^+CD24^{high}CD38^{high}$ Bregs may suppress the disease activity of type 1 AIP, whereas the decreased $CD19^+CD24^{high}CD27^+$ Bregs might be involved in the development of type 1 AIP.[63] These findings suggest that

specific B cell responses may have a pivotal role in the pathogenesis of IgG4-RD such as type 1 AIP and IgG4-SC.

### 7. Th1 and Th2 immune balance

The effector cells in IgG4-RD have been poorly understood. The $CD4^+$ T cells differentiate from naive T cells (Th0) to Th1, Th2, Th17, and Treg cells. In the livers of IgG4-SC patients, a Th2 type immune reaction[38,42] is induced in addition to the Th1 responses.[45,50] Th2 cytokines may be involved in the progression of the disease process, especially the maturation and proliferation of local B cells and plasmacytes.

### 8. Tregs

Foxp3 is a member of the forkhead/winged-helix family of transcriptional regulators, and functions as the master regulator in the development and function of $CD4^+CD25^+$ Tregs classified as naturally occurring naive-Tregs originating in the thymus and adaptively induced memory-Tregs in the periphery by different antigens.[64] In type 1 AIP, circulatory naive ($CD45RA^+$) Tregs are significantly decreased in the peripheral blood, whereas memory ($CD45RA^-$)-Tregs are significantly increased.[39] In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the liver of type 1 AIP and IgG4-SC patients.[40,41] These findings suggest that increased memory-Tregs in the periphery and local tissues may be an inhibitory immune response against inflammation, although decreased naive Tregs may be pathogenic.

### 9. Our hypothesis for the pathogenesis of IgG4-SC

The neonatally thymectomized (nTx)-BALB/c mice models showed that immunization with CA-II or LF induced pancreatitis, cholangitis, and sialadenitis similar to human IgG4-RD.[50] These findings suggest that depletion of naive Tregs may induce macrophage/T cell activation and further proinflammatory reactions during the early stage of the disease as direct cytotoxicity effects through Fas ligand expression. WBN/Kob rat models with congenital decreased peripheral Tregs spontaneously develop sclerotic cholangitis, sialadenitis, thyroiditis, and tubulointerstitial nephritis.[65] These animal models suggest that $CD4^+$/$CD8^+$ T cells play major roles in the development of primary lesions similarly to human IgG4-RD; however, the counterpart of IgG4 in mice IgG subclasses has not been identified.

Based on these findings, we proposed the pathogenesis of type 1 AIP (Fig. 2).[5] The basic concept is the biphasic mechanism of "induction" and "progression." An initial response to unknown disease specific antigens including self-antigens (LF, CA-II, CA-IV, and PSTI) or microorganisms (bacteria or virus) might be induced by decreased naive-Tregs followed by a Th1

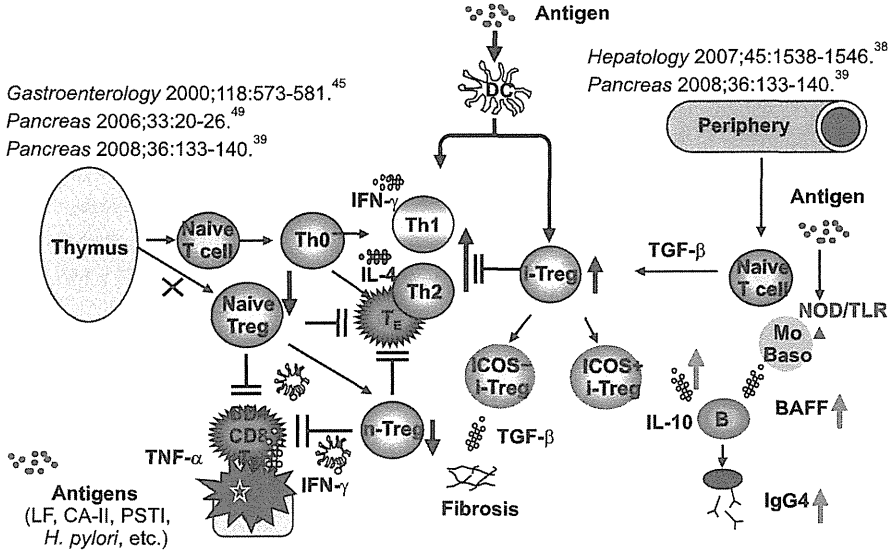


**Fig. 2.** Hypothesis for the pathogenesis of autoimmune pancreatitis (AIP) and IgG4-related disease. In central tolerance, naturally occurring naive regulatory T cells (n-Tregs) derived from the thymus suppress autoreactive CD4 or CD8 cells in the normal state. In the IgG4-related disease, the basic concept is a biphasic mechanism of "induction" and "progression." Initial response to antigens (lactoferrin [LF], carbonic anhydrase II [CA-II], CA-IV, pancreatic secretory trypsin inhibitor [PSTI], α-amylase, plasminogen binding protein peptide of *Helicobacter pylori*, etc.) might be induced by decreased n-Tregs. Th2 immune responses were followed by Th1-type immune responses, with releases of proinflammatory cytokines (interferon γ [IFN-γ], interleukin [IL]-1b, IL-2, tumor necrosis factor α [TNF-α]). In progression, Th2-type immune responses producing IgG, IgG4 and autoantibodies may be involved in pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and transforming growth factor β (TGF-β) secreted from inducible memory-Tregs (i-Tregs), respectively. However, activation of nucleotide-binding oligomerization domain (NOD) receptor or TLRs on monocytes or basophils increases IgG4 via the upregulation of B cell activating factor belonging to the tumor necrosis factor family (BAFF) and IL-13. From Okazaki K, *et al.* J Gastroenterol 2011;46:277-288, with permission from Springer.[5]
DC, ductal cell; TE, effector T cell.

type immune response with the release of proinflammatory cytokines (interferon γ, IL-1β, IL-2, tumor necrosis factor α). In progression, Th2 type immune responses producing IgG, IgG4, and autoantibodies may be involved in pathophysiology. IgG4 and fibrosis may be regulated by increased IL-10 and transforming growth factor β secreted from inducible T cell costimulator (ICOS)-positive and ICOS-negative inducible adaptive Tregs, respectively. Production of IgG4 may be also upregulated by BAFF from monocytes and basophils.

## CONCLUSIONS

Recent advances support the concept of IgG4-RD, a unique clinical entity, in the hepato-bilio-pancreas system. Although the pathogenic mechanism remains unclear, innate and acquired immunity, Tregs, and B cells may be involved in the development of these lesions. Further studies are necessary to clarify the pathogenesis including genetic backgrounds, disease-specific antigens, and the role of IgG4.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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# IgG4 cholangiopathy – Current concept, diagnosis, and pathogenesis

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## Summary

IgG4 related cholangiopathy, a distinctive type of cholangitis of unknown origin, is characterized by increased serum levels of IgG4, massive infiltration of IgG4-positive plasma cells with storiform fibrosis and/or obliterative phlebitis in the thickened bile duct wall, and good response to steroids. Patients with IgG4-cholangiopathy are frequently associated with autoimmune pancreatitis; IgG4-cholangiopathy is recognized as a biliary manifestation of IgG4-related disease. This condition can be diagnosed by a combination of imaging, serology, histopathology, and steroid responsiveness; however, cholangiographic features are often difficult to differentiate from primary sclerosing cholangitis, pancreatic cancer, or cholangiocarcinoma. The Japanese clinical diagnostic criteria for IgG4-related sclerosing cholangitis established in 2012 are useful in the diagnosis of IgG4-cholangiopathy. Although the precise pathogenic mechanism remains unclear, the development of IgG4-cholangiopathy may involve: susceptible genetic factors, abnormal innate and acquired immunity, decreased naïve regulatory T cells, and specific B cell responses.

Further studies on genetic backgrounds, disease specific antigens, and the role of IgG4 are necessary to clarify the pathogenesis.


## Introduction

IgG4 related cholangiopathy is a distinctive type of cholangitis of unknown origin, which is characterized by increased serum levels of IgG4 [1], massive infiltration of IgG4-positive plasma cells with storiform fibrosis and/or obliterative phlebitis in the bile duct wall and good response to steroids [1–3]. Patients with IgG4-cholangiopathy are frequently associated with autoimmune pancreatitis (AIP) [2,3], the concept of which was originally proposed by Yoshida *et al.* [4], and Hamano *et al.* reported increased serum levels of IgG4 in Japanese patients with AIP [1]. Now, it is recognized as a biliary manifestation of IgG4-related disease (IgG4-RD) [2–6]. Clinically, it is important to distinguish IgG4-cholangiopathy from malignancy such as cholangiocarcinoma, pancreas cancer, or a benign counterpart, PSC [2]. The organizing committee of the first international symposium on IgG4-RD in 2009 [6] proposed the nomenclature of "IgG4-related sclerosing cholangitis" (IgG4-SC) instead of "IgG4-associated cholangitis" which was recommended by the European Association for the Study of the Liver (EASL) [6]. Recently, the Japanese clinical diagnostic criteria 2012 for IgG4-SC have been proposed, although the pathogenic mechanisms remain unclear [2]. Here, we introduce the current concept, diagnosis, and recent advances in the pathogenesis of IgG4-SC.

## Current concept and diagnosis of IgG4-SC

### *Classification of sclerosing cholangitis*

Sclerosing cholangitis is classified into a primary type of unknown origin such as PSC or IgG4-SC, and secondary type with obvious pathogenesis (e.g., common bile duct (CBD) stone, cholangiocarcinoma, trauma, operation of biliary tract, congenital biliary anatomy, corrosive cholangitis, ischemic bile duct stenosis, AIDS-related cholangitis, or biliary injury of intra-arterial chemotherapy) (Table 1).

### *Prevalence of IgG4-SC*

The prevalence of IgG4-SC still remains unclear. About 80% of AIP patients suffer complications with stenosis of the distal CBD with wall thickness [2,3,5]. This stricture might be due to both the thickening of bile duct and the effect of inflammation and/or edema of pancreas without CBD wall thickness. Based on these propositions, a recent Japanese national study analyzed 197 PSC and 43 IgG4-SC patients without AIP [7]. The male/female ratio was 106:91 (1.16:1) in PSC and 33:10 (3.3:1) in IgG4-SC and the mean age [min–max] was 48.1 [4.0–86.3] in PSC and 69.3

Keywords: IgG4-related disease; IgG4-cholangiopathy; IgG4-related sclerosing cholangitis; Autoimmune pancreatitis.
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*Abbreviations:* AIP, autoimmune pancreatitis; ANA, anti-nuclear antibody; CA-II, carbonic anhydrase-II; CBD, common bile duct; *CTLA-4*, cytotoxic T lymphocyte antigen-4; ERCP, endoscopic retrograde cholangio-pancreatography; FCRL, Fc-receptor-like; IFN-γ, interferon-γ; IgG4-RD, IgG4-related disease; IgG4-SC, IgG4-related sclerosing cholangitis; IL-4, interleukin-4; LF, lactoferrin; LPSP, lymphoplasmacytic sclerosing pancreatitis; PSC, primary sclerosing cholangitis.

**Table 1. Classification of sclerosing cholangitis.**

| |
|---|
| **Sclerosing cholangitis of unknown origin** |
| Primary sclerosing cholangitis (PSC) |
| IgG4-related sclerosing cholangitis (IgG4-SC) |
| **Secondary sclerosing cholangitis** |
| Biliary lesion in AIDS patients |
| Cholangiocarcinoma |
| CBD stone |
| Postoperative/bile duct injury |
| Congenital biliary disorders |
| Chemical agents/drug-induced cholangitis |
| Ischemic biliary stenosis |
| Others |

[47.6–87.4] in IgG4-SC [7]. Cholangiographic classification of IgG4-SC (Fig. 1) according to the clinical diagnostic criteria of IgG4-SC in 2012 [2] demonstrated that type IV, in which strictures of the bile duct are detected only in the hepatic hilar lesions similar to cholangiocarcinoma was the most common in cases of IgG4-SC without AIP [7].

*Bile duct images of IgG4-SC*

*Cholangiogram*

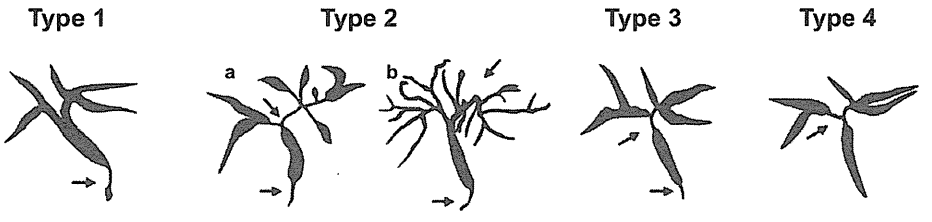
Four types of the characteristic cholangiographic features of IgG4-SC have been proposed based on the regions of stricture (Fig. 1) [2]. Type 1 IgG4-SC shows stenosis only in the distal CBD, which is often observed in pancreas cancer. Type 2 IgG4-SC, in which stenosis is diffusely distributed throughout the intrahepatic/proximal bile ducts, should be differentiated from PSC. Type 3 and type 4 of IgG4-SC show stenosis in the hilar hepatic bile duct similar to hepatic hilar cholangiocarcinoma.

*Circular/symmetric thickening of the bile duct*

Circular and symmetric thickening of the bile duct wall, smooth outer and inner margin, and homogenous internal echo demonstrated by abdominal ultrasonography (US), abdominal computed tomography (CT), abdominal magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), and intraductal ultrasonography (IDUS) are most characteristic images of the bile duct [2]. These characteristic features are recognized not only in the stenotic areas or occasionally in the gallbladder but also in areas without stenosis that appear normal in a cholangiogram [2].

*Characteristic hematological findings*

More than 80% of the patients with IgG4-SC show elevation of serum hepatobiliary enzymes, total bilirubin in cases of obstructive jaundice, and serum IgG4 levels (higher than the upper limit of normal value (ULN) of 135 mg/dl) [1,2]. However, elevation of serum IgG4 levels is not necessarily specific to IgG4-SC; it is also observed in atopic dermatitis, pemphigus, asthma, and some malignant cholangio-pancreatic diseases [2–6]. Cut-off values of serum IgG4 higher than x 2 ULN may be useful for more precisely differentiating IgG4-SC from PSC or cholangiocarcinoma [2,7].

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|---|---|---|---|
| **Differential diagnosis** | | | |
| | Pancreatic cancer<br>Bile duct cancer<br>Chronic pancreatitis | Primary sclerosing cholangitis | Bile duct cancer<br>Gallbladder cancer |
| **Useful modalities** | | | |
| | IDUS* (bile duct)<br>EUS-FNA** (pancreas)<br>Biopsy (bile duct) | Liver biopsy<br>Colonoscopy (R/O co-existence of IBD***) | EUS (bile duct, pancreas)<br>IDUS (bile duct)<br>Biopsy (bile duct) |

**Fig. 1. Classification of cholangiography in IgG4-related sclerosing cholangitis.** The characteristic features of IgG4-SC can be classified into 4 types based on the regions of stricture as revealed by cholangiography and differential diagnosis. Type 1 IgG4-SC shows stenosis only in the lower part of the common bile duct, and it should be differentiated from chronic pancreatitis, pancreatic cancer, or cholangiocarcinoma. Type 2 IgG4-SC, in which stenosis is diffusely distributed throughout the intrahepatic and extrahepatic bile ducts, should be differentiated from PSC. Type 2 is further subdivided into 2 types. Type 2a, with narrowing of the intrahepatic bile ducts with prestenotic dilation and Type 2b, with narrowing of the intrahepatic bile ducts without prestenotic dilation and reduced bile duct branches, which is caused by marked lymphocytic and plasmacyte infiltration into the peripheral bile ducts. Type 3 IgG4-SC is characterized by stenosis in both the hilar hepatic lesions and the lower part of common bile duct. Type 4 IgG4-SC shows strictures of the bile duct only in the hilar hepatic lesions. Cholangiographic findings of type 3 and type 4 need to be discriminated from those of cholangiocarcinoma. *IDUS, intraductal ultrasonography; **EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; ***IBD, inflammatory bowel disease. Modified from Hepatobiliary Pancreat Sci. 2012;19:536–542 [2], Copyright © 2013, with permission.

*Other organ involvements (OOIs)*

Most cases of IgG4-SC (80–90%) are associated with AIP. It is particularly difficult to accurately diagnose IgG4-SC without AIP [3,5]. Occasionally, IgG4-SC is associated with other systemic IgG4-RD such as IgG4-related symmetrical dacryoadenitis/sialadenitis and IgG4-related retroperitoneal fibrosis [5,6]; these are helpful in the diagnosis of IgG4-SC. Unlike PSC, inflammatory bowel disease (IBD) is rarely observed in patients with IgG4-SC [2,6].

*Histopathological findings of bile ducts*

In IgG4-SC, massive infiltration of IgG4-positive plasma cells, storiform fibrosis, and/or obliterative phlebitis in the bile duct wall are characteristic and called lymphoplasmacytic sclerosing cholangitis (LPSC) [2,6]. Such fibroinflammatory involvement is mainly observed in the submucosa of the bile duct wall, whereas the epithelium of the bile duct is intact [8]. Endoscopic transpapillary bile duct biopsy or cytological examinations are useful for differential diagnosis of cholangiocarcinoma, although it is difficult to take enough biopsy samples for characteristic histopathological findings of IgG4-SC [2]. Liver biopsy is sometimes useful in the diagnosis of IgG4-SC in cases of intrahepatic bile duct involvement [2].

*Effectiveness of steroid therapy*

In contrast to PSC or cholangiocarcinoma, the most characteristic feature of IgG4-SC is steroid responsiveness. It is important to make efforts of ruling out malignancy and to take enough biopsy samples. At many institutions, the therapeutic protocol for IgG4-SC follows that for AIP, such as oral prednisolone with the initial dose of 0.5–0.6/kg body weight/day [9]. If lesions do not respond to steroids, re-evaluation to rule out malignancy should be performed. In the refractory cases for oral steroids, it has been reported that steroid mini-pulse therapy [10], immunomodulators [11], and rituximab [12] are useful.

*Diagnosis of IgG4-SC*

In many cases of IgG4-SC, diagnosis can be made by a combination of characteristic biliary images (MRCP, ERCP, and EUS), increased serum levels of IgG4, coexistence of other organ involvements (OOIs), and characteristic histopathological features; however it is sometimes difficult to distinguish from PSC, cholangiocarcinoma, and pancreas cancer [2]. Based on these findings, the Japanese study group for IgG4-SC proposed the clinical diagnostic criteria for IgG4-SC [2] (Table 2). The effectiveness of steroid therapy is an optional diagnostic criterion to ensure accurate diagnosis of IgG4-SC like AIP only after negative workup of malignancy [2].

## Recent advances in the pathogenesis of IgG4-SC

Although the precise pathogenic mechanism remains unclear, susceptible genetic factors, abnormal innate and acquired immunity, decreased naïve regulatory T cells, and specific B cell responses may be involved in the development of IgG4-cholangiopathy [5,3]. The class II antigen haplotype of the human major histocompatibility complex (HLA-DRB1∗0405-DQB1∗0401), polymorphisms of nuclear factor-κB and Fc-receptor-like (FCRL) 3 genes expressed on B cells have been reported in the Japanese patients with AIP [3].

*Innate immunity*

Recently, abnormal innate immunity has been demonstrated in patients with IgG4-RD. Activation of NOD-2 and TLR ligands on monocytes or basophils from patients with IgG4-related AIP enhance IgG4 responses via B cell activating factor (BAFF) and IL-13, although specific pathogens still remain unclear [13]. In animal models, activation of TLR3 (polyinosinic:polycytidylic acid) or TLR4 (LPS) can induce immune-mediated cholangitis, pancreatitis, and sialadenitis similar to human IgG4-RD [14].

*Humoral immunity*

*Role of IgG4 in IgG4-SC*

Although the association of IgE-mediated allergy and IgG4 antibodies is well known, IgG4 characteristics are still poorly understood. IgG4 has non-acting characteristics for immune responses, and is involved in a continuous process referred to as 'Fab-arm exchange', which is a swapping of a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule; this usually results in asymmetric antibodies with two different antigen-combining sites [3]. While these modified antibodies are hetero-bivalent, they behave as monovalent antibodies. Another aspect of IgG4 is that it mimics IgG rheumatoid factor (RF) activity by interacting with IgG [3]. IgG4 seems to be associated with a pathogenic effect in a few situations. In pemphigus, recognition of skin autoantigens (desmogleins) by IgG4 is at the origin of the disease process [3]. In contrast, increased inducible-memory Tregs in the periphery and liver tissues are positively correlated with serum levels of IgG4 [15]. In addition, prominent infiltration of Tregs upregulated IL-10 in livers of the patients with IgG4-SC [16]. These findings suggest that hypersecretory IgG4 from Tregs may be a secondary phenomenon of the development of IgG4-SC, whereas overproduction of IgG4 by BAFF from abnormal innate immunity-related cells such as monocytes or basophils, may be involved with development of IgG4-SC. Further studies are necessary to clarify the role of IgG4 in IgG4-RD.

*The complement system*

Patients in active stages of AIP occasionally show decreased complement (C3, C4) with elevated circulating immune complex as well as serum levels of IgG4 and the IgG4 subclass of immune complexes. However, a recent study showed that the classical pathway through IgG1 may be involved in activation of the complement system rather than mannose-binding lectin or alternative pathways through IgG4 [17].

*Autoantibodies*

Some patients with IgG4-related disease have non-specific antibodies such as an anti-nuclear antibody (ANA). From the view of IgG4 function, the big mystery is whether IgG4-related disease is an autoimmune or an allergic disease. However, the occasional coexistence of OOIs leads us to consider that there may be common target antigens in the involved organs, especially the pancreas, because of high incidence. Among candidate antigens