Immunohistological analysis for immunological response and mechanism of interstitial fibrosis in IgG4 related kidney disease

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Abstract

Objectives Our study aimed to clarify the immunological characteristics and the mechanism of interstitial fibrosis in IgG4 related kidney disease (IgG4-RKD) by the immunohistological analysis.

Methods Immunohistological study was performed in the biopsied renal tissues of 16 IgG4-RKD, 16 Sjögren syndrome (SJS) and 17 idiopathic tubulointerstitial nephritis (ITIN) patients using antibodies against IgG, IgG1, IgG4, CD38, CD3, CXCR3, CCR4, Foxp3, Type I, Type III, Type IV and Type VI collagens and transforming growth factor (TGF)-β1.

Results The ratio of interstitial IgG4+/IgG+ plasma cells was higher in IgG4-RKD than that in SJS and ITIN. The ratio of CXCR3+/CD3+ cells was greater in SJS as compared to that in IgG4-RKD and ITIN. The ratio of CCR4+/CD3+ cells was not different among the three diseases. The ratio of Foxp3+/CD3+ cells was higher in IgG4-RKD in comparison with that in SJS and ITIN. There was a positive correlation between the ratio of Foxp3+/CD3+ cells and that of IgG4+ /IgG+ plasma cells in IgG4-RKD. The ratio of TGF-β1+ cells/total infiltrating cells was greater in IgG4-RKD than that in SJS and ITIN. The significant correlation was found between the ratio of Foxp3+/CD3+ cells and that of TGF-β1+ cells/total infiltrating cells in IgG4-RKD. Foxp3+ cells and TGF-β1+ cells were colocalized in the interstitium in IgG4-RKD. The localization and the distribution of each collagen in the fibrosis region were not different in the three diseases.

Conclusions Our results suggest that Treg cells may play a central role in IgG4 production in the interstitium and TGF-β1 induced by Treg cells may play a pivotal role in the interstitial fibrosis in IgG4-RKD.
Introduction

IgG4-related disease (IgG4-RD) is a newly recognized clinicopathological entity characterized by fibroinflammatory lesions with abundant IgG4 positive plasma cells and fibrosis, and shows usually an elevated serum IgG4 concentration [1-4]. These clinical findings were first described in pancreas, which is now called type 1 autoimmune pancreatitis (AIP) reported by Hamano et al. [5]. IgG4-RD is considered to be a systemic disease involving multi-organs including brain, salivary gland, lymph node, lung, pancreas, alimentary ducts, liver and kidney. IgG4-RD is now diagnosed by a combination of clinical, serological and radiological findings, along with pathological features [1-4].

The dominant feature of the renal involvement is tubulointerstitial nephritis (TIN) with abundant IgG4-positive plasma cell infiltration and fibrosis in the interstitium, along with an increase of serum IgG4 value [6-11]. In addition, glomerular lesions, several radiologically evident lesions including the renal parenchyma and pelvis and extrarenal lesions are involved [6-13]. Therefore, IgG4-related kidney disease (IgG4-RKD) has been proposed as a comprehensive term for the renal lesions associated with IgG4-RD [6-11].

Although the etiology of IgG4-RD remains unknown, Zen et al. have suggested that hyperactivity of regulatory T-cells (Treg) plays a central role in the proliferation of IgG4-producing plasma cells and interstitial fibrosis [14]. Tanaka et al. compared Sjögren’s syndrome (SJS) and Mikulicz’s disease (MD), a typical IgG4-RD for salivary gland, and found that Treg and T-helper type 2 (Th2) cells were involved in IgG4 production in the salivary gland of MD [15]. Nakashima et al. analyzed the mRNA expression of cytokines in
biopsied kidney tissues and reported that IgG4-RKD had significantly higher mRNA expression of Treg cell- and Th2 cell-derived cytokines as compared with other TIN [16]. However, the immunological characteristics of IgG4-RKD and the mechanism of interstitial fibrosis remain unknown. Our study aims to clarify the immunological characteristics and the mechanism of interstitial fibrosis in IgG4-RKD using immunohistological techniques.

Materials and Methods

Patients

Kidney tissues were obtained by means of percutaneous renal biopsy from 16 patients with IgG4-RKD, aged 61 to 83 years, 16 with SJS, aged 20 to 73 years and 17 with idiopathic tubulointerstitial nephritis (ITIN), aged 9 to 72 years between 2000 and 2013. IgG4-RKD and SJS was diagnosed according to the published diagnostic criteria [10]. SJS was diagnosed by the criteria [17], and renal biopsy was performed in those having urinary abnormalities or renal impairment. ITIN was excluded as follows; collagen diseases, ANCA-related TIN, drug associated TIN and postinfectious TIN. Informed consents were obtained from patients before renal biopsy. After approval with the Human Ethics Review Committee of Fukuoka University, this study protocol was implemented.

The clinical records examined at the time of the biopsy including blood pressure, urinalysis, serum protein, estimated glomerular filtration rate (eGFR), serum IgG concentration, serum IgG4 concentration, anti-nuclear
antibody (ANA) titer, CH50 and serum C3 and C4 concentrations were evaluated.

Immunohistological staining

Sections stained with hematoxylin-eosin (HE), periodic acid and Schiff (PAS), Masson-Trichrome (MT) and periodic acid-methenamine silver (PAM) were observed by light microscopy (LM). Routine immunofluorescence microscopy (IF) was performed using fluorescein isothiocyanate (FITC)-labeled anti-IgG, IgA, IgM, C1q, C3c and fibrinogen polyclonal antibodies (Dako, Copenhagen, Denmark).

Monoclonal anti-mouse antibodies of IgG (EMD Millipore Co, Billerica, MA, USA), IgG1 (EMD), IgG4 (EMD), CD3 (Leica, Wetzlar, Germany), CD38 (Leica), CXCR3 (Beckton-Dickinson, Franklin Lakes, NJ, USA), CCR4 (Beckton-Dickinson), and Foxp3 (eBioscience Inc., San Diego, CA, USA) were used. Polyclonal anti-rabbit antibodies of type I collagen (Daiichi Fine Chemical, Toyama, Japan), type III collagen (SouthernBiotech, Birmingham, AL, USA), type IV collagen (SouthernBiotech), type VI collagen (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and TGF-β 1 (Santa Cruz Biotechnology) were used.

Sections were cut at a thickness of three micrometer from paraffinized tissues. After deparaffinization, sections were immersed into phosphate-buffered saline (PBS) three times for 5 minutes. Diluted monoclonal antibodies (IgG, IgG1, IgG4, CD3, CD38, CXCR3, CCR4, Foxp3) and polyclonal antibodies (type I collagen, type III collagen, type IV collagen, type VI collagen, TGF-β 1) were overlaid on the sections. Sections were incubated at 4°C overnight.

Immunohistochemical studies for monoclonal and polyclonal antibodies were performed using avidin-biotin method as previously reported [18,19]. Sections overlaid by monoclonal antibodies were
incubated with biotin-labeled antibody against mouse IgG (Dako), and those overlaid by polyclonal antibodies were incubated with biotin-labeled antibody against rabbit IgG (Dako) at room temperature for 30 minutes. After three washes in PBS, sections were incubated with peroxidase-labeled streptavidin (Dako) for 30 minutes. After three washes in PBS, sections were treated 3,3-diaminobenzidine (Dojindo Laboratory, Kumamoto, Japan). Immunohistochemical specificity was confirmed by an absorption test with antibodies and replacement of primary antibodies to nonimmunized sera according to our previous report [18,19]. As a negative staining control, nonimmune normal serum or PBS was substituted for the primary antibodies according to the previous report [18,19].

Positive cells of IgG4, CXCR3, CCR4, Foxp3 and TGF-β1 were evaluated as the ratio (%) of IgG4-positive plasma cells/IgG-positive plasma cells, CXCR3-positive cells/CD3-positive cells, CCR4-positive cells/CD3-positive cells, Foxp3-positive cells/CD3-positive cells and TGF-β1-positive cells/infiltrating cells. Positive cells were counted at 5 areas at a magnitude of 400. The mean value was evaluated.

Statistical Analysis

The data are expressed as mean±SD. Difference in the mean values between groups was examined for statistical significance using the Mann-Whitney U test. Association of categorical variables was examined by the chi-square test. Kruskal-Wallis test and Steel-Dwass test were used as multiple comparison test. Spearman test was used as coefficient value. P value less than 5% is regarded as a statistically significant difference.
Results

Clinicopathological characteristics of patients with IgG4-related kidney disease

Table 1 shows clinicopathological characteristics of patients with IgG4-RKD. All of them were Japanese (14 men and 2 women) with an average age of 69.9±7.3 years old (61–83 years old) at the time of diagnosis of renal disease. The mean duration from onset to the biopsy was 10.2 months. The durations for the two patients (No. 2 and No. 6) were 15 and 21 days, respectively. Proteinuria was detected for 12 patients and one patient (No.6) presented nephrotic syndrome. All patients had renal impairment, and the serum creatinine level was 1.5-9.0 mg/dl and the estimated glomerular filtration rate (eGFR) was 9.8-63.8 ml/min per 1.73 m² at the time of the biopsy. Serum IgG and IgG4 concentrations were 4012.9±1581.3 mg/dl and 1415.2±1105.7 mg/dl, respectively. Anti-nuclear antibody was positive in 11 patients (69%) and 9 (56%) showed hypocomplementemia. Glomerular diseases were evident in 5 patients; membranous nephropathy (MN) in 3, membranoproliferative glomerulonephritis (MPGN) in one, and Henoch-Schölein purpura nephritis (HSPN) in one. Extrarenal disease was evident in 10; autoimmune pancreatitis (AIP) in 4, bilateral sialadenitis in 4, reactive lymphadenitis in 3 and retroperitoneal fibrosis in one).

The comparison of clinical characteristics among IgG4-RKD, SJS and ITIN is listed in Table 2. The mean age in patients with IgG4-RKD was higher than those with SJS and ITIN. The prevalence of male was higher in IgG4-RKD in comparison with SJS and ITIN. The mean duration from the onset to the biopsy was longer in
SJS than those in IgG4-RKD and ITIN. The value of urinary protein and eGFR were not different among these three groups. Serum IgG concentration was significantly higher in IgG4-RKD than ITIN, but was not different with that of SJS. The prevalence of patients with hypocomplementemia was 56% in IgG4-RKD and was higher than that in SJS (6%) and ITIN (13%). Positivity of ANA was 69% in IgG4-RKD, 100% (15/15) in SJS and 22% (2/9) in ITIN, respectively.

Pathological findings

Pathological findings showed TIN in all cases of IgG4-RKD. As shown in Figs. 1a and 1b, interstitium was significantly expanded in the lesion areas, and prominent fibrosis was noted along with abundant inflammatory cell infiltration including lymphocytes, plasma cells and eosinophils, accompanied by tubular atrophy. Interstitial lesions were relatively well-defined and showed the characteristic fibrosis indicating storiform or bird’s eye proposed by Yamaguchi et al. [20]. Infiltrating inflammatory cells were surrounded by the fibrosis. Tubulitis was absent or very mild. Pathological findings were also recognized in all patients with SJS (Fig. 1c) and ITIN (Fig. 1d). In those with SJS and ITIN, the interstitium was expanded, and interstitial inflammatory cell infiltration including lymphocytes and plasma cells was accompanied by tubular atrophy. However, interstitial fibrosis was weak in SJS and ITIN, and the characteristic finding, storiform or bird’s eye fibrosis observed in IgG4-RKD was not recognized. Some cases with ITIN also showed infiltration of eosinophils. In ITIN, 4 of 17 cases (24%) exhibited mild to moderate tubulitis. A few cases of patients with SJS also had mild tubulitis.
Immunohistological specificity of IgG-4RKD

Immunohistological staining of CD38, IgG, IgG4, CXCR3, CCR4 and Foxp3 shows in Fig. 2. The mean ratio of IgG1-positive plasma cells/IgG-positive plasma cells in the interstitium was 0.9 % in IgG4RKD, 0.4 % in SJS and 1.2% in ITIN, respectively as shown in Table 2. The mean ratio of IgG4-positive plasma cells/IgG-positive plasma cells was 52.2% in IgG4-RKD, whereas that is 0.1% in SJS and 1.3% in ITIN, respectively (Table 2). The mean ratio of IgG4-positive plasma cells/IgG-positive plasma cell in the interstitium in IgG4-RKD was much higher than those in SJS and ITIN.

The ratio of CXCR3-positive cells/CD3-positive cells in SJS was significantly higher than those in the other two diseases (Fig. 3a). The ratio of CCR4-positive cells/CD3-positive cells also tended to be higher in IgG4-RKD than that in the other two diseases, despite no significant difference (IgG4-RKD vs non-IgG4RKD; p = 0.073) (Fig. 3b). There was a significant difference in the ratio of Foxp3-positive cells/CD3-positive cells between IgG4-RKD and the other two diseases (Fig. 3c). A positive correlation (corrected rs = 0.46, corrected p = 0.055) between the ratio of FoxP3-positive cells/CD3-positive cells and that of IgG4/IgG-positive plasma cell was found (Fig. 3d).

The characteristics of fibrosis in IgG4-RKD

In the interstitium in IgG4-RKD, TGF-β1-positive cells were focally observed (Fig. 4a). The ratio of
TGF-β1-positive cells/inflammatory cells in IgG4-RKD had higher significance than those in SJS and ITIN (IgG4RKD vs. SJS; p = 0.0097, IgG4RKD vs. ITIN; p = 0.039) (Fig. 4b). A significant correlation between the ratio of Foxp3-positive cells/CD3-positive cells and that of TGF-β1-positive cells/inflammatory cells in IgG4-RKD was observed (corrected rs = 0.71, corrected p = 0.023) (Fig 4c). Immunofluorescence study revealed the colocalization of Foxp3-positive cells and TGF-β1-positive cells (Fig. 4d).

Concerning the specificity of collagen fibers in interstitial fibrosis, immunostain findings of type I, type III, type IV and type VI collagens in the three diseases showed in Fig. 5. Type I collagen in the interstitium was weakly stained in all three diseases. Type III, type IV and type VI collagens were strongly stained along with the storiform fibrosis in IgG4-RKD, but they were also strongly positive in SJS and ITIN. No characteristic features in collagen fibers were found in IgG4-RKD.

**Discussion**

One of the clinical characteristics of IgG4-RKD is a remarkable male predominance and the age of the patients is high [6, 8-11]. In our study, male was 87.5% in IgG4-RKD and the mean age at diagnosis was 69.9 years old in IgG4-RKD, and this was significantly higher than that in SJS (mean, 53.3 years) and ITIN (mean, 50.2 years). The serum value of total IgG in IgG4-RKD, SJS, and ITIN were 4,013 ± 1,415, 2,896 ± 1,020, and 1,283 ± 562 mg/dL, respectively. IgG4-RKD and SJS showed high value compared with that of ITIN. The positivity of ANA was high in IgG4-RKD, and the prevalence was 78.6% in IgG4-RKD and 100% in SJS as previously reported [6]. Being a rare finding in SJS and ITIN, hypocomplementemia was frequently observed
in IgG4-RKD in 56% (9/16) as previously described [8,9]. The degree of proteinuria and renal function estimated by eGFR at the time of biopsy were comparable among the three diseases. Glomerular lesions associated with IgG4-RKD such as MN, MPGN, HSP nephritis and IgA nephropathy also have been described [3,6,9,12,13,21-24]. In our study, the prevalence of MN was high (19%), and it has been reported that 7-10% of the cases with IgG4-RKD concurrent with MN [3,6,9]. In addition, it was demonstrated that the prevalence of extrarenal lesions in IgG4-RKD was extremely high [6,9]. Saeki et al. reported complicating extrarenal lesions were seen in 42 of 43 cases (97.7%). In our study, the extrarenal lesions were found in 63% (10/16), and it might be the results of overlook for the lesions. When IgG4-RKD is diagnosed, careful investigations for extrarenal lesions should be required.

In our study, the ratio of IgG4-positive plasma cells/IgG-positive plasma cells was predominantly higher in IgG4-RKD than SJS and ITIN as shown in Table 2. In the immunohistological study for chemokine, IgG4-RKD showed significantly lower value in the ratio of CXCR3-positive cells/CD3-positive cells than SJS and ITIN, and showed comparable value for CCR4-positive cells with those. CXCR3 is a chemokine receptor associated with Th1, and CCR4 with Th2 cell. The ratio of IgG4-positive cells/IgG-positive plasma cell was not correlated with that of CCR4-positive cells/CD3-positive cells (data not shown). However, the number of Foxp3-positive cells in IgG4-RKD was significantly higher than that in SJS and ITIN, and a tendency for a positive correlation was observed between the ratio of Foxp3-positive cells/CD3-positive cells and that of IgG4-positive plasma cells/IgG-positive plasma cells. The results of our immunohistological study suggest that Treg cells are involved in the elevation of IgG4 levels in the interstitium of IgG4-RKD. IgG4-RKD also exhibited significantly greater infiltration of TGF-β1-positive cells than SJS and ITIN (Fig. 4a,b), and the ratio
of TGF-β1-positive cells/infiltrating inflammatory cells was correlated with that of Foxp3-positive cells/CD3-positive cells (Fig. 4c). It was also clarified that Foxp3-positive cells and TGF-β1-positive cells were co-localized (Fig. 4d). Therefore, these results suggest that TGF-β1 produced by Treg cells may be involved in interstitial fibrosis formation as previously described [14].

Based on analyzing the mRNA expression of cytokines in the kidneys, IL-4, IL-10, Foxp3 and TGF-β mRNA levels were reported to be higher in IgG4-RKD than those in other TIN, and it was suggested that Th2 and Treg cells play a central role in the IgG4-RKD immune response [16]. MD showed significantly higher IL-4, IL-10, and Foxp3 mRNA levels as compared with those in SJS [15]. IgG4 and IgE are produced by B cells, which are induced to differentiate by Th2 cell-derived IL-4 [25,26]. While Treg cells can produce regulatory cytokines including IL-10 and TGF-β, IL-10 suppresses the switching to IgE and promotes switching to IgG4 [25,26]. Allergy research has elucidated that extended and high-dose exposure to occupational or injected allergens can induce an increase in IgG and IgG4 antibodies with a decrease in IgE antibodies [27,28]. It is accepted that Treg cells are activated by excessive immune reactions to prevent a Th2-type immune response in allergic disease [29,30], and this Th2 response, which suppresses allergic Th2 response, has been called a ‘modified Th2 response’ [31]. Our results suggest that Treg cells are involved in the elevation of serum IgG4 levels in IgG4-RKD. Considering that IgG4-RKD may develop under modified Th2 response, the results that the ratio of IgG4-positive cells/IgG-positive plasma cell was not correlated with CCR4 positive cell, but the correlation with Foxp3 positive cells may be reasonably accepted. In MD, the ratio of IgG4-positive cells/IgG-positive cells showed no correlation with the mRNA expression of CCR4 and IL-4, but it correlated with the mRNA expression of IL-10 and Foxp3 [15].
The pathological characteristics in IgG4-RKD is storiform fibrosis or ‘bird’s eye fibrosis’, accompanied with abundant IgG4-positive plasma cells [6,8-11,20]. We performed immunohistological evaluation with various collagens for the fibrosis presented in the kidneys. Type I collagen staining was weak in any disease, and type III, type IV and type IV collagens were strongly stained in the interstitium. However, the specificity in localization was not apparent in these collagens among the three diseases. Further study is needed to clarify the pathogenesis of the characteristic fibrosis in IgG4-RKD.

In conclusion, the results of our study suggest that in IgG4-RKD, Treg cell-predominated immune response drives the production of IgG4, and that TGF-β1 induced in Treg cells might be involved in the interstitial fibrosis.

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Conflict of interest: None

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viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. J Immunol 151: 1280-1289, 1993


Table 1. Clinicopathological characteristics in the patients with IgG4 related kidney disease

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Table 2. Comparison of clinical findings at the time of renal biopsy in IgG4-RKD, SJS, and ITIN

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<td>IgG4+/IgG+ (%)</td>
<td>52.2 ± 9.8</td>
<td>0.1 ± 0.3#</td>
<td>1.3 ± 1.7##</td>
</tr>
</tbody>
</table>

IgG4-RKD: IgG4-related kidney disease, SJS: Sjögren’s syndrome, TIN: idiopathic tubulo-interstitial nephritis, eGFR: estimated glomerular filtration rate

Kruskal-Wallis test, *p = 0.0030, IgG4-RKD vs SJS, **p = 0.016, IgG4-RKD vs ITIN, #p = 0.012, SJS vs IgG4-RKD, ##p = 0.00002, IgG4RKD vs ITIN, *p=0.0001, IgG4RKD vs SJS, ##p<0.0001, IgG4-RKD vs ITIN
Figure legend

Fig. 1. Light microscopic findings of IgG4-related kidney disease (IgG4-RKD), Sjögren’s syndrome (SJS) and idiopathic tubulointerstitial nephritis (ITIN). a: prominent interstitial fibrosis and abundant inflammatory cell infiltration including lymphocytes, plasma cells and eosinophils. IgG4-RKD, Hematoxylin-Eosin staining 200X, b: characteristic interstitial lesion showing storiform or bird’s eye fibrosis and infiltrating inflammatory cells surrounded by the storiform or birds’ eye fibrosis. IgG4-RKD, Masson Trichrome staining, 200X, c: interstitial fibrosis with inflammatory cell infiltration including lymphocytes and plasma cells. SJS, Masson Trichrome staining 200X, d: interstitial fibrosis with inflammatory cell infiltration including lymphocytes and plasma cells. ITIN, Masson Trichrome staining, 200X. The characteristic storiform or bird’s eye fibrosis found in IgG4-RKD is not observed in SJS and ITIN.

Fig. 2. Immunostaining findings of CD38 (a: IgG4-RKD, 200X), IgG (b: IgG4-RKD, 200X), IgG4 (c: IgG4-RKD, 200X), CXCR3 (d: SJS, 200X), CCR4 (e: IgG4-RKD, 200X) and FoxP3 (f: IgG4-RKD, 200X) positive cells in the interstitium.
Fig. 3. Comparison of the ratio of CXCR3-positive cells/CD3-positive cells (a), CCR4-positive cells/CD3-positive cells (b) and Foxp3-positive cells/CD3-positive cells (c) in the IgG4-RKD, SJS and ITIN. The correlation of the ratio of IgG4-positive plasma cells/IgG-positive plasma cells and the ratio of FoxP3-positive cells/CD3-positive cells (d). a: the ratio of CXCR3-positive cells/CD3-positive cells. Kruskal-Wallis test * p = 0.0004, SJS vs IgG4-RKD, ** p = 0.004, SJS vs ITIN, b: the ratio of CCR4-positive cells/CD3-positive cells. No significant difference in the three diseases by Kruskal-Wallis test, p = 0.073, IgG4-RKD vs non-IgG4RKD c: the ratio of FoxP3-positive cells/CD3-positive cells, Kruskal-Wallis test * p = 0.031, IgG4-RKD vs SJS, ** p = 0.032, IgG4-RKD vs ITIN, d: correlation of the ratio of IgG4-positive plasma cells/IgG-positive plasma cells and the ratio of FoxP3-positive cells/CD3-positive cells, No significant correlation is found despite positive correlation. rs = 0.45, p = 0.055.

Fig. 4. a: immunostaining findings of TGF-β1-positive cells in the interstitium in IgG4-RKD, b: comparison of the ratio of TGF-β1-positive cells/infiltrationg cells in the three diseases, Kruskal-Wallis test, * p = 0.0097, IgG4-RKD vs SJS, ** p = 0.039, IgG4-RKD vs ITIN, c: correlation of the ratio of FoxP3-positive cells/CD3-positive cells and the ratio of TGF-β1-positive cells/infiltrationg cells, Significant correlation is found. rs = 0.71, p = 0.023, d: Immunofluorescence reveals FoxP3-positive cell (left, green), TGF-β1-positive cell (middle, red) and colocalization of FoxP3-positive cell and TGF-β1-positive cell (right, merged). X 200
Fig. 5. Immunostaining findings of type I, type III, type IV and type IV collagens in the IgG4-RKD, SJS and ITIN. X 100
Fig. 1
**Fig. 3**

**(a)** Bar graph showing the percentage of IgG4-RKD, SJS, and ITIN in different conditions. The asterisks indicate statistical significance.

**(b)** Bar graph comparing IgG4-RKD, SJS, and ITIN with error bars indicating variability.

**(c)** Bar graph with a similar comparison as in (a) but with different sample sizes.

**(d)** Scatter plot with a linear trend showing the relationship between Foxp3+ / CD3+ cells (%) and IgG4+ / IgG+ cells (%).
Fig. 4
Fig. 6

Type I collagen                      Type III collagen                   Type IV collagen                Type VI collagen

IgG4RKD

SJS

ITIN