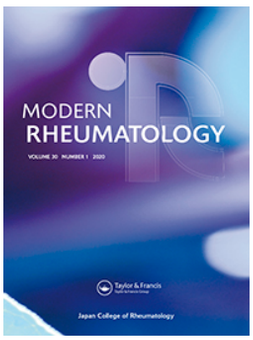

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REVIEW ARTICLE

Pathogenesis of IgG4-related disease. Comparison with Sjögren's syndrome

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ABSTRACT

IgG4-related disease (IgG4-RD) is characterized by lympho-plasmacytic infiltration and fibrosis in multiple organs, accompanied by high serum IgG4 levels. Although both IgG4-RD and Sjögren's syndrome (SS) frequently affect salivary and lacrimal glands, the clinical and pathological features of these two conditions are different. In an attempt to delineate the pathomechanisms of IgG4-RD, we compared the gene expression patterns of various molecules in labial salivary glands (LSGs) between IgG4-RD and SS. First, using quantitative PCR, we demonstrated significantly higher mRNA expression levels of activation-induced cytidine deaminase (AID), IL-10, and TGFβ in LSGs of IgG4-RD than SS and healthy controls (HCs). We propose that the combination of AID and IL-10 contributes to IgG4-specific immunoglobulin class switch recombination, and that TGFβ induces LSGs fibrosis in IgG4-RD. Second, DNA microarray identified 2641 differentially expressed genes (DEGs) in LSGs; with 1321 up-regulated and 1320 down-regulated genes in IgG4-RD, relative to SS. Among the up-regulated DEGs in IgG4-RD, quantitative PCR confirmed significantly higher expression levels of chemokine (C–C motif) ligand 18 (CCL18) and lactotransferrin in LSGs of IgG4-RD than SS and HCs. The former has chemotactic activity on various types of lymphocytes and enhances collagen production from fibroblasts, while lactotransferrin is an iron-binding protein abundantly present in milk and has a wide range of functions, including fibroblast proliferation and maturation of dendritic cells (DCs). Third, immunofluorescence staining confirmed specific upregulation of CCL18 in macrophages, CD11c + and B cells, and plasmacytes of LSGs-IgG4-RD. These pathological findings could help in the identification of disease-specific biomarkers as well as development of novel therapeutic strategies.

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IgG4-related disease; Sjögren's syndrome; labial salivary glands; DNA microarray; chemokine (C–C motif) ligand 18

Introduction

IgG4-related disease (IgG4-RD) is a new disease entity registered by the Japanese in the twenty-first century, which is characterized by lympho-plasmacytic infiltration and fibrosis in multiple organs accompanied by high serum IgG4 levels [1]. Although the pathogenesis of this disease has not yet been fully clarified, many of its clinical and pathological features have been described mainly in Japan [1]. Several types of lymphocytes as well as cytokines produced by these cells, such as T helper 2 (Th2) cells and Th2 cytokines (IL-4, IL-5, and IL-13), regulatory T (Treg) cells and Treg cytokines (IL-10 and TGFβ), and IL-21 produced by Th2 cells and follicular helper T (Tfh) cells, have been shown to contribute to the pathogenesis of IgG4-RD via B cells and plasmacytes activation, enhancement of IgG4 class switch recombination, development of ectopic germinal centers, and induction of fibrosis [2,3]. More recently, other $CD4^+$ T cells subsets, including $CD4^+$ granzyme A^+ cytotoxic T cells, which produce IFN-γ in affected tissues [4], and high proportion of peripheral Tfh1 and Tfh2 cells [5], have recently been demonstrated to play a role in the pathogenesis of IgG4-RD. Importantly, later Tfh2 cells are thought to contribute to plasmablast differentiation and IgG4 production [5]. Furthermore, the roles of various B cell subsets have also been investigated. For regulatory B (Breg) cells, a significantly high proportion of $CD19^+$ $CD24^{high}$ $CD38^{high}$ Breg cells is found in the peripheral blood of type 1 autoimmune pancreatitis (AIP), which is specifically seen in IgG4-RD, but not in healthy controls (HCs) or patients with chronic pancreatitis and pancreatic cancer, whereas $CD19^+$ $CD24^{high}$ $CD27^+$ Breg cells are decreased in AIP [6]. However, the other study demonstrated significantly low counts of peripheral $CD19^+$ $CD24^{high}$ $CD38^{high}$ Breg cells and significantly high counts of $CD19^+$ $CD24^-$ $CD38^{high}$ B cells (which correlate positively with serum IgG4 levels) in patients with IgG4-RD, compared with Sjögren's syndrome (SS) and HCs [7]. Thus, the association between the pathogenesis of IgG4-RD and Breg cells has not been confirmed at present. Moreover, significantly higher numbers of peripheral $CD19^{low}$ $CD38^+$ $CD20^-$ $CD27^+$ plasmablasts are present in IgG4-RD compared with HCs and other inflammatory diseases, and that this finding could be a useful marker for both the diagnosis and the response to therapy [8]. Based on these findings, the pathogenesis of IgG4-RD seems to involve various $CD4^+$ T and B cell subsets.

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Importantly, several kinds of autoantibodies have been found in IgG4-RD, such as anti-pancreatic trypsin inhibitor (PSTI), lactotransferrin, and carbonic anhydrase (CA) antibodies in patients with IgG4-related AIP [9]. Recently, Shiokawa et al. revealed the pathogenic roles and diagnostic utilities of autoantibodies against laminin 511-E8, a truncated laminin 511, one of the extracellular matrix (ECM) proteins, in patients with IgG4-related AIP [10]. Moreover, some new autoantibodies against galactin-3 [11], prohibitin [12], and annexin A11 [13] also have been detected in patients with IgG4-RD. Thus these autoantibodies might be involved in the pathogenesis of IgG4-RD as well as diagnostic markers for this disease.

In addition to these acquired immune cells and responses, research has focused in recent years on cross-talk with innate immune cells, such as macrophages, and dendritic cells (DCs) [2]. It has been demonstrated that monocytes of patients with IgG4-RD produce large amounts of B cell activating factor, belonging to the tumor necrosis factor family (BAFF), after stimulation through the Toll like receptor (TLR) and nucleotide oligomerization domain (NOD)-like receptor (NLR) [14]. Furthermore, the *in vitro* studies also showed that the induction of IgG4 class switch recombination in B cells is dependent on BAFF but independent of T cells [14]. Other studies showed significantly higher serum levels of BAFF and proliferation-inducing ligand (APRIL) in patients with IgG4-RD than HCs [15]. With regard to macrophages, previous studies demonstrated the expression of TLR7 on macrophages of AIP [16], as well as the expression of IL-10 and chemokine (C-C motif) ligand 18 (CCL18) on $CD163^+$ M2 macrophages found in the submandibular glands of IgG4-RD [17], suggesting they play pathogenic roles in IgG4-RD.

To determine the pathogenic mechanisms of IgG4-RD, we compared IgG4-RD and SS. Although both IgG4-RD and SS frequently affect salivary and lacrimal glands, the clinical and histopathological features of these two diseases differ from each other [18]. We were able to obtain labial salivary gland (LSG) tissue samples from patients with the two diseases under minimal invasion, compared with other organs, such as the pancreas, lung, and kidney. We anticipated that the results of comparisons of LSGs of IgG4-RD with those of SS could help in understanding the pathogenic mechanisms of IgG4-RD. In this review, we present our findings on the pathogenesis of IgG4-RD based on comparative analysis of IgG4-RD and SS.

Comparison of clinicopathological features of IgG4-RD and SS

SS is an autoimmune disease that affects exocrine glands, including salivary and lacrimal glands. It is characterized pathologically by lymphocytic infiltration into the exocrine glands, and clinically by dry mouth and dry eyes. SS is subcategorized into primary SS, which is not associated with any other well-defined connective tissue disease (CTD), and secondary SS, which is associated with other well-defined CTD [19]. Primary SS is further subdivided into the glandular form, with involvement of the exocrine glands only, and the extra-glandular form, with involvement of organs other than exocrine glands [20].

Table 1 summarizes the differences and similarities in clinicopathological features of SS and IgG4-related sialadenitis and dacryoadenitis [18,21,22]. Although salivary and lacrimal gland involvement was noted in both SS and IgG4-RD, there are major differences between the two in epidemiological, clinical, immunological, and pathological findings as well as therapeutic responses (recovery of secretory function) to corticosteroids. Thus, SS and IgG4-RD seem to be similar but different diseases.

The presence of sialadenitis of LSGs is one of the main diagnostic criteria of SS, as described in those of the 2016 American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) [23,24], the 1999 revised Japanese Ministry of Health criteria for the diagnosis of SS [25], the 2002 American-European Consensus Group classification criteria for SS (AECG) [19], and the 2012 ACR classification criteria for SS [26]. Accordingly, LSGs biopsy is commonly performed in SS suspected patients. With regard to the diagnosis of IgG4-related sialadenitis and dacryoadenitis, salivary and lacrimal glands biopsy is adopted by both the 2011 comprehensive diagnostic criteria for IgG4-RD [27] and 2008 diagnostic criteria for IgG4-related Mikulicz's disease [28]. LSGs biopsy is also obtained from IgG4-RD suspected patients, especially patients with salivary glands swelling. Although typical pathological changes of IgG4-RD, e.g. $IgG4^+$ plasmacytes infiltration and

Table 1. Comparison of clinicopathological features of SS and IgG4-related sialadenitis and dacryoadenitis [18,21,22].

	SS	IgG4 related sialadenitis and dacryoadenitis
Susceptible age	40–50 years	50–60 years
Gender	Overwhelmingly female dominant	Almost gender equality
Dry eyes and dry mouth	Present	None or mild grade
Swelling of lacrimal and salivary glands	Recurrent, spontaneous regression, solitary submandibular glands swelling is rare	Markedly, persistently, sometimes solitary submandibular glands swelling
Accompanied with allergic diseases (allergic rhinitis, bronchial asthma)	Uncommon	Common
Rheumatoid factor and anti-nuclear antibody	Mostly positive	Mostly negative
Anti SS-A/SS-B antibody	Frequently positive (anti SS-A; 70%, anti SS-B; 30%)	Almost negative
Increased immunoglobulin classes	IgG, IgA, IgM	IgG, IgE
Increased IgG subclasses	IgG1, IgG3	IgG4, IgG2
Increased $IgG4^+$ plasmacytes in tissues	None	Markedly
Lymphoepithelial lesions	Markedly	Rare
Response to corticosteroids (recovery of secretory function)	Poor response	Markedly effective

SS: Sjögren's syndrome; IgG4-RD: IgG4-related disease.

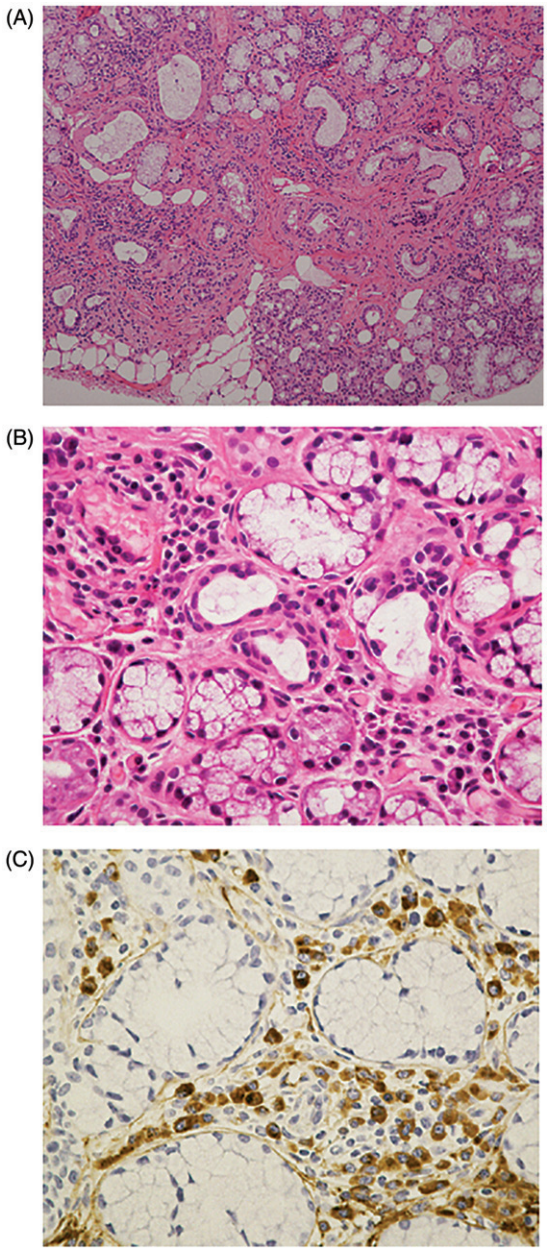

Figure 1. Typical pathological findings in LSGs sample in a representative patient with IgG4-RD. A 61-year-old female was diagnosed with IgG4-RD based on persistent bilateral lacrimal and submandibular glands swelling, high serum IgG4 level (971 mg/dL), and infiltration of typical $IgG4^+$ plasmacytes and fibrosis of the left submandibular gland tissue obtained by needle biopsy. She also underwent LSGs biopsy. (A) Hematoxylin and eosin staining of LSGs (original magnification ×40). Note the lymphoplasmacytic infiltration and mild fibrosis, compared with relative conservation of the ducts and acinar cells. (B) Hematoxylin and eosin staining of LSGs (original magnification ×400). Note the lymphoplasmacytic infiltration around the conserved ducts and acinar cells. (C) Immunohistochemical staining for IgG4 in LSGs (original magnification ×400). Note the marked infiltration of $IgG4^+$ plasmacytes.

fibrosis, are detected in LSGs biopsy samples (Figure 1), this IgG4-RD diagnostic modality is known to be associated with poor sensitivity and high specificity [29]. Despite the low sensitivity, LSGs biopsy seems to be useful for the diagnosis of IgG4-RD based on its high specificity and low invasiveness, compared with biopsies of the pancreas, lung, and kidney. Moreover, standard LSGs samples obtained from patients with IgG4-RD and SS could provide meaningful comparison of the pathogenesis of the two diseases.

Based on the above concepts, we compared IgG4-RD and SS, especially the histopathological and immunohistochemical LSGs findings, to provide the frame work for the pathogenic mechanisms of IgG4-RD.

Comparison of mRNA expression of IgG4-specific and non-specific immunoglobulin class switch-related molecules between IgG4-RD and SS

We compared the mRNA expression levels of IgG4-specific and non-specific immunoglobulin class switch-related molecules in peripheral blood mononuclear cells (PBMCs) and LSGs in IgG4-RD, SS, and HCs [30]. Previous studies confirmed that Th2 cytokines (IL-4 and IL-13) and Treg cytokine (IL-10) induce IgG4 and IgE-specific class switch recombination [31,32], and that tumor growth factor (TGF) β, a Treg cytokine, induces tissue fibrosis [33]. Furthermore, CD40, CD154, BAFF, APRIL, activation-induced cytidine deaminase (AID), interferon regulatory factor 4 (IRF4), and IL-21 are also known to contribute to non-specific immunoglobulin class switch recombination (from IgM to IgG1, IgG2, IgG3, IgG4, IgA, and IgE) [34–36], in conjunction with IL-4, IL-13, IL-10, and TGFβ.

Using quantitative PCR analysis [30], we examined the mRNA expression levels of IgG4-specific immunoglobulin class switch-related molecules, including Treg cytokines (IL-10 and TGFβ), Th2 cytokines (IL-4 and IL-13), transcriptional factors (GATA3 and Foxp3), as well as IgG4-non-specific immunoglobulin class switch-related molecules (CD40, CD154, BAFF, APRIL, AID, and IRF4). Importantly, all LSGs samples from the patients with IgG4-RD used in this study satisfied the histopathological criteria of the 2011 comprehensive diagnostic criteria for IgG4-RD [27]. The mRNA expression levels of Treg cytokines (IL-10 and TGFβ) were significantly higher in LSGs of IgG4-RD than SS and HCs ($p < .05$). Furthermore, IL-4 mRNA expression was significantly higher in LSGs of IgG4-RD than HCs ($p < .05$). However, there were no significant differences in PBMCs expression levels of various cytokines, among the three groups. In LSGs, the expression of GATA3 was significantly lower in IgG4-RD than in SS, and Foxp3 was significantly higher in IgG4-RD and SS than in HCs ($p < .05$). However, there was no significant difference in Foxp3 expression level between IgG4-RD and SS (Figures 2(A) and 3(A)) [30]. The mRNA expression levels of CD40 and CD154 were significantly lower in PBMCs of IgG4-RD than SS ($p < .05$). The expression of BAFF was significantly higher in LSGs of IgG4-RD than HCs ($p < .05$). The expression of APRIL was significantly lower in PBMCs of IgG4-RD than HCs ($p < .05$). The expression of AID was significantly higher in LSGs of IgG4-RD than SS and HCs ($p < .05$) (Figures 2(B) and 3(B)) [30].

Among these observations, we focused on the molecules with different expression levels in IgG4-RD compared with both SS and HCs, with the assumption that these molecules could be IgG4-RD-specific pathogenic factors. Based on this point of view, we demonstrated that the mRNA expression levels of Treg cytokines (IL-10 and TGFβ) and AID were

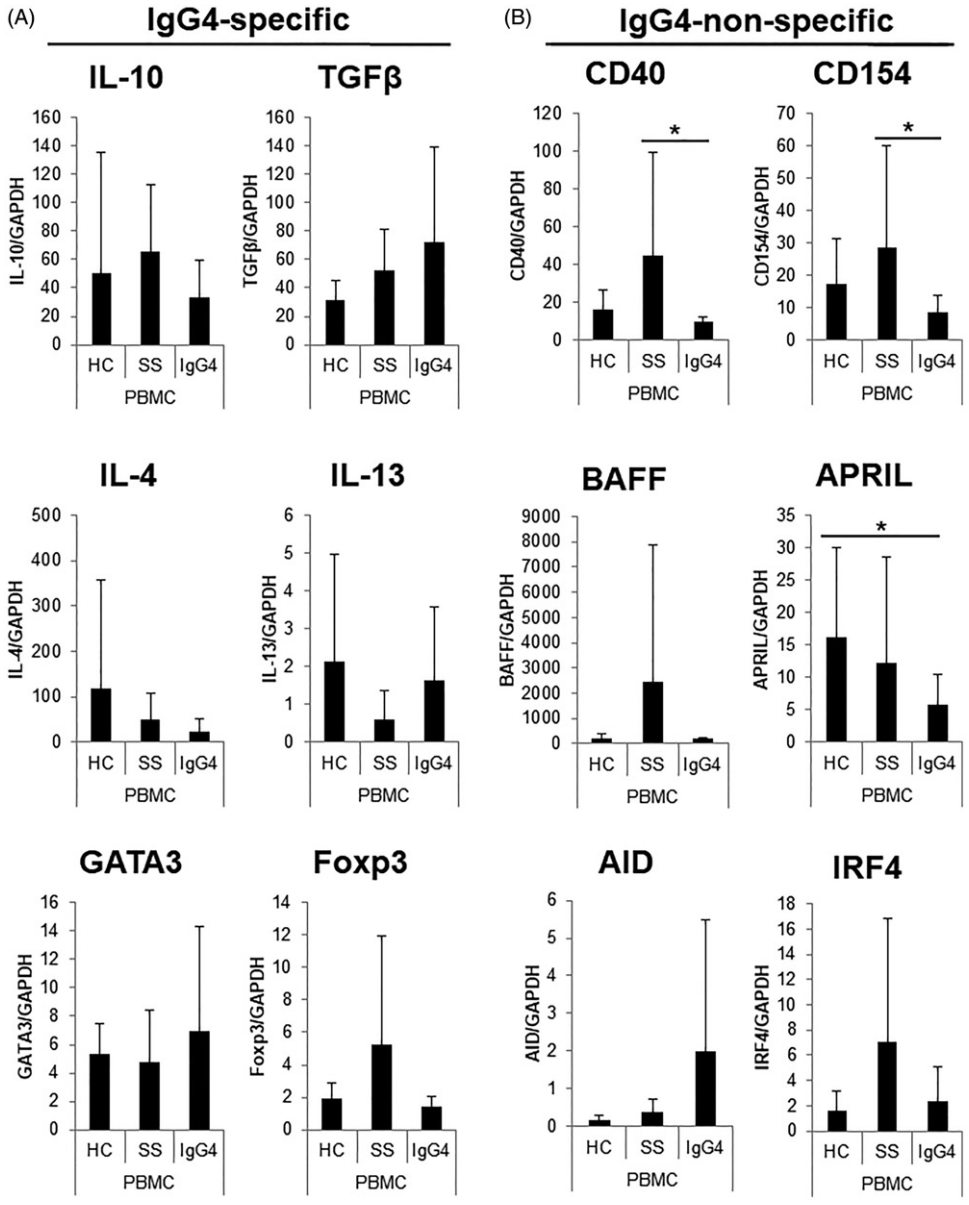

Figure 2. The mRNA expression levels of IgG4-specific and non-specific immunoglobulin class switch-related molecules in PBMCs [30]. The displayed mRNA expression levels in peripheral blood mononuclear cells (PBMC) are relative to the mRNA level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), representing the internal control. (A) IgG4-specific immunoglobulin class switch-related molecules, and (B) IgG4-non-specific immunoglobulin class switch-related molecules. Data are mean ± SD. $p < .05$, by Mann–Whitney's *U* test. HC: healthy control; SS: Sjögren's syndrome; IgG4: IgG4-RD.

significantly higher in LSGs of IgG4-RD than SS and HCs. Considered together, these results suggest that up-regulation of AID accompanied by IL-10 overexpression seem to contribute to IgG4-specific immunoglobulin class switch recombination, and that abundantly produced TGFβ seems to induce fibrosis in LSGs of IgG4-RD. Thus, IgG4 class switch recombination seems to be mainly up-regulated in affected organs [30].

Comparison of gene expression in LSGs between IgG4-RD and SS by DNA microarray

In the next step, we compared the LSGs gene expression pattern among IgG4-RD ($n = 5$), SS ($n = 5$), and HCs ($n = 3$) using DNA microarray. The aim of this analysis was to identify upstream mechanisms that play a role in the infiltration and activation of innate and acquired immune cells, and cross-talk between these cells (http://www.ncbi.nlm.nih.gov/geo/, GEO Series accession number GSE40568) [37]. All LSGs samples of IgG4-RD used in this study also satisfied the histopathological criteria of the 2011 comprehensive diagnostic criteria for IgG4-RD [27]. The principal component analysis (PCA) demonstrated three different clusters of the gene expression patterns in LSGs of IgG4-RD, SS, and HCs, suggesting that the gene expression pattern are broadly different in IgG4-RD, SS, and HCs (Figure 4) [37]. Since PCA demonstrated different gene expression patterns in IgG4-RD and SS versus HCs, we next compared the gene expression, and identified differentially expressed genes (DEGs) between IgG4-RD and SS in pairwise comparisons. Thus, 1771 probe sets (corresponding to

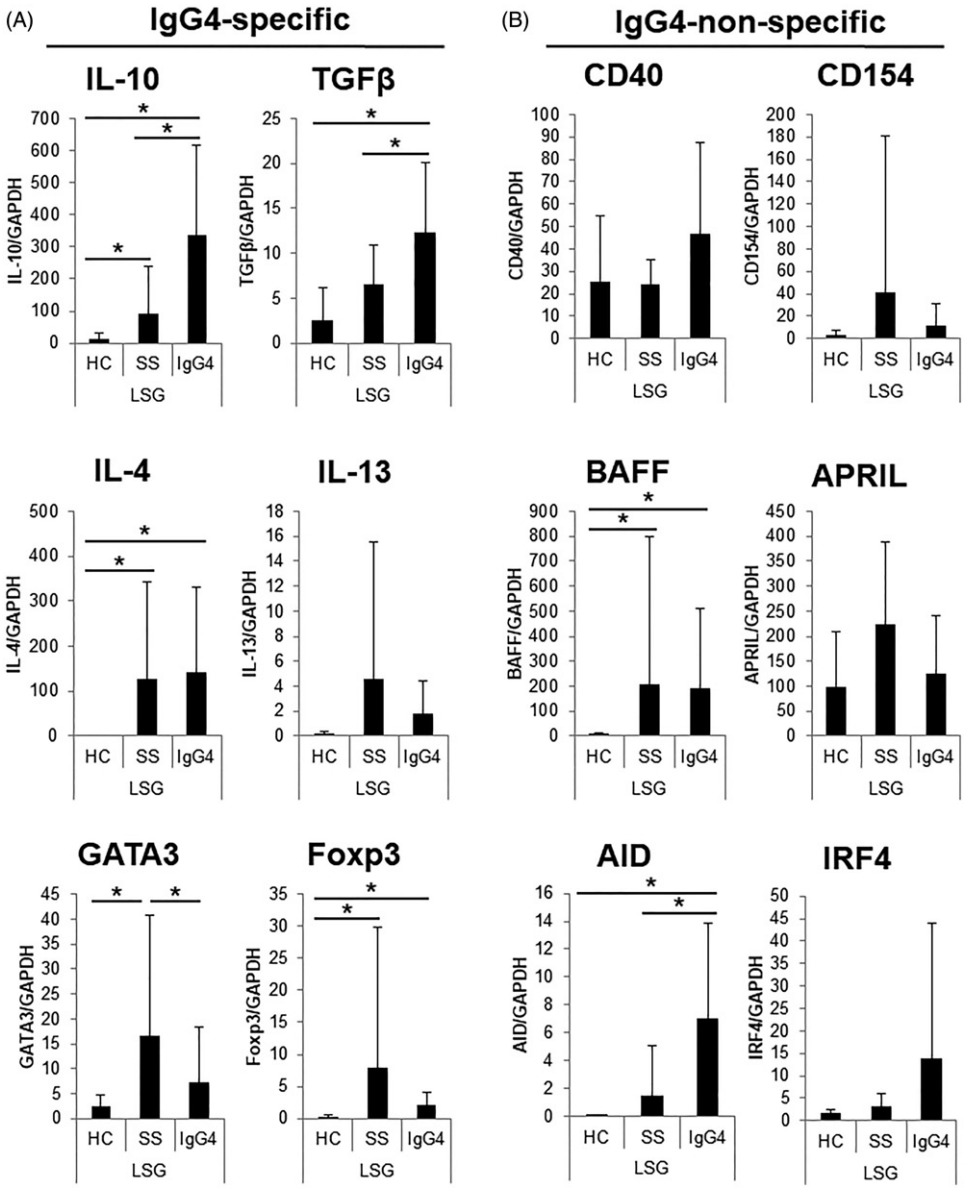

Figure 3. The mRNA expression levels of IgG4-specific and non-specific immunoglobulin class switch-related molecules in LSGs [30]. The displayed mRNA expression levels in labial salivary glands (LSG) are relative to the mRNA level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), representing the internal control. (A) IgG4-specific immunoglobulin class switch-related molecules, and (B) IgG4-non-specific immunoglobulin class switch-related molecules. Data are mean ± SD. $^{*}p < .05$, by Mann–Whitney's *U* test. Abbreviations as in Figure 2.

1321 genes) were identified as up-regulated DEGs in IgG4-RD, compared with SS, by the rank products method, with a false discovery rate (FDR) of <.05 [37]. On the other hand, 1785 probe sets (corresponding to 1320 genes) were identified as down-regulated DEGs in IgG4-RD, compared with SS [37]. The down-regulated DEGs in IgG4-RD (equal to up-regulated DEGs in SS) included many IFN-inducible genes, in agreement with our previous DNA microarray study that showed overexpression of IFN-inducible genes in LSGs of SS relative to that of the control [38].

We also applied gene-annotation enrichment analysis by Gene Ontology (GO) annotation using the web tool DAVID [39]. The analysis showed that the up-regulated set of DEGs in IgG4-RD encoded proteins that function in various biological processes, such as wound healing, response to inorganic substances, skeletal system development, myogenesis, cardiogenesis, angiogenesis, cell morphogenesis and differentiation, cell projection organization, muscle contraction, extracellular matrix organization, actin cytoskeleton organization, cell–matrix adhesion, regulation of cell migration, regulation of cell–substrate adhesion, positive regulation of cell adhesion, regulation of cell proliferation, enzyme-linked receptor protein signaling pathways, regulation of inflammatory response, and translational elongation [37]. On the other hand, the down-regulated set of DEGs in IgG4-RD encoded proteins that function in protein glycosylation, immune response, antigen processing and presentation of peptide antigens via MHC class I, Golgi vesicle transport, cotranslational protein targeting to cell membranes, endoplasmic reticulum (ER) unfolded protein response, and response to viruses [37]. These findings suggest that IgG4-RD is 'a cell and extracellular matrix proliferative disease', whereas SS is 'an autoimmune disease related to IFN signaling'.

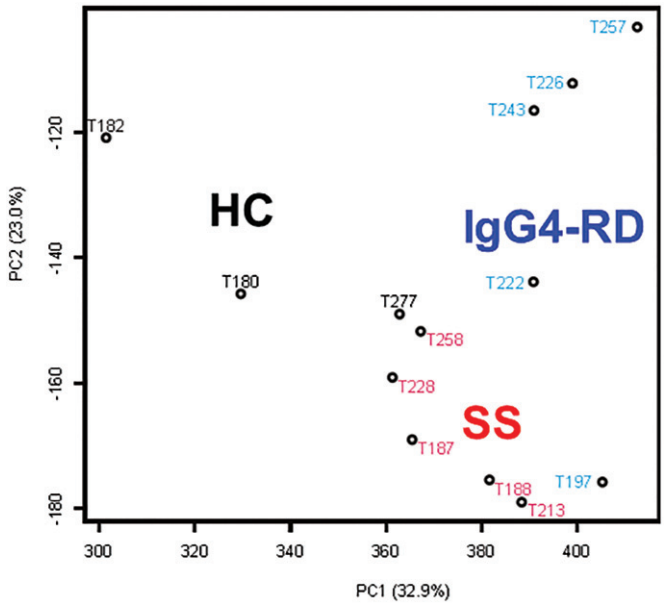

Figure 4. Principal component analysis for gene expression by DNA microarray data [37]. Principal component analysis (PCA) showed different gene expression patterns of IgG4-related disease (IgG4-RD), Sjögren's syndrome (SS), and healthy controls (HCs). The contribution ratio of principal component (PC) 1 was 32.9%, and that of PC2 was 23.0%. T197, T222, T226, T243, and T257; samples from patients with IgG4-RD (blue characters). T187, T188, T213, T228, and T258; samples from patients with SS (red characters). T180, T182, and T277; samples from healthy controls (black characters). IgG4-RD: IgG4-related disease; SS: Sjögren's syndrome; HC: healthy control.

Validation of DEGs in DNA microarray by quantitative PCR, and identification of CCL18 and LTF among upregulated DEGs in LSGs of IgG4-RD compared with SS

In the next step of this study, we selected 10 DEGs from the top 120 up-regulated DEGs in IgG4-RD (relative to SS) for validation by quantitative PCR, based on the following protocol. The selected DEGs had higher rank (higher than 80), smaller FDR (<0.0001), higher fold change (>1.5), higher expression level, smaller dispersion between samples, and functional relationship with immune/inflammatory response, fibrosis, chemotaxis, and cell proliferation. We selected two immune/inflammatory response-related genes [lactotransferrin (LTF, rank 2) and collectin sub-family member 12 (COLEC12, rank 28)], four fibrosis-related genes [decorin (DCN, rank 5), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1, rank 12), EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1, rank 21), and fibronectin 1 (FN1, rank 27)], three chemokines [chemokine (C-X-C motif) ligand 12 (CXCL12, rank 7), chemokine (C-X-C motif) ligand 14 (CXCL14, rank 10), and CCL18 (rank 71)], and a single cell proliferation-related gene [retinoic acid receptor responder 1 (RARRES1, rank 18)] [37]. We performed quantitative PCR to validate the results of DNA microarray analysis, using total RNA extracted from LSGs of 9 patients with IgG4-RD, 10 patients with SS, and 4 HCs other than the cases analyzed by DNA microarray. In the validation study, the mRNA expression level of CCL18 was significantly higher in IgG4-RD than in SS and HCs ($p < .05$). However, there were no significant differences among the three groups with regard to the other chemokines (CXCL12 and CXCL14). Furthermore, the expression level of LTF was significantly higher in IgG4-RD than in SS ($p < .05$). Although the expression levels of the other six genes (COLEC12, DCN, LYVE1, EFEMP1, FN1, and RARRES1) were higher in IgG4-RD than SS and HCs, the differences did not reach statistical significance (Figure 5) [37]. These results confirmed overexpression of CCL18 and LTF in LSGs of IgG4-RD relative to SS [37].

What is known about LTF and CCL18? LTF is an iron-binding protein abundantly present in milk. LTF has a wide range of functions including anti-bacterial and viral activities, anti-cancer activities, wound healing, fibroblast proliferation, and bone growth, in addition to iron binding [40]. Moreover, LTF has been recently reported to stimulate the maturation of DCs and recruit various leukocytes [40]. Interestingly, it was reported that 20.8–76% of patients with type 1 AIP had auto antibodies against LTF [41], indicating the association between immune response against LTF and the pathogenesis of AIP. Thus, LTF or immune response against LTF could be associated with the pathogenesis of IgG4-RD through the activation of innate immune responses and fibroblast proliferation.

On the other hand, CCL18 is a CC chemokine ligand expressed on a broad range of monocytes/macrophages and DCs [42]. Various factors, including lipopolysaccharide (LPS), CD40 ligand, IL-4, and IL-10, can stimulate the production of CCL18 from these cells [42]. Interestingly, IL-10 was up-regulated in LSGs of IgG4-RD as demonstrated in quantitative PCR analysis described above [30], and TLR4, which is a receptor for LPS, was also significantly up-regulated in IgG4-RD compared with SS in our DNA microarray analysis (rank 658) [37]. Previous *in vitro* biological activity studies reported that CCL18 had chemotactic activity on various T cells ($CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, naïve T cells, and memory T cells), B cells (naïve B cells and germinal center B cells), and immature DCs, and that it can induce collagen production from lung fibroblasts [42]. A recent study also indicated that CCL18 recruited human Treg cells [43]. Furthermore, enhanced CCL18 production has been demonstrated in several human diseases, including various malignancies and inflammatory joint, lung, skin, and vessel diseases [42]. In addition to our results, our colleague Moriyama et al. also reported IL-10 and CCL18 expression in $CD163^+$ M2 macrophages in the submandibular glands of IgG4-RD [17]; with CCL18 being up-regulated DEG in the submandibular glands of IgG4-RD by DNA microarray analysis compared with chronic sialadenitis caused by sialolith [44]. A more recent study concluded that serum level of CCL18 could be a useful biomarker for evaluation of not only IgG4-RD disease activity, but also the response to therapy [45]. Collectively, the above findings suggest the importance of CCL18 overexpression in the pathogenesis of IgG4-RD, through its role in fibrosis, recruitment of various cells, including Treg cells, into the affected organs, and association with disease activity and/or response to therapy.

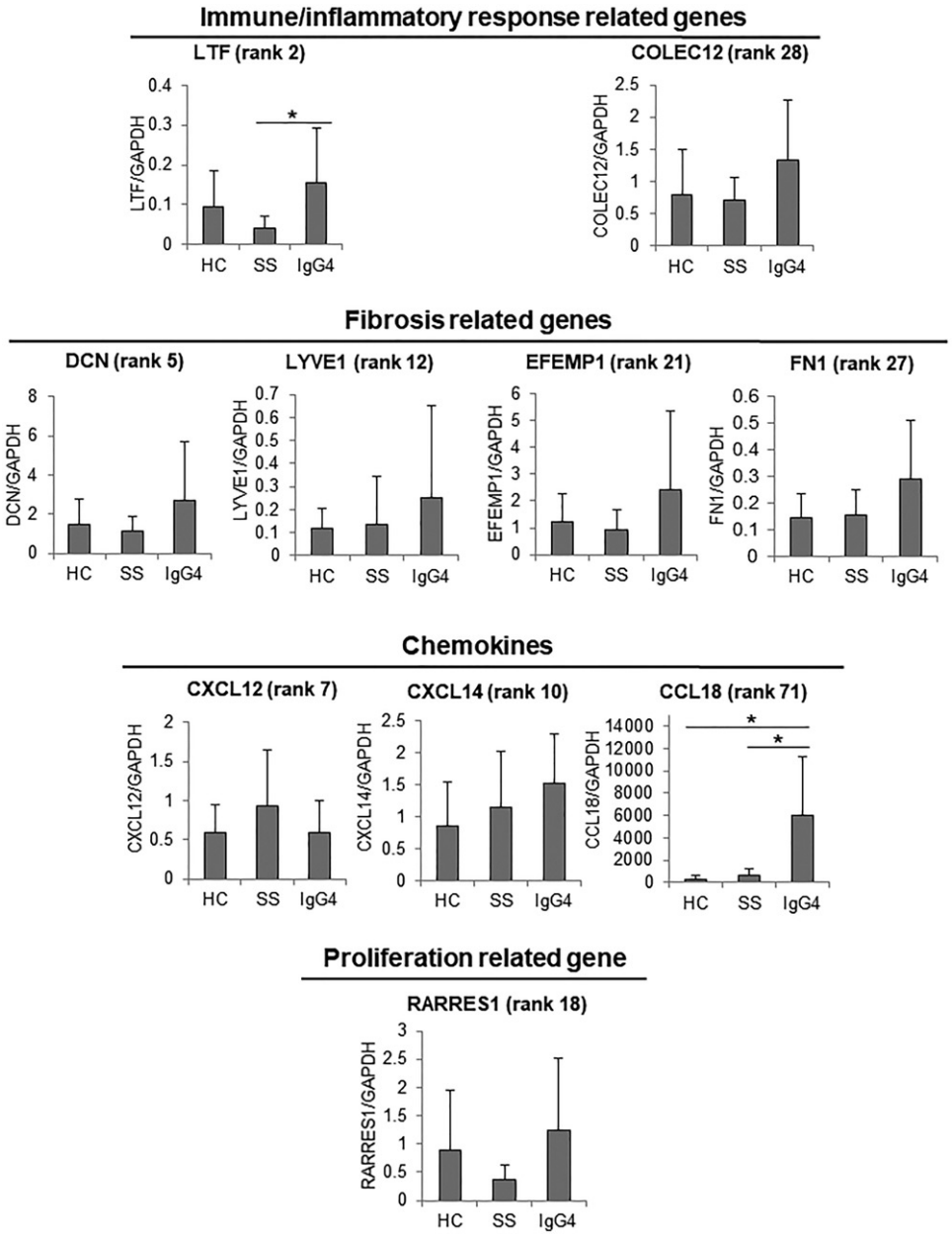

Figure 5. Validation of differentially expressed genes in DNA microarray by quantitative PCR [37]. Quantitative PCR analysis using labial salivary glands (LSGs) of nine patients with IgG4-related disease (IgG4), 10 patients with Sjögren's syndrome (SS) and four healthy controls (HC). These patients were different from those analyzed by DNA microarray. Data are mean ± SD. $^{*}p < .05$, by the Kruskal–Wallis test. LTF: lactotransferrin; COLEC12: collectin sub-family member 12; DCN: decorin; LYVE1: lymphatic vessel endothelial hyaluronan receptor 1; EFEMP1: EGF-containing fibulin-like extracellular matrix protein 1; FN1: fibronectin 1; CXCL12: chemokine (C-X-C motif) ligand 12; CXCL14: chemokine (C-X-C motif) ligand 14; CCL18: chemokine (C–C motif) ligand 18; RARRES1: retinoic acid receptor responder 1.

CCL18 protein expression and CCL18-expressing cells in LSGs of IgG4-RD

The next series of experiments were designed to identify the cells that express CCL18 protein. First, we compared CCL18 protein expression levels in LSGs of patients with IgG4-RD ($n = 3$), primary SS (pSS) ($n = 3$), and HCs ($n = 3$), by immunofluorescence staining. As in our previous studies [30,37], all LSGs samples of IgG4-RD used in this analysis satisfied the histopathological criteria of the 2011 comprehensive diagnostic criteria for IgG4-RD [27]. CCL18 protein expression was strongly detected in LSGs of IgG4-RD, but not in those of HCs, and only a few CCL18-positive cells were detected in LSGs of pSS (Figure 6(A)). Next, we assessed the types of cells that expressed CCL18 protein. Double immunofluorescence staining identified CCL18 expression in macrophages, CD11c + cells, B cells, and plasmacytes in LSGs of IgG4-RD (Figure 6(B)).

As described above, CCL18 is known to be expressed in a broad range of monocytes/macrophages and DCs and induced by a variety of stimuli, such as LPS, CD40L, IL-4, and IL-10 [42]. Accordingly, we speculate that the up-regulated IL-10 and LPS-TLR4 axis could play a role in the up-regulation of CCL18 on macrophages and $CD11c^{+}$ cells in IgG4-RD. Moreover, we propose that CCL18 is specifically expressed on B cells and plasmacytes of IgG4-RD.

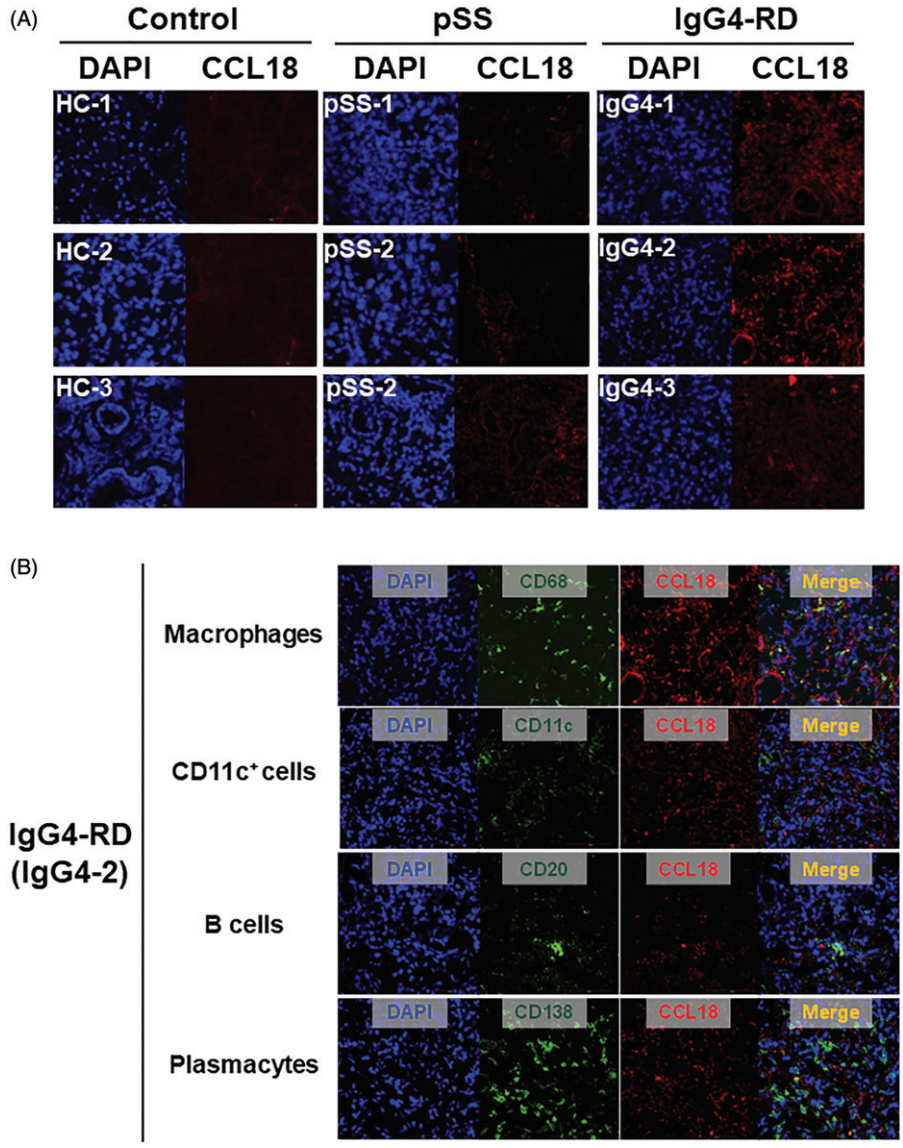

Figure 6. CCL18 protein expression and CCL18-expressing cells in LSGs of IgG4-RD. (A) Comparison of CCL18 protein expression in LSGs of patients with IgG4-related disease (IgG4-RD, $n = 3$), primary Sjögren's syndrome (pSS, $n = 3$), and healthy controls (HC, $n = 3$). Representative fluorescence microscopic images of DAPI/CCL18 staining. Original magnification ×600. IgG4; IgG4-related disease, DAPI; 4′,6-diamidino-2-phenylindole. (B) Fluorescence microscopic images of DAPI/cell surface markers/CCL18/merged of a representative patient (IgG4-2) with IgG4-related disease (IgG4-RD). Original magnification ×600. IgG4: IgG4-related disease; DAPI: 4′,6-diamidino-2-phenylindole.

Possible pathogenic mechanisms of IgG4-RD based on comparison with SS

Our results of comparative analysis of IgG4-RD and SS, based on quantitative PCR [30], DNA microarray [37], and IF staining, allow us to propose the following scenario in the development of IgG4-RD (Figure 7). First, LPS activates several innate immune cells, e.g. macrophages and DCs, via TLR4-mediated signaling. Actually, our DNA microarray analysis showed up-regulated expression of TLR4 in DEGs of IgG4-RD compared with SS [37]. The activated innate immune cells, in addition to B cells and plasmacytes, could produce CCL18, which exert chemotactic effects on T cells, including Treg cells and B cells, leading to their transmigration into the affected tissues. Second, the infiltrating Treg cells produce Treg-related cytokines, such as IL-10 and TGFβ. Third, the Treg cell-produced IL-10 induces IgG4/IgE class switch recombination along with AID, which is highly expressed in affected tissues. Along with CCL18, TGFβ induces fibrosis. Finally, IL-10 induces up-regulation of CCL18 production from innate immune cells. Thus, the above proposed molecular pathological processes form a positive feedback loop via IL-10 production. In addition, LTF could also contribute to the pathogenesis via induction of fibroblast proliferation and maturation of DCs.

Conclusion

The present study was based on the concept that comparison of LSGs of IgG4-RD and SS is suitable for meaningful analysis of the pathogenesis of IgG4-RD. Our comparative study using quantitative PCR, DNA microarray, and IF staining identified the potential roles of several important molecules (e.g. IL-10, TGFβ, AID, LTF, and CCL18) in the pathogenesis of IgG4-RD. Our findings are potentially useful

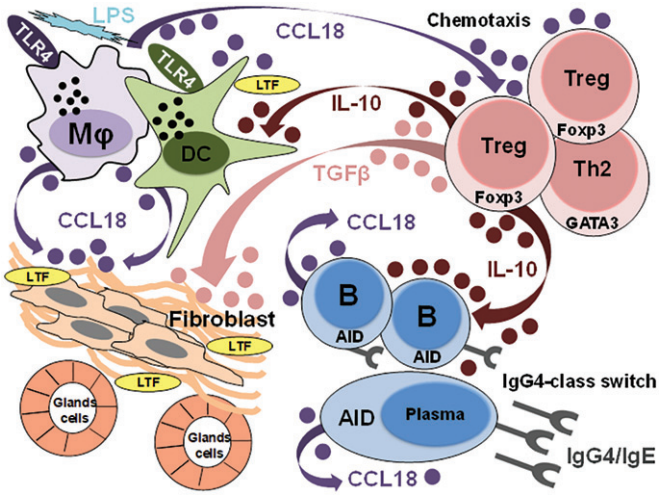

Figure 7. Possible pathogenic mechanisms of IgG4-RD established by comparison of IgG4-RD and SS [37, with modification]. Th2: T helper 2 cells; Treg: regulatory T cells; Mφ: macrophages; DC: dendritic cells; TLR4: Toll-like receptor 4; LPS: lipopolysaccharide; LTF: lactotransferrin; AID: activation-induced cytidine deaminase.

for the identification of disease-specific biomarkers and the development of novel therapies for IgG4-RD.

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Conflict of interest

None.

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