IL-18 is highly expressed in inflammatory infiltrates of submandibular glands in patients with immunoglobulin G4-related disease

著者	小森 岳
著者別表示	Komori Takeshi
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Corresponding Author: Prof. Tomokazu Yoshizaki, M.D., Ph.D.

Corresponding Author's Institution: Graduate school of Medicine, Kanazawa University

First Author: Takeshi Komori, MD

Order of Authors: Takeshi Komori, MD; Satoru Kondo, M.D., Ph.D. ; Naohiro Wakisaka, M.D., Ph.D.; Yousuke Nakanishi, M.D., Ph.D. ; Sayaka Nakanishi-Yagi, M.D., Ph.D. ; Akira Tsuji, M.D., Ph.D. ; Kazuhira Endo, M.D., Ph.D. ; Shigeyuki Muro, M.D., Ph.D. ; Tomokazu Yoshizaki, M.D., Ph.D.

Abstract: Abstract

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a new disease entity characterised by high serum IgG4 concentrations, infiltration of IgG4-positive plasmacytes and fibrosis of various organs. Several groups have reported that IgG4-RD is a unique inflammatory disorder characterised by an immune reaction predominantly mediated by T helper (Th) 2 and regulatory T (Treg) cells. Meanwhile, recent studies have demonstrated that interleukin (IL)-18 has a potential to trigger the production of Th2 cytokines by Th1 cells. We analysed IL-18 expression in submandibular glands of patients with IgG4-RD (20 cases) and controls (19 cases) by immunohistochemical analysis and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). We found that IL-18 was highly expressed in submandibular glands of patients with IgG4-RD than in controls with both protein ($p < 0.05$, chi-square test) and mRNA levels ($p < 0.05$, Mann-Whitney U test). In addition, the expression of IL-18 and IL-13 was correlated in submandibular glands of patients with IgG4-RD. Moreover, by analysing dual immunofluorescence staining, a few numbers of cells were double-positive for IL-13 and interferon (IFN)-03B3 at the inflammatory infiltrates of submandibular glands of patients with IgG4-RD. These data suggest a possibility that IL-13 is produced by Th1 cells. We speculated that IL-18 stimulates Th1 cells producing Th2 cytokines and enhances the immune reaction of Th2 cytokines in pathogenesis of IgG4-RD.

Highlights:

The pathogenesis of IgG4-RD remains largely unknown.

IL-18 was highly expressed in submandibular glands of patients with IgG4-RD.

We speculated that IL-18 acts as a reinforcement of Th2 cytokines in IgG4-RD.

Title: IL-18 is highly expressed in inflammatory infiltrates of submandibular glands in patients with IgG4-related disease

Takeshi Komori MD¹, Satoru Kondo MD, PhD¹, Naohiro Wakisaka MD, PhD¹, Yousuke Nakanishi MD, PhD¹, Sayaka Nakanishi-Yagi MD, PhD¹, Akira Tsuji MD, PhD¹, Kazuhira Endo MD, PhD¹, Shigeyuki Murono MD, PhD¹ and Tomokazu Yoshizaki MD, PhD^{1, *}

¹Divisions of Otolaryngology and Head and Neck Surgery, Graduate School of Medicine, Kanazawa University Division of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan

***Corresponding author:** Division of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan; Phone: +81 76 265 2413; Fax: +81 76 234 4265
E-mail address: tomoy@med.kanazawa-u.ac.jp (T. Yoshizaki)

Keywords: IgG4-RD, IL-18, salivary glands, IL-13, IFN- γ

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Abstract

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a new disease entity characterised by high serum IgG4 concentrations, infiltration of IgG4-positive plasmacytes and fibrosis of various organs. Several groups have reported that IgG4-RD is a unique inflammatory disorder characterised by an immune reaction predominantly mediated by T helper (Th) 2 and regulatory T (Treg) cells. Meanwhile, recent studies have demonstrated that interleukin (IL)-18 has a potential to trigger the production of Th2 cytokines by Th1 cells. We analysed IL-18 expression in submandibular glands of patients with IgG4-RD (20 cases) and controls (19 cases) by immunohistochemical analysis and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). We found that IL-18 was highly expressed in submandibular glands of patients with IgG4-RD than in controls with both protein ($p < 0.05$, chi-square test) and mRNA levels ($p < 0.05$, Mann–Whitney U test). In addition, the expression of IL-18 and IL-13 was correlated in submandibular glands of patients with IgG4-RD. Moreover, by analysing dual immunofluorescence staining, a few numbers of cells were double-positive for IL-13 and interferon (IFN)- γ at the inflammatory infiltrates of submandibular glands of patients with IgG4-RD. These data suggest a possibility that IL-13 is produced by Th1 cells. We speculated that IL-18 stimulates Th1 cells producing Th2 cytokines and enhances the immune reaction of Th2 cytokines in pathogenesis of IgG4-RD.

1. Introduction

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a new disease entity characterised by high serum IgG4 concentrations, infiltration of IgG4-positive plasmacytes and fibrosis of various organs such as the pancreas, bile duct, lung, liver,

kidney, aorta, retroperitoneum and salivary glands [1-4]. The clinical features, including serum abnormalities, organ involvement, diagnosis and the therapeutic approach have been reported [1, 2]. However, the pathogenesis of this disease remains largely unknown.

Several investigators have reported on aberrant immunological findings such as T helper (Th) 2-dominated cytokine production, leading to IgG4 hyperproduction and increase in the number of regulatory T (Treg) cells in IgG4-RD [5-10]. Meanwhile, it has been reported that Th2 cytokines such as interleukin (IL)-4 and IL-13 and Treg cytokines such as IL-10 could induce IgG4- and IgE-specific class-switch recombination. In addition, tumour growth factor (TGF)- β , one of the Treg cytokines, can induce the development of tissue fibrosis, which is a unique pathological finding of IgG4-RD. Moreover, this tissue fibrosis develops in various organs. Thus, IgG4-RD is a unique inflammatory disease characterised by an immune reaction predominantly mediated by Th2 and Treg cells [5].

In contrast, it is widely accepted that Th2 cytokines are principally responsible for inducing bronchial asthma [11, 12]. In particular, some studies have suggested that IL-18, a Th1 cytokine, is involved in the pathogenesis of certain types of bronchial asthma [13]. IL-18 was originally identified as a factor that enhances interferon (IFN)- γ production from Th1 cells [14, 15]. However, recent studies have demonstrated that IL-18 has a potential to induce the production of Th2 cytokines by Th1 cells [16]. Based on this unique function of Th1 cells, Nakanishi *et al.* proposed to designate them as 'super Th1 cells' [16]. Super Th1 cells may be involved in the pathogenesis of certain types of allergic disorders by producing both Th1 and Th2 cytokines.

The objectives of this study were to evaluate IL-18 expression in IgG4-RD that could be closely associated with Th2 cytokines and to investigate the association of

super Th1 cells for the pathogenesis of IgG4-RD.

2. Materials and Methods

2.1. Patients

We obtained 20 specimens of submandibular glands from patients with IgG4-RD who had been diagnosed at the Division of Otolaryngology and Head and Neck Surgery at the Kanazawa University Hospital (Kanazawa, Japan) between 2003 and 2012 (14 males and 6 females; age, 34–77 years). All 20 patients presented with symmetrical swelling of submandibular glands and underwent surgical resection for confirmed diagnosis of IgG4-RD. We used these resected specimens for the experiment in our study. The characteristics of these patients are shown in Table 1. All patients with IgG4-RD satisfied the comprehensive diagnostic criteria for IgG4-RD proposed by the All Japan IgG4 team in 2011 [17]. The diagnosis of IgG4-RD was based on the presence of the following three items: (1) clinical examination showing characteristic diffuse/localized swelling or masses in single or multiple organs; (2) hematologic examination showing elevated serum IgG4 concentrations (135 mg/dL); and (3) histopathologic examination showing (a) marked lymphocyte and plasmacyte infiltration and fibrosis and (b) infiltration of IgG4⁺ plasma cells (ratio of IgG4⁺/IgG⁺ cells >40% and >10 IgG4⁺ plasma cells/HPF).

As controls, we obtained 19 specimens of lymph nodes (LNs) from patients with reactive lymph nodes who underwent LN dissection (9 males and 10 females aged 20–82 years) and 30 specimens of submandibular glands from patients with sialolithiasis and pleomorphic adenoma who underwent surgical resection (17 males and 13 females aged 19–72 years). We labelled the two types of control cases as controls of LN and controls of submandibular glands. We excluded LNs of patients

diagnosed with malignant diseases or lymphoma. The diagnosis was based on clinical data and pathological findings. Reactive lymph nodes had lymphoid follicles that were similar to lymphoid infiltrates of submandibular glands of patients with IgG4-RD according to the pathological finding. Controls of submandibular glands were diagnosed at the the Division of Otolaryngology at Kanazawa University Hospital between 1990 and 2012. Controls of LN were diagnosed between 2013 and 2014.

The study was approved by the Ethics Committee of the Kanazawa University, and informed consent was obtained from each patient before enrolment.

2.2. Laboratory Data

The serum samples were collected from patients at first presentation and stored at -80°C until further processing. Serum Igs (IgG, IgG4, and IgE) were measured routinely.

2.3. Immunohistochemical analysis of submandibular gland and lymph node tissues

Specimens of submandibular glands for both IgG4-RD and controls and specimens of lymph nodes for controls were examined by immunohistochemical analysis of IgG, IgG4, IL-4, IL-13, IL-18 and IFN- γ expression. Three- μm -thick sections were prepared from each block of tissue embedded in paraffin. Deparaffinised sections were treated with 3% hydrogen peroxide for 10 min to inactivate endogenous peroxidase activity. The sections were incubated with protein blocker (Dako, Glostrup, Denmark) for 30 min and incubated at 4°C overnight with rabbit anti-human IgG antibody(1:5000, Dako), mouse anti-human IgG4 antibody (1:2000, Life technologies, Grand Island, NY), rabbit anti-human IL-18 antibody (PM014, 1:2000, Medical and

Biological Laboratories CO., LTD., Nagoya, Japan), rabbit anti-human IL-13 antibody (06-1090, 1:200, MILLIPORE, MA, USA), rabbit anti-human IL-4 antibody (ab9622, 1:200, Abcam, Cambridge, UK) and rabbit anti human IFN- γ antibody (ab9657, 1:500, Abcam) as primary antibodies. The sections were washed three times with phosphate-buffered saline (PBS, pH 7.2). After washing with PBS, the sections were exposed to Envision⁺ secondary antibody (Dako) for 30 min. The reaction products were developed by immersing the sections in a 3'3'-diamidobenzidine tetrahydrochloride solution. The sections were counterstained with hematoxylin.

2.4. Evaluation of the specimens

We first examined IL-18 expression in specimens of submandibular glands of patients with IgG4-RD and control cases. Bombardieri *et al.* reported that IL-18 is expressed in ductal epithelial cells and periductal inflammatory foci of salivary glands in patients with Sjögren's syndrome [18, 19, 20]. In this report, periductal mononuclear infiltrate was defined as an inflammatory focus when at least 50 mononuclear cells with focal organization were present. Meanwhile, it was reported that IL-18 expression was only observed in ductal epithelial cells from submandibular glands of patients with chronic sialoadenitis, but not in periductal inflammatory foci of patients with the same condition [18]. Therefore, we evaluated cytokine expression in inflammatory infiltrates of submandibular glands in the present study. According to similarity of pathological findings between inflammatory infiltrates of submandibular glands of patients with IgG4-RD and lymphoid follicle of LN, we evaluated cytokine expression in lymphoid follicles of LNs in controls of LN.

The stained sections were independently evaluated by two investigators (T. K. and S. K.) who were blinded to the clinical data. After counting both immunoreactive

lymphocytes and total number of lymphocytes at five different high-power fields (HPFs, 400×) with intense inflammation, the average frequency of immunoreactive lymphocytes was calculated. The average percentage of immunoreactive lymphocytes was defined as the expression score and was used for statistical analysis. The cases were classified into negative and positive categories as follows: negative, <10% immunoreactive cells; positive, ≥10% immunoreactive cells.

2.5. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from frozen tissues in cases of IgG4-RD (7 cases) and pleomorphic adenoma of submandibular glands (6 cases in controls of submandibular gland) using the RNeasy Plus Mini Kit (QIAGEN, Valencia, CA, USA). For real-time RT-PCR analysis, total RNA was reverse transcribed using SuperScript III (Invitrogen, Carlsbad, CA, USA). The quantification of gene expression was performed by quantitative RT-PCR using the Mx3000P QPCR System (Agilent Technologies, Santa Clara, CA, USA) and QuantiTect SYBR Green PCR Kit (QIAGEN). Specific primers for IL-4, IL-13, IL-18, IFN- γ and GAPDH were obtained from QIAGEN (QuantiTect Primer Assay). The cycling conditions were as follows: incubation for 5 min at 95°C and 40 cycles of 10 s at 95°C and 30 s at 60°C. The data were normalised to GAPDH. Each experiment was performed in triplicate, and the mean was adopted as the value in each experiment.

2.6. Dual fluorescent immunostaining of IL-13 and IFN- γ

IL-18 has the potential to induce Th2 cytokine production by Th1 cells [21]. In other words, super Th1 cells are capable of producing both Th1 and Th2 cytokines [21].

To further investigate whether cells that produce both Th1 and Th2 cytokines exist in submandibular glands of patients with IgG4-RD, we examined dual immunofluorescence staining of IL-13 as a Th2 cytokine and IFN- γ as a Th1 cytokine in six samples from patients with IgG4-RD.

All formalin-fixed and paraffin-embedded specimens of six cases of IgG4-RD were used for dual fluorescent immunostaining of IL-13 and IFN- γ . The deparaffinised sections were microwaved in citrate buffer (pH 6.0) for 20 min and incubated in protein block solution (Dako) for 20 min. Deparaffinised sections were treated with 3% hydrogen peroxide for 10 min to inactivate endogenous peroxidase activity. Specimens were incubated with a rabbit anti-human IL-13 antibody (06-1090, 1:500, MILLIPORE) and a mouse anti-human IFN- γ antibody (clone25718, 1:10, R&D Systems, MN, USA) overnight at 4°C. The reaction product was visualised with fluorescent goat anti-rabbit Alexa Fluor 488 and anti-mouse Alexa Fluor 594 IgG secondary antibodies (1:500, Invitrogen). Specimens were counterstained with 4', 6-diamidino-2-phenylindole (Invitrogen). No positive staining was observed when the primary antibodies were omitted or replaced with normal serum in the negative controls during staining procedures.

2.7. Statistical analysis

IBM SPSS Statistics version 19 (IBM, Armonk, NY, USA) was used for data analysis. IL-18 expression was analysed using Fisher's exact test and the chi-square test. The relationships between the expression scores of IL-4, IL-13, IL-18 and IFN- γ were analysed with Spearman's rank correlation coefficient.

The differences of mRNA levels of IL-4, IL-13, IL-18 and IFN- γ were analysed using the Mann–Whitney U test. The relationship between the mRNA level of

IL-4, IL-13, IL-18 and IFN- γ was analysed using Spearman's rank correlation coefficient. p values of <0.05 were considered statistically significant.

3. Results

3.1. Main clinical data

Main clinical data of patients with IgG4-RD, controls of LN and controls of submandibular glands are shown in Table 1. Of the 20 patients with IgG4-RD, 15 patients had complications. Aside from submandibular glands, the affected organs were pancreas (five cases), bile duct (one case), lacrimal gland (six cases), parotid gland (three cases), lung (six cases), kidney (three cases), retroperitoneum (one case), aorta (four cases), LN (three cases) and skin (one case). The cases who affected only submandibular gland were five cases.

We confirmed the diagnosis of IgG4-RD based on laboratory data and immunohistochemical analysis (Table 1, Figure 1). The serum IgG4 level (normal range, <135 mg/dL) was increased in all patients with IgG-RD. All the serum IgG4/IgG proportions in IgG4-RD cases were >10%. The serum IgE levels (normal range, <170 IU/mL) was increased in 11 IgG4-RD cases. The ratio of IgG4⁺/IgG⁺ cells in pathological specimens of all IgG4-RD cases were > 40%.

3.2. IL-18 is expressed in inflammatory infiltrates in IgG4-RD

IL-18 was remarkably expressed in inflammatory infiltrates of samples from patients with IgG4-RD. Eventually, 17 of 20 cases were categorized in the positive cohort. On the other hand, in controls of LN, only 9 of 19 cases were categorized in the positive cohort (p < 0.05, Table 1, Figure 2). In controls of submandibular glands, only 8 of 30 were categorized in the positive cohort (p < 0.01, Table1, figures are not

shown).

3.3. IL-18 expression correlates with IL-13 and IFN- γ expression

We next investigated the relationship between IL-18 and either Th1 or Th2 cytokines. In this manner, we evaluated expression scores of IL-4, IL-13 and IFN- γ at inflammatory infiltrates in submandibular glands from IgG4-RD patients and controls of LN.

There was no correlation between IL-18 and IL-4 ($r = 0.477$, $p = 0.08$, Figure 3) in IgG4-RD cases. In contrast, the expression of IL-18 was significantly associated with that of IL-13 ($r = 0.71$, $p < 0.01$, Figure 3). Finally, there was a significant correlation between IL-18 and IFN- γ expression, although the expression level of IFN- γ was weak ($r = 0.493$, $p = 0.027$, Figure 3). On the other hand, in controls of LN, the expression of IL-18 was not associated with that of the other cytokines. There were correlation between IL-4 and IFN- γ in both IgG4-RD cases and control cases.

3.4. Level of IL-18 mRNA increases in submandibular glands of IgG4-RD

We analysed mRNA levels of IL-18, IL-13, IL-4 and IFN- γ in submandibular glands of IgG4-RD and pleomorphic adenoma by qRT-PCR (Figure 4). The IL-18 mRNA levels were significantly higher in submandibular glands of patients with IgG4-RD than in those of pleomorphic adenoma ($p < 0.05$, Mann–Whitney U test). Similarly, both IL-4 and IFN- γ mRNA levels were higher in submandibular glands of patients with IgG4-RD ($p < 0.05$). However, there was no significant difference in the IL-13 mRNA levels between IgG4-RD and pleomorphic adenoma ($p = 0.836$).

In addition, we investigated the relationship among mRNA levels of IL-18 and IFN- γ as Th1 cytokines and IL-4 and IL-13 as Th2 cytokines. As expected, there was a

remarkable correlation between mRNA levels of IL-18 and IFN- γ ($r = 0.893$, $p < 0.01$).

Although there was no correlation between IL-18 and IL-13 ($r = 0.143$, $p = 0.760$), the

expression of IL-18 was closely associated with that of IL-4 ($r = 0.714$, $p = 0.071$).

3.5. Evaluation of IL-13- and IFN- γ -positive cells in submandibular glands

As shown in Figure 5, a few numbers of cells were double positive for IL-13 and IFN- γ , staining at inflammatory infiltrates in submandibular glands of patients with IgG4-RD (Figure 5). Both IL-13- and IFN- γ -positive cells in submandibular glands of IgG4-RD suggest the existence of super Th1 cells.

4. Discussion

To the best of our knowledge, this is the first study to show that IL-18 expression is increased in submandibular glands of patients with IgG4-RD. This finding raises the question of what is the function of IL-18 in IgG4-RD.

IL-18 was originally identified as a factor that enhances IFN- γ production from Th1 cells [14, 15]. In studies of bronchial asthma, it was well known that IL-18 was abundantly stored in epithelial cells of various organs, including bronchial epithelial cells [22, 23, 24]. Sugimoto *et al.* showed that adoptively transferred memory-type Th1 cells induced airway inflammation in the lung without airway hyperresponsiveness (AHR) when stimulated with an antigen (Ag). In contrast, memory Th1 cells induced both severe airway inflammation and AHR when stimulated with Ag and IL-18. IL-18 stimulated antigen-specific cloned Th1 cells to produce IL-13 [13]. These cloned Th1 cells produced IL-13 dependent on the doses of IL-18 used for stimulation. However, these Th1 cells did not produce IL-4 when they were stimulated with IL-18. Notably, cloned Th2 cells in this study did not produce either IL-4 or IL-13 when they were

stimulated with IL-18. Surprisingly, Ag plus IL-18-stimulated Th1 cells produced IFN- γ and IL-13, suggesting that they played a relevant role in the induction of bronchial asthma [13]. Moreover, Terada *et al.* demonstrated that Th1 cells induced intrinsic atopic dermatitis by the production of Th1 and Th2 cytokines and chemokines [25]. Based on this unique function of Th1 cells, Nakanishi *et al.* proposed to designate them as 'super Th1 cells' [16]. According to these studies, super Th1 cells produced IL-13 and not IL-4 [16].

Our present study showed that IL-18 was highly expressed in submandibular glands of patients with IgG4-RD with both protein and mRNA levels. In addition, there was a correlation between the expression of IL-18 and IL-13 but not IL-4 in IgG4-RD with protein levels (Figure 3). However, there was no correlation between mRNA levels of IL-18 and IL-13. We speculate that this discrepancy can be explained by the small number of samples that is insufficient to analyse the relationship between mRNA levels of IL-18 and IL-13. It has been previously reported that there were very few IL-18 receptors on Th2 cells [26]. Therefore, we hypothesized that Th1 cells were stimulated by IL-18 to produce IL-13. These Th1 cells that produce Th2 cytokines have been named super Th1 cells. Our data suggest that IL-13 is produced by super Th1 cells and not Th2 cells. Moreover, in our dual immunofluorescence staining data, a few numbers of cells were double-positive for IL-13 and IFN- γ staining at inflammatory infiltrates in patients with IgG4-RD (Figure 4). This finding is assured that a possible existence of super Th1 cells in submandibular glands of patients with IgG4-RD.

There was a correlation between the expression of IL-4 and IFN- γ in submandibular glands of IgG4-RD patients and in reactive LNs of control cases with protein levels. This phenomenon indicated that submandibular glands of IgG4-RD and reactive LNs have a similar environment for immune reactions of Th1 and Th2 cells.

However, there were difference of IL-18 expression and difference of correlative relationship of IL-18 and other cytokines between IgG4-RD and reactive LNs. Because of this reason, IL-18 is considered to be related to the pathogenesis of atypical inflammation of IgG4-RD.

In the present study, IL-18 stained strongly in specimens from submandibular glands of patients with IgG4-RD. We speculate that IL-18 acts as a reinforcement of the immune reaction of Th2 cytokines in IgG4-RD. In addition, super Th1 cells may play a role in the pathogenesis of IgG4-RD from the perspective of Th2 cytokine production.

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Conflicts of Interest

There are no conflicts of interest.

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Figure Legends

Figure 1.

IgG and IgG4 expression in inflammatory infiltrates in the submandibular glands of patients with IgG4-RD

Immunostaining of IgG (A) and IgG4 (B) in submandibular glands of IgG4-RD. Magnification (A, B) $\times 200$. All patients with IgG4-RD satisfied the comprehensive diagnostic criteria for IgG4-RD proposed by the All Japan IgG4 team in 2011.

Figure 2.

IL-18 expression in inflammatory infiltrates in the submandibular glands of patients with IgG4-RD

Immunostaining of IL-18 in submandibular glands of IgG4-RD (A, B) and controls of LN (C, D). Magnification (A, C) $\times 100$, (B, D) $\times 200$. IL-18 was strongly expressed in inflammatory infiltrates of submandibular glands of patients with IgG4-RD. In contrast, there was sparse expression of IL-18 in inflammatory infiltrates of controls of LN.

Figure 3.

IL-18 expression correlates with IL-13 and IFN- γ expression in submandibular glands of patients with IgG4-RD

Immunostaining of IL-4 (A, D), IL-13 (B, E) and IFN- γ (C, F) in submandibular glands of patients with IgG4-RD (A-C) and controls of LN (D-F). Magnification, $\times 200$ (A-F).

There was a strong expression of IL-4 and IL-13 in inflammatory infiltrate of submandibular glands of patients with IgG4-RD (A, B). The relationships between the expression score of IL-18, IL-4, IL-13 and IFN- γ were analysed with Spearman's rank correlation coefficient (G-N).

(G) Correlation between IL-18 and IL-4 expression scores in IgG4-RD. (H) IL-18 and IL-13 in IgG4-RD. (I) IL-18 and IFN- γ in IgG4-RD. (J) IL-4 and IFN- γ in IgG4-RD. (K) IL-18 and IL-4 in controls of LN. (L) IL-18 and IL-13 in controls of LN. (M) IL-18 and IFN- γ in controls of LN. (N) IL-4 and IFN- γ in controls of LN.

Figure 4.

Level of IL-18 mRNA increases in submandibular glands of IgG4-RD

Expression levels of IL-18, IL-13, IL-4 and IFN- γ were examined in submandibular glands of IgG4-RD or pleomorphic adenoma. The relative expression level of samples from P1, one of IgG4-RD cases, was defined as 1. Black columns: seven cases of IgG4-RD; white columns: six cases of control. Levels of IL-18 (A), IL-13 (B), IL-4 (C) and IFN- γ (D) mRNAs are shown. *: $p < 0.05$.

Levels of IL-18 mRNA were significantly higher in submandibular glands of patients with IgG4-RD than in those of pleomorphic adenoma. Similarly, levels of IL-4 and IFN- γ mRNA were higher in submandibular glands of patients with IgG4-RD. There was no statistically significant difference in levels of IL-13 mRNA between IgG4-RD and pleomorphic adenoma ($p = 0.836$).

Figure 5.

Evaluation of IL-13- and IFN- γ -positive cells in submandibular glands

Dual-fluorescent immunostaining of IL-13 (A), IFN- γ (B) and DAPI (C) in submandibular glands of IgG4-RD. IL-13 and IFN- γ were co-expressed in inflammatory infiltrates of submandibular glands. Original magnification: $\times 200$. Both IL-13 and IFN- γ -positive cells in submandibular glands of IgG4-RD suggest existence of super Th1 cells (D).

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448 **Tables**

449 **Table 1. Patient characteristics**

450 n.d., not determined.

Figure 1.

Figure 2.

Figure 3.

Figure 3.

Figure 3.

Figure 4.

Figure 5.

Table(s)

Variables	IgG4-RD	controls of lymph node	P-value	controls of submandibular gland	P-value
Age at diagnosis (years ± SD)	60.3 ± 11.0	52.4 ± 19.9	0.171	53.6 ± 16.9	0.331
Gender					
Female	6	10		17	
Male	14	9	0.200	13	0.064
Serum IgG4 (mg/dL)	663.1 ± 475.1	n.d		n.d	
Serum IgG (mg/dL)	1870.2 ± 702.8	n.d		n.d	
Serum IgE (IU/mL)	1207.0 ± 1024.7	n.d		n.d	
IgG4/IgG in pathological specimens (%)	65.9 ±20.0	n.d		n.d	
IL-18 expression score					
IL-18 positive	17	9		8	
IL-18 negative	3	10	< 0.05	22	< 0.01