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Triptolide inhibits tonsillar IgA production by upregulating FDC-SP in IgA nephropathy

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Running heads: Triptolide inhibits tonsillar IgA production by FDC-SP
ABSTRACT

IgA nephropathy (IgAN) is primarily resulted of qualitative abnormality of IgA. The occurrence of IgAN is associated with affected tonsils which enhances the IgA production via IgA class switching and immuno-activation. Follicular dendritic cell-secreted protein (FDC-SP) was found to be a negative effect for IgA production in tonsil. The previous studies suggested that Triptolide might reduce IgA production by its immunosuppression role. Given this background, this study investigated the mechanisms underlying the role of Triptolide and FDC-SP in the generation of IgA and IgA class switching in tonsil of IgAN patients. Immunohistochemistry and reverse transcription-polymerase chain reaction revealed that the expression of FDC-SP was increased in the tonsils of IgAN patients with Triptolide treatment compared with those without treatment. Meanwhile, the expression of FDC-SP was negatively correlated with IgA inducing cytokines in the tonsils of IgAN patients treated with Triptolide, due to the significant decreased IgA-bearing cells. The expression of FDC-SP in tonsillar tissue was confirmed by double immunofluorescence. Importantly, Triptolide promoted FDC-SP secretion, and correlated negatively with decreased IgA production in isolated FDC-associated clusters, which had been isolated from patients without TW treatment previously. Our study demonstrated that Triptolide might have an impact on FDC-SP production and downregulation of IgA synthesis in the tonsils of IgAN patients, which could be a promising strategy for therapeutic intervention in IgAN patients.
Keywords: IgA nephropathy; Triptolide; follicular dendritic cell-secreted protein; tonsil; IgA class switching; IgA production

1. Introduction

Immunoglobulin A nephropathy (IgAN) is a very common primary glomerular disease in the world since it was first described by Berger in 1968. IgAN is characterized by IgA deposition and enhancement of circulating aberrant IgA in the renal mesangium (Bellur et al., 2019). It is suggested that IgA of tonsillar origin was related to the pathogenesis of IgAN (Su et al., 2017). Previous studies have shown that the number and relative percentage of IgA-containing cells in the tonsils of IgAN patients were significant (Meng et al., 2016). The germinal center (GC) is the primary zone of B cell proliferation, and follicular dendritic cells (FDCs) support IgA class switching (Koutsakos et al., 2019). B cells functionally interact with FDCs in the GC and undergo several critical functional processes, including proliferation, apoptosis, somatic hypermutation, selection for high-affinity antigen binding, isotype switching, and differentiation into plasma cells or memory cells. Upon activation by antigen and accessory signals, tonsillar GC naive IgM IgD B cells may acquire IgA expression by undergoing class switch recombination (CSR) (Proietti et al., 2019).

FDC-secreted protein was firstly identified in primary FDCs which had been isolated from human tonsils (Marshall et al., 2002). The previous study has reported that FDC-SP regulates GC and antibody responses and modulates B cell activity (Liu et al., 2016). The recently study has also shown that FDC-SP regulates IgA production in tonsils in individuals with IgAN (Hou et al., 2014).

Triptolide, a diterpene triepoxide, is the major biologically active compound
isolated from a traditional Chinese medicinal herb Tripterygium wilfordii Hook F. (also named Lei Gong Teng) (Guo et al., 2019). Triptolide exhibits multiple pharmacological effects, especially in anti-autoimmune diseases including anti-IgAN, anti-rheumatoid arthritis, anti-psoriasis and anti-lupus by its immunosuppression effect (Sun et al., 2019). Moreover, the benefits of Tripterygium wilfordii for IgAN patients suggested that Tripterygium wilfordii might be closely related to tonsillar production of IgA (Liang et al., 2018; Wang et al., 2019). However, the role of Triptolide in tonsillar IgA production in IgAN is unknown due to the complexity of traditional Chinese medicine ingredients. Furthermore, the molecular and cellular mechanisms remain unknown.

The aims of this study was to evaluate the role of Triptolite involved in tonsillar IgA production and to investigate the underlying molecular mechanism of Triptolide in regulation of the synthesis of IgA and IgA class switching in IgAN patients. Our study demonstrated that Triptolide might inhibit IgA production and tonsillar IgA class switching and by upregulating FDC-SP synthesis.

2. Materials and Methods

2.1. Research subjects

Sixty patients were enrolled in the present study. Among those, 20 IgAN patients diagnosed by biopsy were treated with Tripterygium wilfordii before tonsillectomy, 20 patients with biopsy-proven IgAN without Tripterygium wilfordii treatment, and 20 patients with chronic tonsillitis but not renal disease or a history of hematuria after
Indications for tonsillectomy in hematuria-type IgAN patients were demonstrated in previous studies, especially those presenting hematuria after tonsillar infection; with a baseline creatinine level of $\leq$ 2mg/dl. Patients were recruited at the First Affiliated Hospital of HeiLongjiang University of Chinese Medicine (Harbin, China) and Harbin Medical University Cancer Hospital (Harbin, China) from January 2000 to December 2016. IgAN patients with TW treatment received 60mg/d for 60 days of dosing before tonsillity. The patients have been informed about the possible side effects of the TW therapy. The patients had no obvious side effects because of the short duration of medication. Patients with Henoch–Schönlein purpura, systemic lupus erythematosus, liver cirrhosis, palmoplantar pustulosis, rheumatic arthritis and ossification, or other systemic diseases were excluded. Palatine tonsil tissues were sectioned from enrolled IgAN and non-IgAN chronic tonsillitis patients during tonsillectomy.

2.2. Ethics statement

This study was performed according to the principles of the Declaration of Helsinki. All participants were informed and consented to the study. Approval for this study was obtained from the Medical Ethics Committees of First Affiliated Hospital of HeiLongjiang University of Chinese Medicine (HZYLLBA201714).
2.3. Antibodies, IHC, and immunofluorescence

For IHC, tonsil tissues were fixed with formalin, then were embedded in paraffin, subsequently were sectioned by microtome. 4 µm sections were blocked with 1% H$_2$O$_2$ and then subjected to antigen retrieval in trypsin for 30 min at 37°C; followed by immersion in citrate buffer (pH 6.0; Mitsubishi Chemical Medience, Tokyo, Japan) for 20 min at 120°C in an autoclave. IHC was performed using either the streptavidin-biotin-peroxidase complex (strept-ABC) or the alkaline phosphatase anti-alkaline phosphatase (APAAP) method as previously reported (Meng et al., 2016). Sections were then blocked with Protein Blocking Agent (Streptavidin-Biotin Universal Detection System; Beckman Coulter, Marseille, France) and incubated with the following primary antibodies overnight at 4°C: rabbit anti-human FDC-SP (1:100, IgG, Abcam, Cambridge, UK), rabbit anti-IgA (1:100, Nichirei, Tokyo, Japan), mouse anti-IgG (1:100, A57H; IgMκ, Nichirei), rabbit anti-IgE (1:100, Dako, Japan), or rabbit anti-IgM (1:60, IgG, Covance). This was followed by incubation with secondly antibodies from the Streptavidin-Biotin Universal Detection System (Beckman Coulter). Sections were visualized using DAB. Specific isotype control antibodies and phosphate-buffered saline (PBS; omitting primary antibodies) were used as negative controls. The number of cells staining positive by IHC and immunofluorescence were scored as 0 (absent), 1+ (<25% of GC cells), 2+ (25–50% of GC cells), 3+ (50–75% of GC cells), or 4+ (> 75% of GC cells). The scoring was done in a blinded fashion.

FDC-associated clusters were cultured with RPM1 1640 containing 10% FBS in Millicell EZ 4-well glass slides (EMD Millipore Corporation, Billerica, MA, USA)
before staining. After 7 days FDC-associated clusters were rinsed in PBS then fixed
with 4% paraformaldehyde in PBS for 20 min, then subjected to IHC procedure as
described above, excluding the dewaxing and antigen retrieval steps.

For multiple immunofluorescence labeling, the procedure of formalin-fixed and
paraffin-embedded sections were performed as previously described. Briefly,
immosaver (pH 7.4; Nissin EM) was used for dewaxing and antigen retrieval for
45 min at 98°C. Sections were washed in PBS and rinsed in PBS containing 1% BSA
and 2% fetal calf serum, then incubated with primary antibodies overnight at 4°C
followed by incubation with other primary antibodies for 1-2 h at room temperature.
The primary antibody incubation was followed by incubation with
fluorochrome-conjugated secondary antibodies. Sections were washed in PBS
between each step. No cross reactivity was observed with the antibodies.
Phosphate-buffered saline without primary antibodies was used as negative control.
Slides were mounted with Fluoromount (Diagnostic BioSystems, Pleasanton, CA,
USA) and analyzed under a microscope (BX53; Olympus) using a BX3-URA
fluorescence system (Olympus).

2.4. Laser-capture microdissection (LCM) of tonsillar GCs

LCM was performed to collect tonsillar GCs tissues as previously described
(Meng et al., 2016). Fresh palatine tonsils were surgically removed and fixed with
RNAlater RNA Stabilization Reagent (abcam) for 12 h at 4°C, then embedded in
optimal cutting temperature compound (Sakura Tissue-Tek 4583; Sakura Finetek USA,
Inc., Torrance, CA, USA), then sectioned into 8-µm-thick sections by a freezing microtome. Subsequently, sections were placed on cooled PEN Membrane Glass Slides (LCM0522, Applied Biosystems, Carlsbad, CA, USA). GCs were captured by LCM using a PALM Microlaser System (PALM Microlaser Technologies AG, Bernried, Germany) according to procedure manuals. Two thousand GC components were captured from thirty sections for each tonsil and collected into a 0.5 mL RNase-free microcentrifuge tube (PALM Microlaser Technologies AG) containing RNAlater and immediately used for RNA extraction or frozen at -80°C until RNA extraction.

2.5. RNA extraction and RT-PCR analysis

Total RNA was extracted from LCM-captured cells using an RNeasy Micro kit (Qiagen, Hilden, Germany), then treated with DNaseI according to the manufacturer’s instructions. Complementary DNA (cDNA) was synthesized using a QuantiTect Reverse Transcription Kit (Qiagen). The PCR was performed using cDNA as a template. The forward- and reverse-specific primers, amplicon sizes, and annealing temperatures were as follows:

- β-actin: 5′-CAGAGCAAGAGAGCATCCT-3′ (forward) and 5′-ACGTACATGGCTGGGGTG-3′ (reverse);
- FDC-SP: 5′-ACGTACATGGCTGGGGTG-3′ (forward) and 5′-TACTTTTCGCTAGGAAGGGGAGTTG-3′ (reverse);
- AID: 5′-TCGGCGTGAGACCTACC-3′ (forward) and 5′-TCGGCGTGAGACCTACC-3′ (forward) and
5′-CGAAGATAACCAAAGTCCAGTG-3′ (reverse), 81bp, 56°C; and germline Iα-Cα mRNA 5′-CCAAGGTCTTCCCGCTGAG-3′ (forward) and 5′-CCATCTGGCTGGGTGCTG-3′ (reverse), 43bp, 56°C. For nested PCR was for switch circle Iα-Cμ mRNA, primers and temperatures were as follows: forward primer for first round, 5′-CACAGCCAGCGAGGCGAGAGCG-3′; reverse primer for first round, 5′-ACGAAGACGCTCACTTTGGG-3′; annealing temperature for first round, 51°C; forward primer for second round, 5′-TGAGTTGGACCTGCATGACG-3′; reverse primer for second round, 5′-CGTCTGTGCCTGCATGACG-3′; amplicon length, 349bp; annealing temperature for second round, 58°C. PCR products were analyzed by 4% agarose gel electrophoresis and stained by ethidium bromide.

2.6. Quantitative real-time PCR analysis

50 ng RNA were reverse transcribed using a QuantiTect RT kit (Qiagen). cDNA was amplified with a Fast SYBR Green Master Mix (Applied Biosystems) according to the manufacturer’s instructions, and samples were subjected to PCR on a 7500 Fast Real-Time PCR System (Applied Biosystems). Primers for β-actin, FDC-SP and AID were as described in the RT-PCR section. Relative expression was determined using the relative standard curve method. Data were normalized to β-actin expression.

2.7. Preparation of Triptolide

Triptolide (Best Technologies, Inc.) was dissolved in DMSO at a stock concentration of 100 mM. The use of HPLC as a biological assay was utilized for the
quality measurement of Triptolide extract.

2.8. Isolation and identification of FDC-associated clusters from tonsillar GCs, and Cell culture to assess IgA production

FDC-associated clusters were isolated from tonsillar GCs of IgAN patients as described previously (Meng et al., 2016). For IgA production analysis, FDC-associated clusters were cultured with the base cell culture media supplemented with 0, 0.5, 1, 5, 10 µg/mL Triptolide for 7 days. After collection of supernatants, FDC-SP and IgA were qualitatively analyzed with Human FDC-SP ELISA Kit (C4orf7, MyBioSource, USA) and IgA Human ELISA Kit (ab137980; Abcam).

2.9. Statistical analysis

Statistical analyses were performed with the Mann-Whitney U test, Spearman’s correlation analysis (SAS Institute Inc., Cary, NC, USA) as described in details in figure legends. Differences with $p$-values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical parameters

The clinical data of the patients were shown in Table 1, including gender, age, and kidney functional parameters. The kidney functional parameters of IgAN patients were significantly decreased after treated by Triptolide.
3.2. Ratio of IgA-containing cells was decreased in the tonsils of IgAN patients treated with Tripterygium Wilfordii treatment

IHC analysis revealed that the number of IgA-containing cells was significantly decreased among all Ig-containing cells in IgAN patients treated with TW (Figure 1). Compared to IgAN patients treated with Tripterygium Wilfordii and non-IgAN chronic tonsillitis, the percentage of IgM-containing cells was reduced in IgAN patients without treatment. Meanwhile, the relative percentage of IgA-containing cells was significantly lower in the tonsils of IgAN patients with Tripterygium Wilfordii treatment than of IgAN patients without treatment (Figure 1, 2).

3.3. Increased The expression of FDC-SP was increased in the tonsils of IgAN patients with TW treatment

In contrast to the reduced IgA-containing cells, the numbers of FDC-SP-positive cells were increased in the tonsillar GCs of IgAN patients treated with Tripterygium Wilfordii compared to those without treatment (Fig. 2). The result displayed a negative correlation between IgA and FDC-SP expression levels in tonsils of IgAN patients (R = 0.86, and p < 0.05 for Spearman’s correlation; Figure 2C).

3.4. The expression of FDC-SP was increased in tonsillar GCs of IgAN patients treated with TW

The GC is the primary zone of IgA class switching in tonsils. The previous studies indicated that FDC-SP was expressed by activated FDCs and that it can bind
to the surface of B lymphoma cells (Marshall et al., 2002). To understand the mechanisms of IgA down-regulation in the tonsils of IgAN patients with Tripterygium Wilfordii treatment, we assessed the expression of FDC-SP in FDCs (marked by CD23) and B cells (marked by CD20) using double immunofluorescence staining.

Coexpression of CD23 with FDC-SP and CD20 with FDC-SP was detected in both IgAN and non-IgAN patients by double immunofluorescence assays (Fig. 3). Additionally, coexpression levels of CD23 with FDC-SP and CD20 with FDC-SP were remarkable higher in tonsillar GCs of IgAN patients treated with Tripterygium Wilfordii than those without treatment \((p < 0.01\), Student’s \(t\)-test).

3.5. Expression of mRNA encoding FDC-SP, AID, and IgA class switching in the tonsils of IgAN patients and non-IgAN patients

To evaluate molecular changes in FDC-SP and IgA class switching, RT-PCR and Real-time PCR analysis were performed in the present study. Tonsillar GCs that had been isolated by laser microdissection (Figure 4A). AID and \(\text{I} \alpha-\text{Ca}\) GLTs are required for CSR initiation. The result showed that the transcripts of \(\text{AID}, \text{I} \alpha-\text{Ca}, \text{I} \alpha-\text{C} \mu\) genes were detected in GCs from both the IgAN and non-IgAN groups (Figure 4C). Meanwhile, the results displayed that that the transcript levels of \(\text{FDC-SP}\) were increased in GCs in the IgAN patients with TW treatment compared with those without treatment \((p < 0.05\); Figure 4D), and correlated negatively with decreased AID mRNA levels and IgA class switching level.
3.6. Triptolide induces FDC-SP secretion, but inhibits IgA synthesis in FDC-associated clusters

FDC-associated clusters consist of CD10+ GC cells and CD21+ FDCs, each FDC-associated cluster contains about 1 FDC per 10 lymphocytes. To investigate the dose dependent effect on FDC-SP secretion and IgA production, FDC-associated clusters were treated with gradient concentration of Triptolide for 7 days. Interestingly, exposure to Triptolide induced FDC-SP secretion, but inhibited IgA secretion in FDC-associated clusters which had been isolated from patients without TW treatment previously (Figure 4G, H).
4. Discussion

IgAN is characterized by IgA deposition and enhancement of circulating aberrant IgA in the renal mesangium. Immunoglobulin A nephropathy (IgAN) is a very common primary glomerular disease in the world since it was first described by Berger in 1968. IgAN is characterized by IgA deposition and enhancement of circulating aberrant IgA in the renal mesangium (Mariani et al., 2018). Recent studies revealed that mucosal immunity was related to the pathogenesis of IgAN (Huang et al., 2019; Chen et al., 2018). Palatine tonsils are part of nasopharynx-associated lymphoid tissue and play a major role in mucosal immunity in human airways. It is suggested that IgA of tonsillar origin was crucial to the pathogenesis of IgAN (Lai et al., 2019). O-glycosylation of IgA1 molecules (galaktose-deficient IgA1) is crucial for pathophysiology of IgA nephropathy which leads to the productions of autoantigens. IgG autoantibodies target O-glycans in the hinge region and this cause formation and deposition of immune complexes in the kidney with the local inflammatory response. Moreover, the shift from mucosal IgA1 producing cells to the bone marrow and the subsequent excess in the systemic circulation contributes to the disease progress (Lai et al., 2019).

The previous studies indicated that IgAN patients acquired good benefits from TW treatment (Liang et al., 2018). However, the mechanisms of TW improving renal outcomes are still unclear. TW (treatment) is usually used to target tonsillitis, while little is known about the involvement of TW in IgA production in the tonsils. Moreover, because of the complexity of traditional Chinese medicine ingredients, the
role of Triptolide (the major active compound of TW) in tonsillar IgA production in
IgAN is unknown. The recent trials of IgAN treatment have suggested that the risks
associated with immunosuppressive therapy outweigh the benefits, which may shift
the treatment paradigm of this disease. Accordingly, Leflunomide (LEF) appears to
improve renal function while decreasing loss of urine protein. Combination regimens
including LEF were better and safer compared with corticosteroids or ACEI alone or
combinations including CTX (He et al., 2016).

Tonsillar GCs act as vital zone for mucosal B cell responses which are supported
by FDCs and a few number of follicular helper T cells (He et al., 2016). The activated
B cells may undergo IgA class switching, then differentiate into IgA⁺ plasma cells or
directly migrate to systemic sites (Han et al., 2018). The previous study reported that
IgA class switching was upregulated in the tonsils of IgAN patients, meanwhile, IgAN
patients with hematuria-type IgAN, especially those presenting hematuria after
tonsillar infection acquired benefits from tonsillectomy (Feriozzi et al., 2016).

The present study indicated that both the numbers and percentage of IgA-
containing cells were significantly decreased among immunoglobulins in IgAN
patients treated with TW (Figure1 and 2). Therefore, IgAN patients might benefit
from TW treatment by downregulation of IgA production in the tonsils. When CSR,
Iα-Cα GLTs and Iα-Cμ switch circles have short half-lives, and detection of the targets
indicate ongoing CSR. In our study, the percentage of IgA⁺ cells was significantly
decreased, while that of IgM⁺ cells was increased in IgAN patients with TW treatment
compared with those without treatment, similar to Non-IgAN. These results indicate
that TW may inhibit the class switching from IgM to IgA.

FDC-SP is a tissue specific protein, tonsillar crypts and its synthesis is induced by activated FDCs (Iwai et al., 2018). The previous studies demonstrated that FDC-SP could directly bind to the surface of B cells and regulate the induction of B cell responses inside and outside GCs (Liu et al., 2016). AID is the crucial protein that promotes DNA double-strand breaks, an essential mechanism of class switch recombination (CSR) (Yewdell et al., 2017). The expression of FDC-SP protein (Figure 2A) and mRNA (Figure 4C) in GCs was increased in IgAN patients with TW treatment compared to those without treatment, corresponding to the inhibited AID expression and IgA class switching observed in the tonsils of IgAN patients. Additionally, the present study indicated a correlation between tonsillar FDC-SP expression and IgA concentrations in IgAN patients. Thus, these data suggested that FDC-SP and AID might be involved in IgA production in tonsils of IgAN patients treated with TW.

We found that coexpression of FDC-SP and B cells in GCs was higher in IgAN patients with TW treatment compared to those without treatment and non-IgAN patients. The recent studies suggested that B cell migration was stimulated by FDC-SP in cooperation with CXC chemokines, while their migratory responses could be blunted by chronic exposure to high levels of FDC-SP (Al-Alwan et al., 2007). Chronic exposure to high levels of FDC-SP, B cells in GCs in the tonsils of IgAN patients may present depressed migratory responses from the dark zone to the light zone, where they undergo isotype switching, and differentiation into IgA⁺ plasma.
cells or memory cells. Similarly, in our data, we observed increased expression of FDC-SP in tonsillar GCs of IgAN with Triptolide treatment, which correlated with the decreased expression of IgA (Figure 4G, H). Based on the present and previous data, a possible model was proposed whereby Triptolide induce the increased expression of FDC-SP and decreased expression of AID by tonsillar FDCs, inhibiting the generation of IgA⁺ B cells and IgA⁺ plasmablasts.

The present study provided the first evidence that the increased expression of FDC-SP in tonsillar FDCs correlated with depressed IgA expression in the tonsils of IgAN patients with TW treatment compared with those without treatment. Together with the findings that Triptolide induce the increased expression of FDC-SP and correlated with the decreased expression of IgA in tonsillar GCs cells. This was the first study to indicate that Triptolide might inhibit IgA class switching in IgAN patients through the cooperative roles of FDC-SP, which might represent a promising strategy for therapeutic intervention for IgAN patients.

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Conflicts of Interest

All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.
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Figure Legends

Figure 1. Rates of IgA was decreased among immunoglobulin classes in the tonsils of IgAN patients with Tripterygium Wilfordii treatment.

Immunohistochemistry of IgA, IgG, IgM and IgE on tonsillar serial sections of IgAN patients showed the presence of Ig-bearing cells in the follicular germinal centers (GCs), reticular crypt epithelium (Ep), and subepithelial area. Bars, 500 μm. GC, germinal center. Positive cells were counted in low-power (100× magnification) fields for each patient. The slides were analyzed in blinded manner by two independent investigators. n = 20 for IgAN patients with Tripterygium Wilfordii treatment, n = 20 for IgAN patients without treatment and n = 20 for non-IgAN patients with chronic tonsillitis. Error bars indicate SEMs. *, P< 0.05 (Mann-Whitney U test).

Figure 2. IgAN patients with Tripterygium Wilfordii treatment exhibited increased numbers of FDC-SP and decreased numbers of IgA-bearing cells in their tonsils.

Immunohistochemistry of FDC-SP and IgA in the tonsils of IgAN patients and non-IgAN patients with chronic tonsillitis showed the presence of FDC-SP and IgA-bearing cells in the follicular germinal centers (GCs), reticular crypt epithelium (Ep), and subepithelial area. Bars, 200 μm. The number of FDC-SP and IgA-bearing cells in the tonsils was counted in 10 randomly chosen, low-power (100× magnification) fields for each patient. The slides were analyzed in blinded manner by
two independent investigators. n = 20 for IgAN patients with Tripterygium Wilfordii treatment, n = 20 for IgAN patients without treatment and n = 20 for non-IgAN patients with chronic tonsillitis. Error bars indicate SEMs. *, P< 0.01 (Mann-Whitney U test). The Y axis label on the graph (third row) showed “the number of IgA bearing cells in tonsillar GCs”, and the X axis label showed “the levels of FDC-SP expression in tonsillar GCs”. Correlation between IgA and FDC-SP was analyzed by Spearman’s correlation.

Figure 3. Coexpression of FDC-SP and CD23, CD20 on FDCs and germinal centers (GC) B cells increased in the tonsils of IgAN patients with Tripterygium Wilfordii treatment compared with that without treatment.

Double immunofluorescence for CD23 (green), CD20 (green) and FDC-SP (red) showing double-positive cells localized in the GCs (magnification: ×200).

Figure 4. Increased expression of FDC-SP mRNA and decreased expression of AID mRNA in tonsillar germinal centers (GCs) of IgAN patients.

I. Laser capture microdissection was performed to extract GC components from tonsils. HE staining of a sample before (A) and after (B) laser capture microdissection of the GC components in tonsils fixed by RNAlater. II. Using RT-PCR, mRNA levels of genes encoding β-actin and FDC-SP were detected in tonsillar GCs of both IgAN patients (IgAN) and non-IgAN chronic tonsillitis (non-IgAN) (C). The expression levels of the FDC-SP mRNA were higher in the GCs of IgAN patients with
Tripterygium Wilfordii treatment than in those of without treatment and non-IgAN chronic tonsillitis patients (D). The expression levels of the AID and CRS mRNA were lower in the GCs of IgAN patients with Tripterygium Wilfordii treatment than control (D). Error bars indicate SEM. *, $P < 0.05$ (Student’s $t$-test). **Triptolide induce FDC-SP secretion, but inhibit IgA production in FDC-associated clusters.** (E) Morphology and CD21$^+$ FDCs in FDC-associated clusters isolated from tonsillar GCs of IgAN patients. (F) FDC-associated clusters are composed of CD 10$^+$ GC cells and CD21$^+$ FDCs, with about 1 FDC per 10 lymphocytes in each FDC-associated cluster. (G, H) IgA and FDC-SP concentrations in the supernatants of FDC-associated clusters. IgA and FDC-SP were quantified in the supernatants using ELISA. Combined data (mean ± SD) from experiments using FDC-associated clusters from 3IgAN patients are presented.
### Table 1. Profiles and clinical parameters of patients

<table>
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<th>Parameter</th>
<th>IgAN group (n = 20)</th>
<th>IgAN group with TW treatment (n = 20)</th>
<th>Chronic tonsillitis group (n = 20)</th>
<th>Healthy volunteer group (n = 20)</th>
<th>Normal value (reference)</th>
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<td>Age (years)</td>
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<td>N.D.</td>
<td>0–0.15</td>
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<td>26.07 ± 21.54</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.96 ± 0.28</td>
<td>0.62 ± 0.48</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.47–0.79</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.63 ± 1.12</td>
<td>3.46 ± 1.66</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.4–5.6</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.97 ± 0.84</td>
<td>0.44 ± 0.45</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0–0.24</td>
</tr>
<tr>
<td>Serum complement (U/mL)</td>
<td>49.58 ± 16.42</td>
<td>36.68 ± 12.23</td>
<td>N.D.</td>
<td>N.D.</td>
<td>28–44</td>
</tr>
</tbody>
</table>

Note: values are the means ± SDs. hpf = high-power field; N.D. = no data
A

IgAN

IgAN with Tripterygium Wilfordii treated

Non-IgAN

B

C

Number of bearing cells/ Lower power field

IgA

IgG

FDC-SP

IgA bearing cells in tonsillar GCs

FDC-SP bearing cells in tonsillar GCs