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A proliferation-inducing ligand (APRIL) induced hyper-production of IgA from tonsillar mononuclear cells in patients with IgA nephropathy

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ABSTRACT

IgA nephropathy (IgAN) is a tonsil-related disease. We previously showed that oligodeoxynucleotides with CpG (CpG-ODN) and B-cell activation factor (BAFF) are involved in hyperproduction of IgA from tonsillar mononuclear cells of patients with IgAN (IgAN-TMCs). In this study, we focused on a proliferation-inducing ligand (APRIL), homologous to BAFF. IgAN-TMCs produced more APRIL than non IgAN-TMCs in the presence of both CpG-ODN and control-ODN. TLR9 expression was higher in B-cells of IgAN-TMCs, and treatment with CpG-ODN enhanced transmembrane activator and CAML interactor (TACI) expression. IgA production from IgAN-TMCs was inhibited by APRIL neutralization antibody or TACI blocking antibody, and enhanced by co-treatment of APRIL and CpG-ODN. Serum APRIL levels were higher in patients with IgAN, and decreased after tonsillectomy. These findings suggest that APRIL is involved in the hyperproduction of IgA from IgAN-TMCs, and that CpG-ODN enhanced APRIL-induced IgA production by increasing TACI expression on B-cells of IgAN-TMCs.

1. Introduction

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide [1]. Initially, the course of IgAN was considered benign, but it is now recognized that progression to end-stage renal failure often occurs in these patients over a period of 20 years [2]. The disease often worsens after upper respiratory tract infections, particularly acute tonsillitis. There is a report that tonsillectomy had no impact on renal outcome 10 years after biopsy [3], however, otolaryngologist including us have believed that tonsillectomy is an effective treatment for patients with IgAN [4]. Recently, a 10 year observation and clinical comparison studies between patients who underwent tonsillectomy and those who did not showed that the renal survival rates of the tonsillectomy group were significantly higher than those of the non-tonsillectomy group, demonstrating that tonsillectomy is an independent factor for long-term renal survival in patients with IgAN [5,6]. Therefore, IgAN is currently recognized as a tonsil-related disease.

Because IgAN is characterized by mesangial IgA deposits, circulating IgA is thought to play a key role in the pathogenesis of IgAN. In fact, it is well-known that serum IgA and IgA immune complex levels are elevated in patients with IgAN [7,8]. Because IgA-positive plasma cells are increased in the tonsils of patients [9,10], IgA production from tonsillar mononuclear cells (TMCs) is increased in these patients *in vitro* [11] and serum IgA levels after tonsillectomy are decreased in patients [3]. The tonsils are thought to be a source of pathogenic IgA. This dysregulation of IgA production may be caused by novel immune responses to oral indigenous bacteria, particularly *Haemophilus parainfluenzae* [12–14]. *H. parainfluenzae* is more commonly isolated from the tonsils of patients [12] and produces TMCs which proliferate and produce increased amounts of IgA *in vitro* [13,14]. However, *H. parainfluenzae* is not always detected in the tonsils of patients. Therefore, common factors may exist for inducing a novel immune response.

As a candidate, we found that oligodeoxynucleotides (ODN) with CpG (CpG-ODN) are present in microbial DNA and mimic immunostimulatory activity [15]. CpG-ODN provokes innate immune responses via Toll-like receptor (TLR)-9, resulting in hyper-production of IgM, IgG, and IgA [16]. We previously demonstrated that TMCs from patients with IgAN was hyper-responsive to CpG-ODN and produced large amounts of IgA through B-cell-activation factor (BAFF) [17], a

Abbreviations: IgAN, IgA nephropathy; CpG-ODN, Oligodeoxynucleotides with CpG; BAFF, B-cell activation factor; APRIL, A proliferation-inducing ligand; TACI, Transmembrane activator and calcium modulator and cyclophilin ligand interactor

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stimulator of B-cell proliferation and immunoglobulin production without the help of *T*-cells [18]. CpG-ODN stimulation significantly increased IgA production from the TMCs of patients and the production was inhibited by an anti-BAFF neutralizing antibody [17]. However, this inhibition was not complete, and we predicted that other factors are also involved in the hyper-IgA production of TMCs stimulated by CPG-ODN in patients with IgAN.

A proliferation-inducing ligand (APRIL) is a member of the tumor necrosis factor superfamily, which is homologous in both structure and function to BAFF [18,19]. APRIL and BAFF, derived primarily from myeloid cells such as dendritic cells (DCs), polymorphonuclear cells such as neutrophils, and epithelial cells, mediate this CD40-independent isotype switching from IgG to IgA [18,19]. B-cell maturation antigen (BCMA) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) are known as APRIL receptors [19]. In fact, APRIL production by monocyte-derived DCs and TACI expression on B-cells were increased by treatment with CpG-ODN [20,21]. These findings suggest that APRIL is also involved in hyper-IgA production of TMCs stimulated by CPG-ODN in patients with IgAN. APRIL is a key factor in the pathogenesis of IgAN according to studies showing that the serum APRIL level was significantly higher in patients with IgAN than in healthy controls [22]. The level was correlated with the severity of IgAN [23], and albuminuria, serum IgA levels, and glomerular IgA deposition were significantly decreased in a mouse model of IgAN by anti-APRIL Ab treatment [24]. Moreover, Muto et al. have already found that higher APRIL expression was demonstrated in germinal center B cells of tonsil from patients with IgAN by immunohistochemical analysis [25].

In this study, we focused on the role of APRIL in IgA production of TMCs stimulated with CpG-ODN in patients with IgAN. Using TMCs from patients with IgAN and non-IgAN, we investigated the (i) production of APRIL in the presence of CpG-ODN, (ii) production of IgA in the presence of CpG-ODN and TACI blocking antibody, (iii) TACI expression in the presence of CpG-ODN, (iv) production of IgA in the presence of CpG-ODN and APRIL, and (v) serum APRIL levels before and after tonsillectomy.

2. Materials and methods

2.1. Patients and samples

Sixty-four patients undergoing tonsillectomy from 2007 to 2012 at the Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical University, were enrolled in this study. The patients were divided into 2 groups of 32 patients each: the IgAN and non-IgAN group. The number, age, and gender of patients enrolled in the total and each examination are summarized in Table 1. The age and gender distribution were not significantly different between the groups.

IgAN was diagnosed by nephrologists based on renal biopsy findings including the presence of predominant deposition of IgA in the mesangium area and proliferation of mesangial cells and matrix. No patients were treated with anti-platelet drugs, anti-inflammatory drugs, corticosteroids, and/or immunosuppressants before tonsillectomy.

The non-IgAN group included patients undergoing tonsillectomy because of recurrent episodes (three or more times per year) of acute tonsillitis and/or persistent sore throat due to chronic tonsillitis. No patients in the non-IgAN group had renal disease.

All patients signed informed consent forms for therapy and tissue studies. The Institutional Review Board approved this study.

2.2. Cell preparation

Tonsils obtained by tonsillectomy were manually cut into small pieces in phosphate-buffered saline (PBS) with 100 U/mL of penicillin and 100 μg/mL of streptomycin (Life Technologies, Carlsbad, CA, USA), and the cells were passed through a cell strainer (BD Biosciences, Franklin Lakes, NJ, USA). TMCs and peripheral blood mononuclear cells (PBMCs) were isolated by the gradient centrifugation method using Ficoll Paque Plus® (Amersham Pharmacia Biotech, Amersham, UK) as described previously [26,27]. The cells were washed 5 times with sterile PBS and counted. The viability of the cell suspensions was over 95%.

To isolate B cells, a Dynabeads Untouched Human B Cells kit was used (Invitrogen Dynal AS, Carlsbad, CA, USA). According to the protocol, TMCs were incubated with beads conjugated with antibody mix recognizing TMCs other than B-cells. Negatively selected B-cells were used in the following experiments.

Table 1
Distribution of the patients enrolled in each study.

Study	Disease	Number of cases	Gender male:female	Age range, median
Total	IgAN	32	21:11	17–65, 45
	non-IgAN	32	13:19	18–60, 38
APRIL ELISA (culture supernantent)	IgAN	15	9:6	18–65, 48
	non-IgAN	10	6:4	35–46, 38
APRIL ELISA (preoperative serum)	IgAN	11	6:5	25–65, 45
	non-IgAN	12	8:4	24–61, 36
APRIL ELISA (postoperative serum)	IgAN	9	5:4	21–65, 45
	non-IgAN	10	6:4	24–51, 35
Flow cytometry (TRL9, TACI)	IgAN	11	8:2	21–64, 48
	non-IgAN	9	6:3	30–58, 45
IgA ELISA (APRIL stimulation)	IgAN	11	8:2	21–64, 48
	non-IgAN	9	6:3	30–58, 45
IgA ELISA (APRIL neutralization)	IgAN	8	2:6	19–48, 39
	non-IgAN	8	2:6	23–55, 45
IgA ELISA (TACI inhibition)	IgAN	9	7:2	17–63, 40
	non-IgAN	10	5:5	18–60, 41

2.3. Cell culture

The culture conditions were described previously [17]. Briefly, TMCs were suspended at a concentration of 1×10^6/mL in 1 mL of RPMI 1640 medium (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies), 100 U/mL of penicillin, and 100 μg/mL of streptomycin.

TMCs and tonsillar B-cells were cultured with 1 μM of CpG-ODN (5′-tcgtcgttttcggcgcgcgccg-3′; ODN2395; Hycult Biotech, Uden, Netherlands) or Control-ODN (5′-gcttgatgactcagccggaa-3′; NON-CpG DNA; Hycult Biotech) in 24-well culture plates in an atmosphere with 5% CO_2 at 37 °C. After 3 days, the culture supernatant and the cells were collected. For subsequent assays, TMCs were cultured with 5 μg/mL of TACI blocking antibody/Mouse IgG1 isotype control (R&D Systems, Minneapolis, MN, USA), 2 μg/mL of APRIL neutralizing antibody/Mouse IgG1 isotype control (R&D Systems), or 8 μg/mL of recombinant APRIL (Enzo Life Sciences, Farmingdale, NY, USA) for 3 days.

2.4. Flow cytometry

Flow cytometric analysis was performed as described previously [17]. For TACI expression, 5×10^5 of tonsillar B-cells were reacted with 20 μL of phycoerythin (PE)-labeled anti-TACI antibody (R&D Systems) or 5 μL of phycoerythin (PE)-labeled anti-BCMA antibody (BioLegend, San Diego, CA, USA). Isotype mouse IgG2a (Biodesign International, Saco, ME, USA) was used as a control. The cells were washed three times and then subjected to flow cytometric analysis using a FACS Calibur analyzer and CellQuest software (BD Biosciences). The data are displayed as the mean fluorescence intensity (MFI).

For intracellular staining with TLR9, the IntraStain kit (DAKO,

Carpinteria, CA, USA) was used. First, 5×10^5 of tonsillar B-cells were incubated with 100 μL of Dako Intrastain Reagent A for 15 min. The cells were washed and stained with 100 μL of Dako Intrastain Reagent B and 20 μL of FITC-labeled anti-TLR9 antibody or mouse IgG1-FITC isotype control (IMGENEX, San Diego, CA, USA) according to the manufacturer's protocol.

2.5. Enzyme-linked immunosorbent assay

Commercialized enzyme-linked immunosorbent assay (ELISA) kits were used to measure IgA (Bethyl Laboratories, Montgomery, TX, USA) and APRIL (Bender MedSystems, Vienna, Austria). The culture supernatants were added to the wells and analyzed according to manufacturer's instructions as described previously [26,27]. Briefly, the 96-wells flat-bottomed plates coated with mouse anti-human IgA or APRIL antibodies were incubated with PBS containing 4% bovine serum albumin for 2 h at 37 °C. The wells were washed and incubated overnight with 100 μL of supernatant culture fluids at an adequate dilution at 4 °C. Each sample was assayed in duplicate. After washing, the plates were incubated with 100 μL of biotinylated anti-human IgA or anti-APRIL antibodies, followed by peroxidase-conjugated streptavidin. After 10 min of the reaction with substrate solution, the optical density of each well was measured with an automated spectrophotometer (SLT-Labinstruments, Grödig, Austria) at 450 nm. A serial diluted standard solution was evaluated in each plate and the concentration in the sample was determined from the standard curve.

2.6. Statistical analysis

Data are expressed as the median: minimum and maximum values. Two groups were compared using nonparametric test procedures such as Mann-Whitney *U* test and Spearman rank correlation coefficient. The Wilcoxon rank-sum test was used for continuous variables. Statistical tests were based on a level of significance of $p < 0.05$.

3. Results

3.1. Clinical characteristics and outcome of patients

According to the histologic classification predicting the long-term renal outcome of IgA nephropathy [28], 5, 12, 10, and 5 patients were classified into groups I, II, III, and IV, respectively. After tonsillectomy, patients with IgAN were followed from 1 to 48 months with median periods of 12 months. Steroid pulse therapy was provided in 20 of 32 patients. In 15 (47%) patients, urinary abnormality disappeared during the follow-up period. No patients showed renal death or required dialysis during the observation period.

3.2. APRIL productions from TMCs treated with CpG-ODN

APRIL levels in the culture supernatant of TMCs without treatment were significantly higher in patients with IgAN (1050: 700–1400 pg/mL) than in patients with non-IgAN (775: 400–1050 pg/mL, $p < 0.01$, Fig. 1ab). Similarly, a difference in APRIL levels was found after treatment with control-ODN (IgAN group; 1020: 790–1560 pg/mL, non-IgAN group; 770: 400–1040 pg/mL, $p < 0.01$, Fig. 1ab) or CpG-ODN (IgAN group; 1010: 700–1450 pg/mL, non-IgAN group; 815: 543–1100 pg/mL, $p < 0.01$, Fig. 1ab). In contrast, there was no significant difference in the APRIL level between CpG-ODN and control-ODN treatment in either patient group (Fig. 1ab).

3.3. TLR9, BCMA and TACI expression of tonsillar B-cells

TLR9 expression was significantly higher in tonsillar B-cells from patients with IgAN (MFI 500: 151–980) than in those from patients with non-IgAN (MFI 254: 125–775, $p < 0.05$, Fig. 2a). BCMA expression on tonsillar B-cells was faintly found in the presence of control- or CPG-ODN in either patient group (Fig. 2b). TACI expression on tonsillar B-cells from patients with IgAN was significantly higher in the presence of CPG-ODN (MFI 73: 48–85) than in the presence of control-ODN (MFI 62: 36–76, $p < 0.01$, Fig. 3b). No difference in TACI expression was found on tonsillar B-cells from patients with non-IgAN (Fig. 2c).

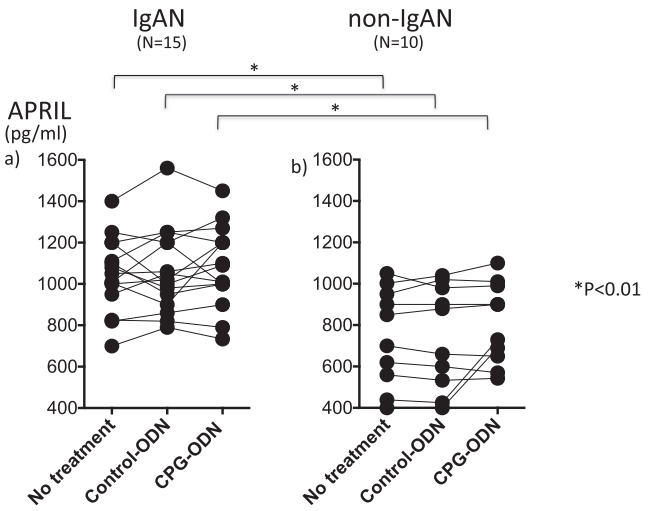

Fig. 1. APRIL production by TMCs stimulated with CpG-ODN. a) TMCs stimulated with CpG-ODN produced APRIL at the same level as that stimulated by control-ODN in patients with IgAN. b) Similarly, in patients with non-IgAN, there was no difference in APRIL production by TMCs between the stimulations. In contrast, TMCs from patients with IgAN produced larger amounts of APRIL compared to that with non-IgAN under conditions of both CpG-ODN and control-ODN stimulation.

3.4. IgA production of TMCs treated with APRIL neutralizing antibody

In patients with IgAN, IgA levels in the culture supernatant of TMCs treated with APRIL neutralizing antibody (1944: 1140–2550 ng/ml) were significantly lower than in those treated with isotype control (2215: 1402–2747 ng/mL, $p = 0.02$, Fig. 3a). In patients with non-IgAN, no difference was observed following APRIL neutralizing antibody or isotype control treatment (Fig. 3a).

3.5. IgA production of TMCs treated with CpG-ODN and TACI blocking antibody

In patients with IgAN, IgA levels in the culture supernatant of TMCs treated with control-ODN and TACI blocking antibody (713: 333–2461 ng/ml) were significantly lower than in those treated with control-ODN and isotype control (1768: 278–2634 ng/mL, $p = 0.04$, Fig. 3b). Similarly, a difference in IgA levels was found following treatment with CpG-ODN (1160: 314–2391 ng/mL, 1841: 389–2998 ng/mL, $p < 0.01$, Fig. 3b). In patients with non-IgAN, no difference was observed following control-ODN or CpG-ODN treatment (Fig. 3c).

3.6. IgA production of TMCs treated with CpG-ODN and APRIL

In patients with IgAN, IgA levels in the culture supernatant of TMCs treated with CpG-ODN and APRIL (459: 141–2694 ng/mL) were significantly higher than in those treated with CpG-ODN alone (330: 135–1856 ng/mL, $p < 0.01$, Fig. 4a). However, no difference was found following treatment with control-ODN. In patients with non-IgAN, no difference was found following control-ODN or CpG-ODN treatment (Fig. 4b).

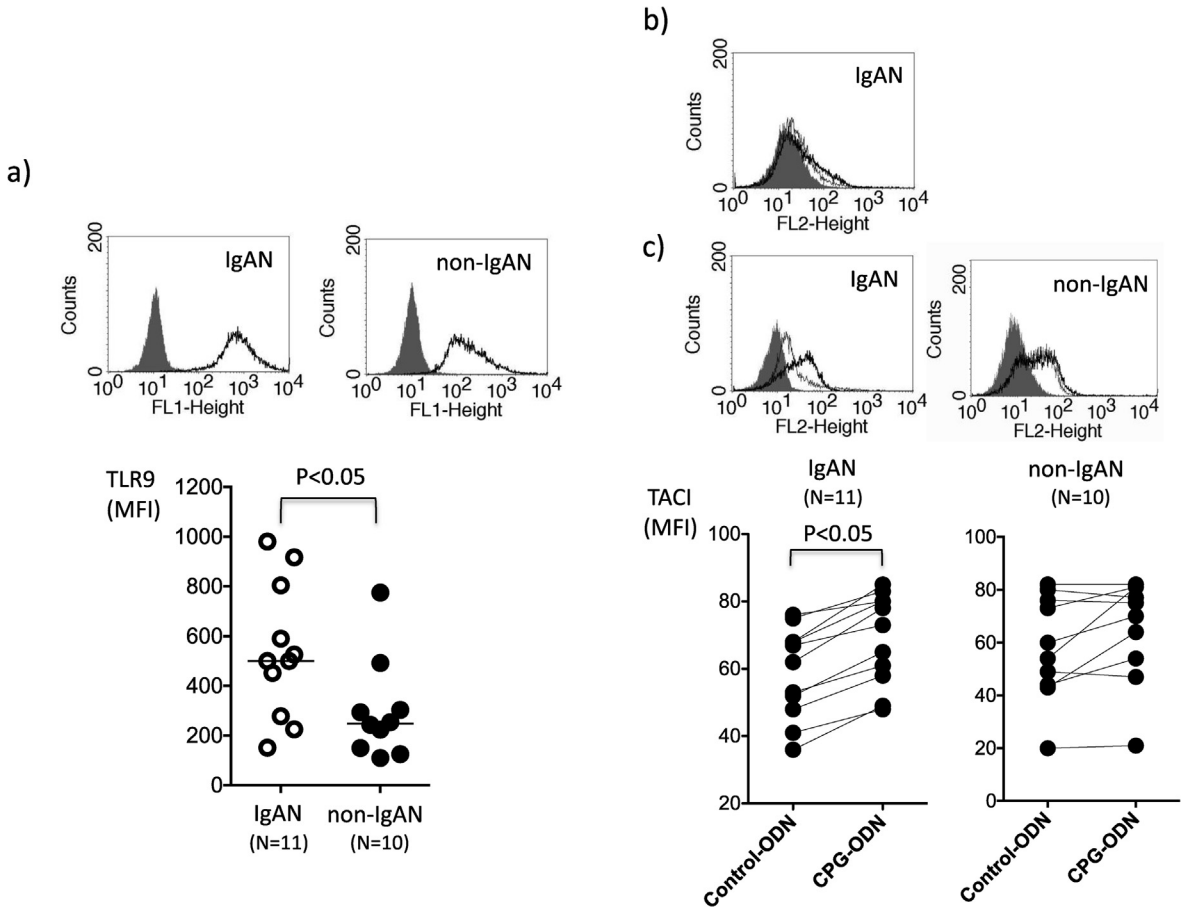

Fig. 2. TLR9 and TACI expression in tonsillar B-cells. a) TLR9 expression was measured by flow cytometric analysis. Full line showed expression of TLR9. Filled histograms showed isotype control signals. TLR9 expression was significantly higher in tonsillar B-cells of patients with IgAN compared to in patients with non-IgAN. b) BCMA expression was measured by flow cytometric analysis. Thin and bold line showed expression of BCMA on the cells treated with control-ODN and CPG-ODN, respectively. Filled histograms showed isotype control signals. BCMA expression was not found on tonsillar B-cells of patients with IgAN. c) TACI expression was measured by flow cytometric analysis. Thin and bold line showed expression of TACI on the cells treated with control-ODN and CPG-ODN, respectively. Filled histograms showed isotype control signals. TACI expression on tonsillar B-cells of patients with IgAN was enhanced by CPG-ODN but not by Control-ODN. This enhancement was not observed on tonsillar B-cells from patients with non-IgAN.

3.7. Serum APRIL levels before and after tonsillectomy

The serum APRIL level was significantly higher in patients with IgAN (11.3: 4.9–31.2 ng/mL) than in patients with non-IgAN before tonsillectomy (5.0: 2.8–12.5 ng/mL; $p < 0.05$, Fig. 5ab). Additionally, the level was significantly decreased after tonsillectomy in patients with IgAN (before; 11.3: 4.9–31.2 ng/mL, after; 5.1: 0.1–28.8 ng/mL; $p < 0.05$, Fig. 5a). However, no decrease was observed in patients with non-IgAN (Fig. 5b).

4. Discussion

We showed that TMCs from patients with IgAN produced greater amounts of APRIL than those from patients with non-IgAN in the presence of not only CpG-ODN but also control-ODN. Therefore, spontaneous APRIL production by TMCs from patients with IgAN was higher than that by TMCs of patients with non-IgAN. This agrees with Muto's study showing that tonsillar APRIL mRNA expression in patients with IgAN was significantly higher than that in patients with chronic tonsillitis [25]. Moreover, they also reported that percentage of germinal centers (GC)–containing APRIL–producing cells was significantly higher in total tonsillar GCs from patients with IgAN [25]. APRIL mRNA was reported in monocytes, macrophages, and DCs after stimulation with interferon-γ (IFN-γ) [29], which was spontaneously produced at high levels by TMCs from patients with IgAN [17,30]. Therefore, IFN-γ may be involved in hyper-production of APRIL from the TMCs of patients with IgAN. We previously reported that IFN-γ enhanced IgA production by the TMCs of patients with IgAN through BAFF [17]. According to the above results, APRIL and BAFF mediated IFN-γ and IgA production. In an experimental IgAN mouse model, administration of IgA immune complex with IFN-γ led to diffuse proliferative glomerulonephritis with proteinuria and hematuria [31]. Treatment with an anti-IFN-γ receptor antibody improved the disease [31]. These results together with our results indicate that IFN-γ acts as a trigger in the pathogenic process of IgAN. Further immunologic analyses are necessary to resolve this hypothesis.

BCMA and TACI are known to bind to APRIL [19]. Moreover, BAFF also bind to these receptors. In these binding, binding of APRIL to TACI reportedly increased IgA production, and binding of BAFF to TACI downregulates the production in human B cells [32]. In addition, the TACI gene is associated with IgA deficiency [33]. Moreover, TACI-deficient mice exhibit a reduction in serum IgA [34], but BCMA-deficient mice did not [35]. BCMA was also expressed on tonsillar B-cells, however, expression level is lower than that of TACI according to our results and other investigator [17,36]. These results suggested that binding of APRIL to TACI has predominant role for IgA production in tonsil. We showed that inhibition of IgA production by blocking of the TACI in the presence of CpG-ODN was more effective than that in the presence of Control-ODN in patients with IgAN, and that treatment of APRIL enhanced IgA production in the presence of CpG-ODN in patients

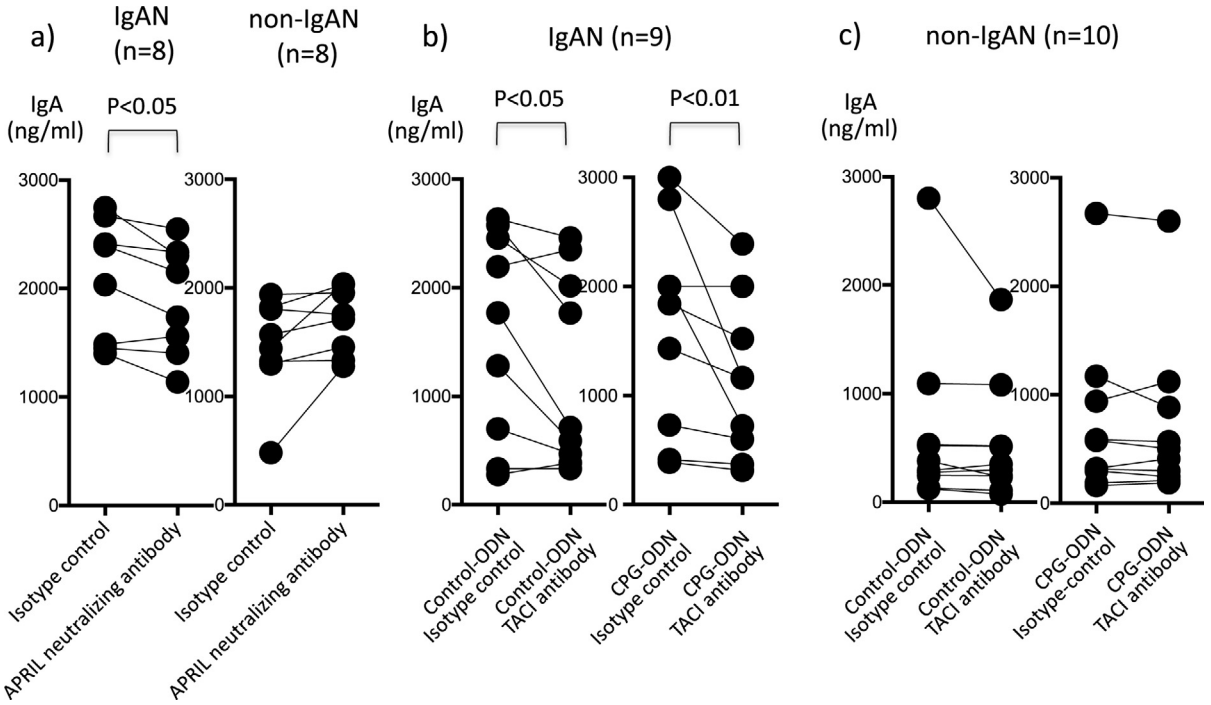

Fig. 3. IgA production by TMCs treated with APRIL neutralization antibody and co-treated with CpG-ODN and anti-TACI blocking antibody. a) Treatment with APRIL neutralization antibody significantly reduced IgA production by TMCs in patients with IgAN. b) Treatment with TACI blocking antibody significantly reduced IgA production by TMCs stimulated with both control-ODN and CpG-ODN in patients with IgAN. In the presence of CpG-ODN, a TACI blocking antibody inhibited IgA production more effectively. c) Treatment with a TACI blocking antibody did not affect IgA production from TMCs stimulated with either CpG-ODN or control-ODN in patients with non-IgAN.

with IgAN. Therefore, CpG-ODN may enhance APRIL-dependent IgA production. According to our results, this enhancement may have been caused by up-regulation of TACI expression on tonsillar B-cells stimulated with CpG-ODN rather than up-regulation of APRIL production from TMCs stimulated with CpG-ODN. Muto et al. reported that CpG stimulation of TMCs obtained from patients with IgAN up-regulated the expression of APRIL compared with control [25]. Unexpectedly, we could not find the up-regulation. A discrepancy of the results may be caused by differences in culture environment and detection method. With regard to up-regulation of TACI expression, the finding is consistent with a study showing that CpG-ODN induced the expression of TACI on human peripheral B-cells [21] and human tonsillar B-cells [25]. The difference in TACI expression on tonsillar B-cells stimulated by CpG-ODN between patients with IgAN and patients with non-IgAN may have been caused by the difference in TLR9 expression in tonsillar B-cells between the two patient groups. The difference was also described by Muto, et al. at the mRNA level [25]. The relationship between TLR9 and IgAN was observed in several studies. In an IgAN-prone mouse model, Suzuki et al. [32] reported that nasal challenge with CpG-ODN aggravated renal injury with elevated levels of albuminuria, serum IgA level, and mesangial IgA deposition. They [37] identified a single-nucleotide polymorphism in TLR9 as an important

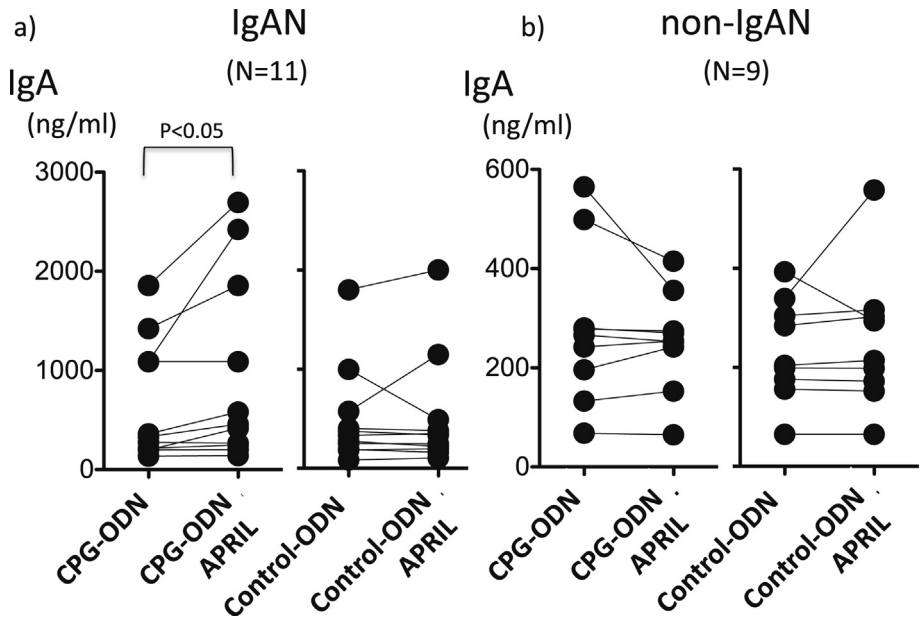

Fig. 4. IgA production by TMCs treated with CpG-ODN and APRIL. a) IgA production by TMCs stimulated with CpG-ODN was significantly enhanced by treatment with APRIL in patients with IgAN, but IgA production by control-ODN was not increased. b) In patients with non-IgAN, the enhancement was not observed under conditions of either CpG-ODN or control-ODN stimulation.

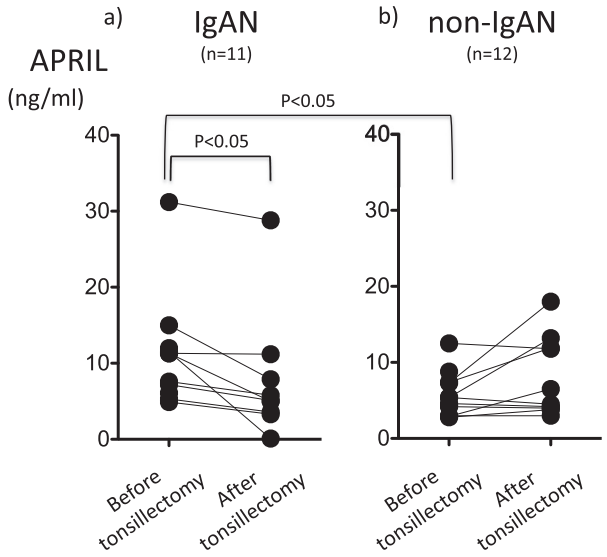

Fig. 5. Serum APRIL levels before and after tonsillectomy. a) Serum APRIL level was significantly decreased after tonsillectomy in patients with IgAN. b) The decrease was not observed in patients with non-IgAN. Additionally, the level before tonsillectomy in patients with IgAN was significantly higher than that in patients with non-IgAN.

risk factor for disease progression in patients with IgAN. They further demonstrated that some patients with IgAN exhibited relatively high expression of tonsillar TLR9 mRNA (TLR9 high group), and this group developed earlier clinical remission by tonsillectomy with steroid pulse therapy than the group with low expression (TLR9 low group) [38]. These reports indicate that TLR9 expression in the tonsils is involved in the pathogenesis of IgAN. In this study, we first demonstrated how was evaluated the effects of TLR9: high expression of TLR9 caused tonsillar B-cells to overreact to CpG-ODN to enhance TACI expression, overreact to APRIL, and produce an excessive amount of IgA.

Recently, APRIL has been shown to play an important role in the pathogenesis of IgAN. Kim et al. [24] reported that albuminuria, serum IgA levels, and deposition of glomerular IgA were significantly decreased in a mouse model of IgAN by anti-APRIL Ab treatment. Moreover, Zhai et al. [23] showed that plasma APRIL levels were higher in patients with IgAN and correlated with the degree of proteinuria and estimated glomerular filtration rate. Furthermore, Muto et al. reported that TLR9 and APRIL mRNA were higher in tonsillar B-cells of patients with IgAN than in patients with non-IgAN [25]. These results suggest that APRIL contributes to the development of IgAN. In this study, we clearly demonstrated that APRIL-dependent IgA hyperproduction occurs in the tonsils of patients with IgAN. Recently, increasing evidence has indicated that aberrantly glycosylated IgA1 molecules, mainly circulating galactose-deficient IgA1 (Gd-IgA1), trigger mesangial deposition and subsequent kidney injury in IgAN [39]. Zhai et al. also reported a positive correlation between the plasma APRIL level and Gd-IgA1 levels in patients with IgAN, and that Gd-IgA production by PBMCs was significantly higher in patients with IgAN than in healthy controls after APRIL stimulation [23]. Because Gd-IgA1 production was reported to be higher in the tonsils of patients with IgAN than in those of chronic tonsillitis [40], APRIL may induce the production of Gd-IgA1 from TMCs of patients with IgAN.

In summary, we demonstrated that: (i) spontaneous APRIL production was higher by TMCs from patients with IgAN, (ii) neutralization of APRIL or blocking of TACI inhibited IgA production by TMCs, and the inhibition by blocking of TACI was more potent in the presence of CpG-ODN, (iii) TLR9 expression was higher in B-cells of TMCs, and treatment with CpG-ODN enhanced the expression of TACI on B-cells, (iv) treatment with APRIL enhanced IgA production by TMCs in the presence of CpG-ODN, and (v) the serum APRIL level was higher in patients with IgAN, which decreased after tonsillectomy. These results suggest that hyper-production and hyper-responsiveness of APRIL lead to hyperproduction of IgA by TMCs of patients with IgAN. CpG-ODN is thought to be involved in the hyper-responsiveness of APRIL caused by over-expression of TACI. Such immune responses in the tonsils may be involved in the pathogenesis of IgAN.

5. Conclusion

In conclusion, we showed that APRIL in the tonsils is involved in the pathogenesis of IgAN. These results confirm the relevance of tonsillectomy as a treatment for IgAN.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

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