Release from Th1-type immune tolerance in spleen and enhanced production of IL-5 in Peyer's patch by cholera toxin B induce the glomerular deposition of IgA

Takahiro Yamanaka a, Hidekazu Tamauchi b,e,*, Yusuke Suzuki a, Hitoshi Suzuki a, Satoshi Horikoshi a, Masazumi Terashima c, Kazuya Iwabuchi b, Sonoko Habu d, Ko Okumura d, Yasuhiro Tomino a

a Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan
b Division of Cellular Immunology, Kitasato University Graduate School of Medical Science, Kitasato, 1-15-1, Minami, Sagamihara, Kanagawa 252-0373, Japan
c Ehime Plant, Dainippon Sumitomo Pharma Co., Ltd., Sobrals-cho, Niihama, Ehime 792-0001, Japan
d Department of Immunology, Juntendo University School of Medicine, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan
e Division of Fundamental Medical Technology, Department of Immunology, Ehime Prefecture University of Health Sciences, Takoua 543, Tobe-cho, Iyo-gun, Ehime 791-2101, Japan

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ABSTRACT
We examined the pathogenesis of glomerular damage in Th2 type-dependent GATA-3 transgenic (GATA-3 Tg) mice with IgA nephropathy (IgAN). GATA-3 Tg mice were immunized orally using OVA plus cholera toxin B (CTB), and measurement of the serum IgA antibody level and histopathological examination were performed. Marked increases in the serum levels of OVA-specific IgA antibody, IgA and IgG, C3 deposits analogous to those seen in IgAN, and expansion of the matrix in association with mesangial cell proliferation were observed. Furthermore, glomerular IgA deposits were co-localized with mannan-binding lectin (MBL) deposits, which might actually have been abnormal IgA deposits. In GATA-3/TCR-Tg mice that had been orally sensitized with CTB plus OVA and were re-stimulated with OVA in vitro, cultured Peeyer’s patch cells from the enhanced production of IL-5 and supernatants from cultures of spleen cells showed a reduction of TGF-β production with a simultaneous increase in IL-2 production and the recovery of IFN-γ formation. The amount of TGF-β produced by the spleen cells was found to be correlated with the amount of IFN-γ and IL-IL-2 produced by the cells. Also, the percentage of regulatory T cells (Treg) in the spleens of mice sensitized with OVA plus CTB was lower than that in mice orally sensitized with OVA alone. These results suggest that the increased production of IL-5 from Peeyer’s patch cells (PPc) and the restored Th1-type immune response might cause the production of abnormal IgA and might induce the deposition of IgA in glomeruli.

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1. Introduction

Immunoglobulin A (IgA) nephropathy was first described in 1968 (Berger and Hinglais, 1968) and is now generally regarded worldwide as the most common form of primary glomerulonephritis. IgA nephropathy (IgAN) accounts for approximately half of the cases of primary glomerulonephritis in Japan, with the disease progressing to end-stage renal failure in about 30–40% of all cases (Wakai et al., 1999).

The cause of primary IgAN is unknown. No consistent infectious or environmental agents have been identified that can be considered as being definitively responsible for the IgA-antibody response. IgAN occurs sporadically, and there is no evidence to suggest any familial pattern of inheritance of this condition. Nevertheless, immunogenetic factors may predispose some individuals to IgA nephropathy, as familial clustering has been reported (Scolari, 1999; Scolari et al., 1999; Song and Wei, 2003; Shin and Lee, 2008). Primary IgAN is a form of immune-complex-mediated glomerulonephritis and is characterized immunohistologically by
the presence of IgA deposits in the glomeruli, accompanied by a variety of histological lesions. The identification of the mechanisms responsible for the development and progression of IgAN is urgently needed, as the disease often progresses to end-stage renal disease. IgAN that is characterized by the deposition of IgA and complement C3 in the glomerular mesangial region is generally thought to be an inflammatory-immune disease associated with IgA or IgA immune complex deposits and complement activation, and its pathogenesis has been investigated from both the aspects of humoral immunity and cell-mediated immunity (Holdsworth et al., 1999; Kitching et al., 2003).

Clinical and histopathologic observations suggest that the mucosal immune system plays an important role in the pathogenesis of some forms of human immune complex-associated glomerulonephritis. For example, many studies of idiopathic recurrent hematuria and glomerulonephritis associated with alpha-1-antitrypsin deficiency, in which IgA is the predominant or only immunoglobulin deposited in the glomeruli, have reported a clinical association between kidney damage and viral-like respiratory and gastrointestinal syndromes (Roy et al., 1973; Katz et al., 1976; Hernandez et al., 1997). Furthermore, much of the IgA in the body is derived from the gut and respiratory-associated lymphoid tissue, and an expansion of specific IgA-synthesizing cells to the mesenteric lymph nodes, spleen, and secretory mucosa, together with a significant elevation in the levels of IgA in the serum and exocrine fluids, can occur in response to oral immunization (Ruedl et al., 1996). Thus, the onset of IgAN may be associated with infections of the upper respiratory tract. Accordingly, IgAN may result from hyperreactivity of the mucosal immune system.

In recent years, abnormal IgA glycosylation has been discussed in relation to the pathogenesis of IgAN. The induction of abnormal IgA glycosylation by Th2 cytokines and the involvement of these cytokines in Th2-dependent modifications of the sugar chain in the tonsillar and gastrointestinal mucosa have also been demonstrated (Chintalacharuvu and Emancipator, 1997; Chintalacharuvu et al., 2001, 2008; Kobayashi et al., 2002; Yamada et al., 2010). We examined the pathogenesis of glomerular IgA deposition using a double-transgenic mouse model, in which GATA-3 transgenic mice were crossed with OVA-recognition T cell receptor transgenic mice, to determine the contribution of mucosal immunity to the pathogenesis of IgAN. Furthermore, the relation to systemic immunity was also examined.

2. Materials and methods

2.1. Mice

GATA-3 gene-introduced transgenic (GATA-3-Tg) mice were prepared by introducing a 7.2-kb Not I linearized fragment with a lck distal promoter into an uncleaved fertilized ovum from a C57BL/6 mouse. The hemizygous GATA-3-Tg mice were then crossed with homozygous OVA233-298 peptide-specific T cell receptor gene-introduced transgenic (TCR-Tg) mice in the context of I-Ad restriction. These double-transgenic and non-GATA-3/TCR transgenic mice were designated as GATA-3/TCR-Tg and WT/TCR-Tg mice, respectively (Tamauchi et al., 2005). The mice were used in the experiments at about 10 weeks of age. Each experimental group consisted of at least 5 mice. All the experiments were conducted in accordance with the Guidelines for Animal Experimentation published by the Japanese Association for Laboratory Animal Science (1987). The protocol of this study was approved by the Animal Care and Use Committee for Experimental Animals of Kitasato University. Mice were maintained under specific pathogen-free conditions in our animal facilities.

2.2. Immunization and challenge protocol

GATA-3/TCR-Tg and WT/TCR-Tg mice were immunized orally with cholera toxin B (CTB; 10 μg; Calbiochem, Germany) alone (protocol b) or with OVA (OVA grade V; 5 mg; Sigma–Aldrich, St. Louis, USA) alone (protocol c) or with CTB plus OVA (5 mg; protocol a) three times at one-week intervals. The animals were titrated with an additional oral dose of OVA (50 mg) seven days after the last OVA plus CTB administration, followed by the collection of samples 4 days after the last OVA administration. As a control group, we administered 0.2 mL of phosphate-buffered saline (PBS) instead of the OVA challenge.

2.3. Histopathological assessment of renal tissues

Kidney tissue specimens were quickly frozen in liquid nitrogen and fixed in 10% buffered formalin. The formalin sections were stained with hematoxylin and eosin or periodic acid Schiff. Thin sections of the frozen tissues (about 3 μm thick) were cut using a cryostat, and fluorescent staining was performed at room temperature. For direct immunofluorescence, FITC-labeled goat anti-mouse IgG and C3 antibodies (Capel, Aurora, Ohio), FITC-labeled goat anti-mouse IgA antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and FITC-labeled rat anti-mouse IgG1 and IgG2a antibodies (BD Pharmingen, San Diego, CA) were used. Biotin-labeled mannan-binding lectin (MBL) antibody was provided by Dr. M. Matsushita (Tokai University School of Engineering, Hiratsuka Kanagawa). The stained preparations were examined using a Zeiss dark-field fluorescence microscope. Staining for MBL was also performed using the enzyme-labeled antibody method and the avidin-biotinylated peroxidase complex technique. The sections were pretreated using the Avidin/Biotin Blocking Kit (Vector, UK) before staining. The levels of renal damage were then assessed using PAS-stained sections.

2.4. Measurement of OVA-specific IgA antibody in sera

OVA-specific IgA antibodies in the sera of immunized mice were measured using a sandwich enzyme-linked immunosorbent assay (ELISA), as described previously (Tamauchi et al., 2005). Each serum sample, as well as serial dilutions of the control sample, was incubated in a 96-well microtiter plate coated with OVA (100 μg/mL; Sigma). After incubation, biotin-labeled rat monoclonal antibodies (mAb) against mouse IgA (2ymed Laboratories, South San Francisco, CA) were incubated with streptavidin-horseradish peroxidase (BD Pharmingen, San Diego, CA). Finally, the plates were subjected to a peroxidase reaction in a tetramethylbenzidine (TMB) substrate solution for color development and analyzed at 450 nm using an ELISA plate reader.

2.5. Cytokines production assay of culture supernatants

GATA-3/TCR-Tg mice and WT/TCR-Tg mice were orally immunized with OVA or OVA plus CTB three times at one-week intervals. Four days after the last immunization, the animals were sacrificed and the Peyer’s patch and spleen were removed and used to make cell suspensions. The Peyer’s patch and spleen cells (5 × 10⁶/well) were then cultured for 48 h at 37 °C under 5% CO₂ in 96-well flat-bottomed plates containing the indicated concentrations of purified OVA grade V (Sigma Chemical Co., St. Louis, MO). The levels of IFN-γ, IL-2, IL-4, and IL-5 in the supernatants obtained from the cultured cells were determined using ELISA assay kits (Endogen, Cambridge, MA) as well as TGFB-β (R&D Systems, UK). Cells with a survival rate of 95% or more were used.
Table 1
Immunostaining of IgA, IgG1, IgG2a and C3 in Kidney of the OVA-plus-CTB immunized GATA-3/TCR Tg mice.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Immunization</th>
<th>Challenge</th>
<th>No. of positive mice/No. of total mice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT/TCR</td>
<td>CTB</td>
<td>OVA</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>WT/TCR</td>
<td>OVA</td>
<td>OVA</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>WT/TCR</td>
<td>OVA + CTB</td>
<td>OVA</td>
<td>2/10 (20)</td>
</tr>
<tr>
<td>GATA-3/TCR</td>
<td>CTB</td>
<td>OVA</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>GATA-3/TCR</td>
<td>OVA</td>
<td>OVA</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>GATA-3/TCR</td>
<td>OVA + CTB</td>
<td>OVA</td>
<td>9/10 (90)</td>
</tr>
</tbody>
</table>

Note: GATA-3/TCR-Tg mice and WT/TCR-Tg mice were treated with OVA, CTB alone orally or OVA plus CTB orally. OVA and the adjuvant were administered three times at one-week intervals, followed by the oral administration of OVA seven days after the last immunization. Four days after the OVA challenge, the renal tissues were frozen in liquid nitrogen. Sections were then stained with immunofluorescent antibodies for IgA, IgG1, IgG2a or C3. The sections were observed at a magnification of ×200.

2.6. Flow-cytometric analysis

Three days after the oral OVA challenge of GATA-3/TCR-Tg mice sensitized with OVA plus CTB, the spleen and Peyer’s patches of the mice were removed and cell suspensions were prepared. The Treg cells were stained with the Mouse Regulatory T cell Staining kit (eBioscience, San Diego CA), washed with Foxp3 staining buffer, and analyzed using FACScan.

2.7. Statistical analysis

Statistical significance was determined using an unpaired t-test (comparison of two groups) or a one-way ANOVA (comparison of three or more groups). The results are expressed as the mean ± S.D. Differences of P < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Immunopathological findings in the kidney and their correlations with serum OVA-specific IgA antibody titers

At first, we examined GATA-3 Tg mice using the immunological method shown in Fig. 1 to determine the immunological mechanism underlying the deposition of IgA in the glomeruli in IgA nephropathy.

The renal tissues of the GATA-3/TCR-Tg mice orally immunized with OVA-plus-CTB showed marked deposition of IgA, IgG2a, and C3 and the expansion of the matrix associated with mesangial cell proliferation in the kidney; these observations were analogous to those seen in clinical cases of IgAN in humans. The deposition of IgA, C3, and IgG2a was observed in 90% of the OVA-plus-CTB-immunized GATA-3/TCR-Tg mice. In the WT/TCR-Tg mice, however, the deposition of IgA, C3, and IgG2a was observed in less than 20% of the OVA-plus-CTB-immunized mice (Table 1).

Regarding IgA deposition, the OVA-plus-CTB-immunized/OVA-challenged WT/TCR-Tg mice exhibited a much weaker deposition than the OVA-plus-CTB-immunized/OVA-challenged GATA-3/TCR-Tg mice (Fig. 2). The deposition of Th1-dependent IgG2a was not observed in the glomeruli of the IgA deposition-negative OVA-plus-CTB-immunized/OVA-challenged WT/TCR-Tg mice. However, the marked deposition of Th2-dependent IgG1 was found in the glomeruli of both the OVA-plus-CTB-immunized/OVA-challenged GATA-3/TCR-Tg and WT/TCR-Tg mice (Fig. 2 and Table 1). No histological changes were found in the glomeruli of mice immunized with CTB or OVA alone (Table 1).
We measured the OVA-specific IgA antibody titers in serum samples obtained from OVA-plus-CTB-immunized/OVA-challenged mice and found that the OVA-specific IgA antibody titer was approximately five times higher in the GATA-3/TCR-Tg mice than in the WT/TCR-Tg mice (Fig. 3). Although the data are not shown, we did not find any differences in the changes in the level of OVA-specific IgA antibody in the serum following the administration of OVA alone. We were also unable to recognize any differences in the antibody titers between the GATA-3/TCR-Tg and WT/TCR-Tg mice following the administration of only CTB three times. Furthermore, the antibody titers were similar in the non-immunized GATA-3/TCR-Tg and WT/TCR-Tg mice (OD<sub>450</sub> 0.9  1.0). In addition, no significant difference in the production of IgA was observed between the non-immunized GATA-3/TCR-Tg mice and the WT/TCR-Tg mice.

We examined the deposition of IgA and MBL because the deposition of MBL, which consists of molecules participating in the lectin pathway, has been reported in IgAN. We found that IgA and MBL deposits were colocalized in the mesangial region in the OVA-plus-CTB-immunized/OVA-challenged GATA-3/TCR-Tg mice. However, in the OVA-plus-CTB-immunized/OVA-challenged WT/TCR-Tg mice, no deposits of either IgA or MBL were found (Fig. 4).

Oral antigen sensitization using CTB may have resulted in a carbohydrate chain abnormality in the IgA produced in GATA-3/TCR-Tg mice, in which the Th1/Th2 balance of the immune system was strongly tilted towards Th2-type immunity.

3.2. Production of Th1 and Th2 cytokines from the spleen cells and Peyer’s patch cells following oral immunization

We obtained spleen cells from GATA-3/TCR-Tg mice sensitized orally with OVA alone or OVA plus CTB and re-stimulated the cells with OVA antigen in vitro to further examine the cytokine production. The production of IFN-γ and IL-2, which are Th1-type cytokines, was inhibited (Fig. 5a) in the GATA-3/TCR-Tg mice immunized with OVA alone, compared with that in non-immunized GATA-3/TCR-Tg mice. For IFN-γ production, a notably significant difference was recognized. On the other hand, no changes in the production of IL-5, which is a Th2-type cytokine, or TGF-β were observed (Fig. 5b and c). However, the production of IL-4 was about 1.5–2 times higher in the immunized GATA-3/TCR-Tg mice than in similarly immunized WT/TCR-Tg mice. When stimulated with OVA concentrations from 500 to 1000 µg/ml, a significant difference was found in the level of IL-4 production from the spleen cells of GATA-3/TCR-Tg and WT/TCR-Tg mice. In the mice treated with OVA plus CTB, IL-2 and IFN-γ production was restored, compared with that observed in the mice treated with OVA alone, and the production levels of these cytokines were similar to those seen during a normal murine response. A more moderate suppression of the production of IL-4 and TGF-β was observed, compared with that in the untreated mice. IL-4 production was inhibited to the same extent as that in the untreated mice, whereas the IL-2 and IFN-γ production levels were restored to the same extent as that in the untreated mice (Fig. 5b and c).

In the PPC obtained from the GATA-3/TCR-Tg mice, a three-fold to four-fold increase in the production of IL-5 and an approximately 1.5-fold increase in the production of IL-4 were observed in animals treated orally with CTB plus OVA, compared with that in PPC from OVA-plus-CTB-immunized WT/TCR-Tg mice. In PPC obtained from OVA-plus-CTB-immunized or OVA-alone-immunized GATA-3/TCR-Tg mice, the degree of IL-2 and IFN-γ production was the same as that in PPC from WT/TCR-Tg mice. As for the quantity of TGF-β produced by the PPC, no differences were observed between the groups. TGF-β was produced regardless of the presence or absence of antigen sensitization. However, when combined with CTB, the quantity of TGF-β production increased, but this response was not specific to GATA-3/TCR-Tg mice. In mice sensitized with OVA alone, the profile of TGF-β produced from the spleen was similar to that for PPC from GATA-3/TCR-Tg and WT/TCR-Tg mice. However, the amount of TGF-β produced by the spleen cells from the CTB-plus-OVA-immunized GATA-3/TCR-Tg mice was antigen-dependent, except in the group treated orally with OVA (Fig. 5c).

3.3. Analysis of Treg populations in the spleen and Peyer’s patch

We measured the numbers of CD4<sup>+</sup> Foxp3<sup>+</sup> cells in the spleen and Peyer’s patch of GATA-3/TCR-Tg mice sensitized with OVA plus CTB using a FACSscan. The results revealed that after the administration of OVA, both the GATA-3/TCR-Tg mice and the WT/TCR-Tg mice showed an approximately 1.5-fold increase in the number of CD4<sup>+</sup> Foxp3<sup>+</sup> cells in the spleen, compared with the levels in respective non-treated Tg mice. In the Peyer’s patches, on the other hand, the percentage of CD4<sup>+</sup> Foxp3<sup>+</sup> cells decreased in both the GATA-3/TCR-Tg and WT/TCR-Tg mice following the oral administration of OVA. These changes in the numbers of CD4<sup>+</sup> Foxp3<sup>+</sup> cells (Fig. 6) were similar to those observed for TGF-β production, as shown in Fig. 5.

The addition of CTB was suggested to control the Treg cells. Also, both the spleen and Peyer’s patches of the OVA-plus-CTB-immunized mice showed equivalent percentages of Treg cells, compared with the percentages for the spleen and Peyer’s patches of untreated GATA-3/TCR and WT/TCR mice. The percentages of Treg cells were also similar in untreated and OVA-alone-treated mice of both genotypes. From these results, the cytokine production and proportion of Treg cells appeared to show contradictory responses in the spleen and Peyer’s patch, and CTB could be responsible for regulating the immune response.

4. Discussion

Recently, tonsillectomy plus steroid pulse therapy for the treatment of IgAN has received considerable attention, with the
therapeutic response reportedly varying with the interval from the onset of the abnormal urinalysis findings until the initiation of treatment (Leiri et al., 2007). The remission rate is high in patients with early treatment and declines with an increasing interval between disease onset and treatment initiation. Based on these observations, we suggested that the cells responsible for the development of IgAN may be present in the mucosa, including the tonsils, during the early phase of the disorder and may migrate from the mucosa to the secondary lymph nodes as well as the bone marrow and spleen in the later phases. Thus, immune responses in the mucosa are likely to be vital in the development of IgAN.

The present study was conducted using GATA-3-Tg mice that constantly express the GATA-3 gene, tilting the Th1/Th2 balance towards Th2 dominance, with the aim of examining the pathogenesis of IgAN by clarifying the mechanism of the production and deposition of IgA in these animal models (Tamauchi et al., 2004).

In mice orally immunized with OVA plus CTB, IgA deposits were evident in the kidney, and these IgA deposits were colocalized with MBL deposits (Fig. 4). OVA-specific IgA has been suggested to consist of abnormally glycosylated IgA (Oortwijn et al., 2006; Hashimoto et al., 2012).

The Th2-type cytokines IL-4 and IL-5 have been reported to be involved in the glycosylation of IgA. As a cause of the deposition of IgA in the kidney, the abnormal glycosylation of IgA has been suggested, and the Th2-type cytokine IL-4 is reportedly important for such abnormal glycosylation (Chintalacheruvu et al., 2001; Yamada et al., 2010). At the time, however, an association between the Th2-type cytokine IL-5 produced by PPC and the deposition of IgA had been suggested. Regarding the production of IL-4, we recognized a statistically significant difference in the level of IL-4 produced by PPC from WT/TCR-Tg, compared with that produced by PPC from GATA-3/TCR-Tg mice. However, the production of IL-5 was thought to play an important role in IgA deposition in the glomerulus because the production of IL-5 was much higher than that of IL-4 in PPC. Recently, glycosyltransferase of α-N-acetylgalactosaminidase α-2,6-sialyltransferase 2 and β-1,3-galactosyltransferase were reported to induce the deposition of IgA in the glomerulus in response to IL-6 (Suzuki et al., 2014).

In addition, IFN-γ, a Th1-type cytokine, has also been reported to be involved in glycosylation (Chintalacheruvu et al., 2001). Oral immunization with either OVA or CTB alone did not evoke any significant changes in the serum IgA antibody titers or the degrees of IgA deposition in the kidney, indicating that the oral co-administration of CTB as a mucosal adjuvant together with the antigen (OVA) was important for inducing IgA deposition in the kidney. With a view to further clarifying the mechanism of production of the deposited IgA, PPC and spleen cells obtained from mice that had been orally immunized with OVA alone or with OVA plus CTB three times at one-week intervals were re-exposed to the antigen (OVA) in vitro, followed by assays of the culture supernatants collected after 48 h of incubation and analyzed using ELISA for Th1 and Th2 cytokines (IFN-γ, IL-2, IL-4, IL-5, and TGF-β) to assess the Th1/Th2 balance. A marked enhancement of IL-5 production was noted in the 48-h cultures of PPC obtained from GATA-3/TCR-Tg mice immunized with OVA plus CTB. Oral sensitization with OVA inhibited the production of IL-2 and IFN-γ from the spleen cells. However, in the cultures of spleen cells obtained from GATA-3/TCR-Tg and WT/TCR-Tg mice orally immunized with OVA plus CTB, TGF-β production was suppressed, while IL-2 production remained stable and IFN-γ production tended to be restored. A previously published report described the role of the immune response to CTB (Elson and Ealing, 1984; Iijima et al., 1998; Marinaro et al., 1995). We have also reported that IL-2-dependent energy was induced by the oral administration of OVA (Tamauchi et al., 2005). In vitro, the production of IFN-γ and IL-2 from the spleen cells of OVA-inoculated mice was remarkably inhibited, compared with that of untreated mice, when the murine spleen cells were stimulated with OVA. However, we clarified that the production of IFN-γ was restored by the addition of IL-2 during OVA stimulation (Tamauchi et al., 2005).

We were able to show that the IgA antibody responses in mucosal lymphoid tissues, such as Peyer’s patches, generally involve a predominance of IgA production (data not shown). An increase in IgA production was observed in the spleen of the GATA-3/TCR-Tg mice after the oral administration of OVA plus CTB in this experimental system (Fig. 3). A proliferation of TGF-β-positive cells was also observed in this model (data not shown). TGF-β is an inhibitory cytokine secreted by regulatory T cells, which include CD4+Foxp3+ -regulating T cells as endogenous constitutive regulatory T cells, and Th3 and Tr1 cells as inductive regulatory T cells. Immune tolerance arising from IL-2-dependent energy was induced in the spleen of the GATA-3/TCR Tg mice by oral sensitiza-
Fig. 5. Production of cytokines in spleen and Payer’s patch cells from OVA-plus-CTB-immunized/OVA-challenged GATA-3/TCR-Tg mice. Spleen and Payer’s patch cells were obtained from GATA-3/TCR-Tg and WT/TCR-Tg mice that had been orally immunized with OVA plus CTB three times at one-week intervals; the cells were re-stimulated with OVA in vitro for 48h, and the supernatants were recovered and assayed using ELISA kits for (a) IFN-γ and IL-2, (b) IL-4 and IL-5, and (c) TGF-β. Data are the mean ± S.D. of three independent experiments. Significant differences in the cytokine levels produced by the spleen and Payer’s patch cells of GATA-3/TCR-Tg and WT/TCR-Tg mice sensitized with OVA plus CTB were observed. *P < 0.05 and **P < 0.01.
tion with OVA, indicating that the TGF-β-positive cells were highly likely to be CD4+Foxp3+ -regulating T cells. When the lymphocytes of the Peyer’s patches of the GATA-3/TCR-Tg mice sensitized with OVA plus CTB were challenged with OVA, the amount of TGF-β produced by the cells was the same as that produced by Peyer’s patch lymphocytes from WT/TCR-Tg mice sensitized with OVA plus CTB and then challenged with OVA.

TGF-β is reportedly produced by Treg (CD4+Foxp3+) (Smaldini et al., 2015). Also, we confirmed that CD4+CD25+Foxp3+ cells and TGF-β+cells were equivalent in this study using immunohistological studies (data not shown). The results of this study clearly show that TGF-β in the periphery controls IFN-γ production. Moderate TGF-β production was observed in PPC from OVA-alone-inoculated mice, compared with untreated mice, but the difference was not statistically significant. In in vitro studies, the production of TGF-β increased according to the antigen quantity, similar to the production of TGF-β in the spleen in mice not treated with OVA. However, in the OVA-treated group, the production of a fixed level of TGF-β was always observed regardless of the quantity of antigen used for stimulation. As for the level of TGF-β production from PPC, the same level was always produced regardless of the quantity of antigen used for stimulation and regardless of the administration of OVA. While the reason for this observation remains uncertain, stimulation with enteric bacteria or food might always produce the same results.

The PPC from the GATA-3/TCR-Tg mice used in the present study exhibited mainly IL-5 production after the oral immunization of the mice with OVA plus CTB. Furthermore, the results suggested that with the addition of CTB to OVA, the amount of IgA produced was enhanced by the increased production of TGF-β associated with the recovery of the percentage of CD4+Foxp3+ cells.

Because other groups have also described the importance of IL-5 and TGF-β in IgA antibody production, our models appeared to be suitable for eliciting the efficient production of IgA (Sonoda et al., 1989). The onset trend for the deposition of isotypic antibody in the glomeruli, the cytokine-producing profiles, and the percentages of Treg cells indicated that the clinical condition arises as a result of alterations in the Th1/Th2 balance. So far, other groups have suggested the contribution of Th1/Th2 balance to the pathogenesis of IgAN (Chintalacharuvu et al., 1997, 2001, 2008; Kobayashi et al., 2002).

Recently, Kuwahara et al. (2012) reported that TGF-β inhibited Th2-type immune responses. However, we were not able to show that the enhanced production of TGF-β following immunization with OVA plus CTB suppressed Th2-type cytokine production in PPC from GATA-3/TCR-Tg mice. Furthermore, TGF-β production was suppressed in spleen cells from GATA-3/TCR-Tg mice following
immunization with OVA plus CTB. However, the production of IL-2 and IFN-γ, both Th1-type cytokines, recovered to the same extent as that from the spleen in the untreated mice, despite the absence of the suppression of Th2-type cytokine production. We previously reported that a contradiction might exist between the peripheral immune responses in the spleen and mucosal immune responses in the mesenteric lymph nodes, especially in this experimental system involving the use of OVA (Tamauchi et al., 2005).

The existence of both T cell-dependent (TD) and T cell-independent (TI) pathways has been reported for the production of IgA. Plasmacytoid dendritic cells (pDCs) in Peyer’s patches are involved in IgA production, and pDCs in mesenteric lymph nodes (MLN) are involved in TI IgA production. The expressions of a proliferation-inducing ligand (APRIL) and B cell-activating factor of the tumor necrosis factor family (BAFF) in pDCs are important, and these factors are reported to control IgA antibody production (Tezuka et al., 2011).

Also, there is a report suggesting that in BAFF-overexpressing Tg mice, IgA antibody production against commensal bacteria and the deposition of IgA in the glomeruli are enhanced, and these findings suggest that mucosal immunity is involved in the deposition of IgA in the glomeruli in IgAN (McCarthy et al., 2011).

In the present study, we did not analyze pDCs. However, the contribution of pDCs was suggested, since OVA, which was used as the antigen, is a TD antigen. It is unknown whether APRIL and BAFF in pDCs play important roles in the deposition of IgA in the glomeruli even though they are closely involved in the production of IgA. Also, the possible involvement of TI in our experimental system cannot be excluded.

These findings suggest that the deposition of IgA in the glomeruli in IgAN is induced by a Th2-type cytokine (IL-5) involved in mucosal immunity and a Th1-type cytokine (IL-2 and IFN-γ) production involved in systemic immunity, and the possible involvement of TGF-β production in the development of IgAN has also been suggested (Border et al., 1990; Chihara et al., 2006). Regarding T-dependent IgA antibody production, mutual interactions between CD40 and CD40L, which are co-stimulatory molecules, are reportedly important (Stavnezer, 1996).

As for the deposition of IgA antibody in the glomeruli, it was suggested that the recovery of the Th1-type immune response through the failure of systemic acquired tolerance and the enhanced IL-5 production associated with the Th2-type mucosal immune response may be involved.

In this model, hematuria and albuminuria were not detected in the GATA-3/TCR-Tg mouse model. Therefore, which factors may be necessary to induce hematuria and albuminuria should be examined in detail.

Conflicts of interests

1. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

2. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

3. We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of animal bodies and that such approvals are acknowledged within the manuscript.

Author contributions

Conceived and designed the experiments: TY, HT, YS, HS, MT, KI, SH, KO, and YT. Performed the experiments: TY and HT. Analyzed the data: TY, HT, YS, HS, MT, KI, SH, KO, and YT. Contributed reagents/materials/analysis tools: TY, HT, YS, HS, MT, KI, SH, KO, and YT. Wrote the paper: TY, HT, YS, and YT.

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References


