—Original—

Th2-biased GATA-3 transgenic mice developed severe experimental peritoneal fibrosis compared with Th1-biased T-bet and Th17-biased RORγt transgenic mice

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Abstract: Encapsulating peritoneal sclerosis is one of the most serious complications of long-term peritoneal dialysis. The pathogenesis of encapsulating peritoneal sclerosis has not been elucidated, but several putative factors necessary for the development of peritoneum fibrosis (PF) have been reported. However, the roles of T helper (Th) cells in the progression of PF are unknown. The purpose of this study was to clarify the roles of Th1, Th2, and Th17 cells in the progression of PF. T-bet, GATA-3, and RORγt are Th1, Th2, and Th17 lineage commitment transcription factors, respectively. We previously generated Th1-biased (T-bet transgenic (Tg)) mice, Th2-biased (GATA-3 Tg) mice, and Th17-biased (RORγt Tg) mice. In this study, Th1, Th2, Th17-biased, and wild-type mice were administered chlorhexidine gluconate (CG) intraperitoneally and analyzed on day 21. CG-injected GATA-3 Tg mice showed a distended intestinal tract and developed marked thickening of the submesothelial space compared with the other groups. CG-injected GATA-3 Tg mice also showed significant expression of α-SMA positive cells, macrophages, and collagen III in the submesothelium. In contrast, CG-injected T-bet Tg mice only developed mild peritoneal fibrosis. Cytokines analysis in peritoneal fluid showed that IFN-γ was significantly increased in CG-injected T-bet Tg mice and that IL-13 was significantly increased in CG-injected GATA-3 Tg mice. Moreover, intraperitoneal administration of IFN-γ improved PF in GC-injected wild-type mice. Our results suggest that Th2 cells may play roles in the development of experimental PF and that Th1 cells may alleviate the severity of experimental PF.
Key words: IFN-γ, peritoneal fibrosis, T helper cell type 1, T helper cell type 2

Introduction

Encapsulating peritoneal sclerosis (EPS) is a severe complication of long-term peritoneal dialysis (PD). EPS is characterized by progressive and excessive fibrotic thickening of the peritoneum, leading to encapsulation of the bowels and intestinal obstruction [18]. Several reports have shown an increase in the incidence of EPS, especially in long-term PD patients [3, 12, 13]. Kawanishi *et al.* reported an overall incidence of 2.5%, with

a higher incidence of 17.2% and a mortality rate of 100% for patients suffering from PD for more than 15 years [13]. A recent study by Johnson *et al.* showed that the respective cumulative incidences of peritoneal sclerosis at 5, and 8 years were 0.8, and 3.9%, respectively [12]. These studies showed that EPS is a rare condition; but that it has a high mortality rate. Previous studies have provided insight into the pathophysiology of EPS and identified possible risk factors. Several putative causative factors for the development of EPS were reported in epidemiologic or animal studies, including peritonitis caused by bacterial or fungal organisms and exposure to plasticizers, acidic pH, and glucose degradation products in PD solutions [30].

In PD patients with peritonitis, an inflammatory process occurs, but the roles of T cells and the patterns of cytokine secretion also have not yet been clarified. CD4+ T helper (Th) cells are a subcategory of T lymphocytes that play a central role in modulating immune responses. Three major subtypes of effector T helper cells have been identified, Th1, Th2, and Th17 cells [8, 25, 29]. Th1 cells induce cellular immunity and granuloma formation and protect against intracellular pathogens [16, 31]. The Th2 subset favors production of the various immunoglobulin classes that shape or help humoral immunity [16]. Th17 cells participate in the development of autoimmunity; and play an important role in host defense against infection [36]. These three polarized T helper subsets can be identified by the cytokines they secrete. Th1 cells produce interleukin (IL)-2 and interferon-γ (IFN-γ), Th2 cells produce IL-4, IL-5, and IL-13; and Th17 cells produce IL-17, IL-21, and IL-22 [24]. Klínger *et al.* reported that patients on PD with or without peritonitis showed immune activation *per se* and high production of pro-inflammatory cytokines accompanied by a strong pattern of Th2 cytokines and a deficiency of IFN-γ production. This indicated immunodeviation towards a Th2 response and Th1 immunodeficiency [16].

Experimental models of EPS have been described, and several agents have been used to induce EPS, including chlorhexidine gluconate (CG) [34], acidic glucose solution (pH 3.8) [26], and acidic glucose solution (pH 5.0) supplemented with methylglyoxal [9, 30]. CG, a chemical irritant, is the most commonly used agent, and repeated injections of CG in rats or mice disrupt mesothelial cell integrity and cause subsequent injury in subserosal tissue, leading to inflammatory responses and subsequent excessive fibrosis. The progression and macroscopic and histological findings induced by CG are similar to EPS in human patients [30].

T-bet, GATA-3, and retinoic acid-related orphan receptor gamma-t (RORγt) are Th1 [35], Th2 [46, 47], and Th17 lineage commitment transcription factors [11, 40], respectively. We previously generated Th1-biased (T-bet transgenic (Tg)) mice [10], Th2-biased (GATA-3 Tg) mice [44], and Th17-biased (RORγt Tg) mice [43] to clarify relationships in several diseases using Th1, Th2, and Th17 mouse backgrounds [1, 10, 14, 15, 17, 23, 32, 43, 44]. In this study, we used Th1, Th2, and Th17-biased mice to elucidate the roles of T helper cells in a CG-induced peritoneum fibrosis (PF) model.

Materials and Methods

Animals

T-bet Tg, GATA-3, and RORγt Tg male mice on the C57BL/6J background and their wild-type littermates were used. Transgenic mice overexpressing T-bet, GATA-3, or RORγt under the control of the CD2 promoter were generated in our laboratory, as previously described [10, 43, 44]. Mice were fed a normal diet comprised of commercial laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and were maintained under specific pathogen-free conditions in the Laboratory Animal Resource Center of the University of Tsukuba. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals at the University of Tsukuba, and the study was approved by the Institutional Review Board of the university.

Mice PF model

Ten-week-old mice were administered 0.1% CG (0.01 ml/g body weight) in 15% ethanol intraperitoneally three times a week. Control mice were injected with 15% ethanol in 2 ml of saline intraperitoneally. After 21 days, mice were sacrificed, and peritoneal tissues were obtained for the study.

Intraperitoneal administration of IFN-γ and anti-IL-13 antibody

Mice were administered IFN-γ (R&D Systems, Minneapolis, MN, USA) (100 unit/g body weight), goat anti-mouse IL-13 antibody (R&D) (1μg/g body weight), and normal goat IgG control (R&D) (1μg/g body weight) intraperitoneally two times a week. IFN-γ, anti-IL-13 antibody, and normal IgG control injection started one

week prior to CG administration. The period of IFN-γ, anti-IL-13 antibody, and normal IgG control injection was four weeks. Mice were also administered 0.1% CG (0.01 ml/g body weight) in 15% ethanol intraperitoneally three times a week for three weeks.

Histopathological analysis and immunohistochemistry

Organs were fixed with 10% formalin in 0.01 M phosphate buffer (pH 7.2) and embedded in paraffin. Sections (3 μm) were stained with hematoxylin and eosin (H&E) for histopathological examination by light microscopy. We used a monoclonal anti-α smooth muscle actin antibody (α-SMA) (Sigma, St Louis, MO, USA), a rat anti-mouse macrophage (F4/80) antibody (Cedarlane Labs, Burlington, ON, Canada), and a rabbit anti-collagen III antibody (LSL Co., Ltd., Tokyo, Japan). Staining for α-SMA was performed using Histofine Simple Stain Max PO (mouse) (Nichirei, Tokyo, Japan). Anti-collagen III and F4/80 staining were performed using and Histofine Simple Stain Max PO (rat) (Nichirei).

Morphometric analysis of histology and immunohistology

Morphometric analyses were performed using a BIO-REVO BZ-9000 fluorescence microscope (Keyence, Osaka, Japan). Fibrotic change was evaluated by measuring the full thickness of the submesothelium in Masson's trichrome-stained sections oriented perpendicular from the top of the muscle layer to the serosal surface in 3 randomly selected fields. The stained area of α-SMA and collagen III was assessed in a predetermined field (magnification ×40) of the submesothelial zone in 3 randomly selected fields. The number of cells positive for F4/80 (magnification ×100) in the submesothelial zone was counted in 3 randomly selected fields.

Peritoneal lavage fluid

The peritoneal cavity was lavaged with 4 ml of sterile saline, and this peritoneal lavage fluid was collected. The peritoneal lavage fluid was then stored at –20°C for assessment of cytokine levels.

Measurement of cytokines

Appropriate ELISA kits were used to determine the levels of IFN-γ, IL-4, IL-5, and IL-13 (R&D Systems), in accordance with the manufacturer's instructions.

Statistical analysis

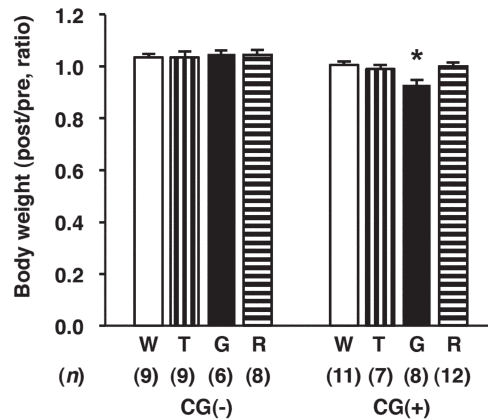All data are expressed as means ± SEM. Multiple data

Fig. 1. Body weight changes of control mice and chlorhexidine gluconate (CG)-injected mice. The ratios of body weight change between the weights on day 21 and before treatments are shown. W, wild-type mice; T, T-bet transgenic (Tg) mice; G, GATA-3 Tg mice; R, RORγt Tg mice. Data represent means ± SEM. *$P<0.01$, CG-injected GATA-3 Tg mice vs. control GATA-3 Tg mice, CG-injected wild-type mice, and T-bet Tg and RORγt Tg mice.

comparisons were performed by one-way analysis of variance (ANOVA). *P* values<0.05 were considered statistically significant.

Results

GATA-3 Tg mice developed significant body weight loss caused by CG administration

We injected CG into wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice and analyzed body weight on day 21. Body weight ratio was calculated by comparing the posttreatment body weight with the pretreatment body weight (Fig. 1). There were no significant changes in the control groups. In the CG treatment groups, the body weight ratio in GATA-3 Tg mice (0.93 ± 0.02) was significantly decreased compared with wild-type (1.01 ± 0.02), T-bet Tg (0.99 ± 0.02), and RORγt Tg mice (1.00 ± 0.01). The body weight ratio in CG-treated GATA-3 Tg mice was also significantly decreased compared with control GATA-3 Tg mice (1.05 ± 0.02).

CG-injected GATA-3 Tg mice developed a distended intestinal tract and surface bleeding

Mice were sacrificed on day 21. All mice developed peritonitis. However, CG-injected GATA-3 Tg mice developed the most severe peritonitis compared with the
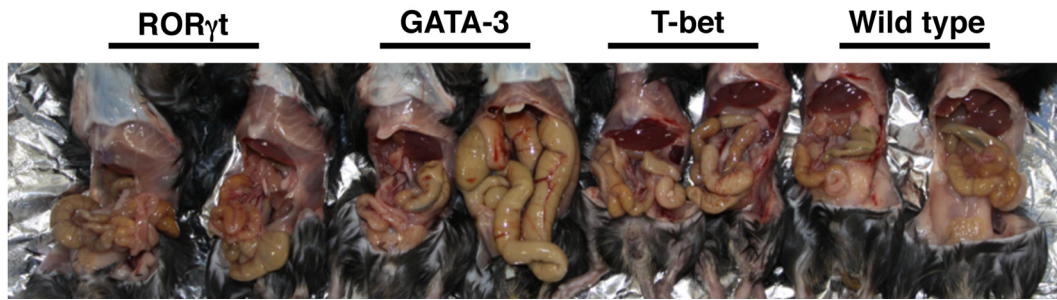
Fig. 2. Macroscopic findings of the peritoneum of CG-injected mice. CG-injected mice were sacrificed on day 21 and observed macroscopically. Representative mice are shown. CG-injected GATA-3 Tg mice developed severe distended intestinal tract and surface bleeding. The gastrointestinal loop in the CG-injected GATA-3 Tg mouse shows adhesion and bleeding.

other mice (Fig. 2). Distended intestinal tract and predominant bleeding on the surface of the intestinal tract were only detected in CG-injected GATA-3 Tg mice.

CG-injected GATA-3 Tg mice developed a thickened peritoneum

Masson's trichrome staining was used to analyze peritoneal thickness (Fig. 3). In the control groups, the peritoneal tissue consisted of a peritoneal mesothelial monolayer (Fig. 3A). Compared with the control group, peritoneal tissue of the GC-injected groups showed significant thickening of the submesothelial compact zone (Fig. 3B and C). The mean peritoneal thicknesses of the control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 24.3 ± 2.4 μm, 25.4 ± 3.9 μm, 25.0 ± 2.0 μm, and 25.3 ± 1.4 μm, respectively. The mean peritoneal thicknesses of the CG-injected wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 114.5 ± 9.0 μm, 76.3 ± 16.9 μm, 145.3 ± 16.5 μm, and 104.2 ± 11.5 μm, respectively. The thickness of the submesothelial compact zone in CG-injected GATA-3 Tg mice was significantly greater than in the other CG-injected groups.

Increased expression of α-SMA in the submesothelial zone of CG-injected GATA-3 Tg mice

The expression of α-SMA was rarely observed in the submesothelial zone in control mice (Supplementary Fig. 1A). The mean areas of positive α-SMA staining of the control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were $13.7 \pm 2.5 \times 10^3$ μm^2, $12.0 \pm 1.0 \times 10^3$ μm^2, $15.9 \pm 4.0 \times 10^3$ μm^2, and $16.0 \pm 2.8 \times 10^3$ μm^2, respectively (Supplementary Fig. 1B). The mean areas of positive α-SMA staining of the CG-injected wild-type,

T-bet Tg, GATA-3 Tg, and RORγt Tg mice were $77.7 \pm 5.2 \times 10^3$ μm^2, $51.4 \pm 8.6 \times 10^3$ μm^2, $124.3 \pm 29.2 \times 10^3$ μm^2, and $71.2 \pm 19.7 \times 10^3$ μm^2, respectively (Fig. 4A and Supplementary Fig. 1B). In the CG-injected group, increased expression of α-SMA was observed, and the positive areas in CG-injected GATA-3 Tg mice were significantly larger than those in the other CG-injected groups.

Increased expression of collagen III in the submesothelial zone of CG-injected GATA-3 Tg mice

In the control groups, the expression of collagen III was minimal in the submesothelial zone (Supplementary Fig. 2A). The mean areas of positive collagen III staining of the control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were $22.7 \pm 2.6 \times 10^3$ μm^2, $23.6 \pm 5.8 \times 10^3$ μm^2, $21.3 \pm 2.9 \times 10^3$ μm^2, and $21.7 \pm 3.3 \times 10^3$ μm^2, respectively (Supplementary Fig. 2B). The mean areas of positive collagen III staining of the CG-injected wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were $106.7 \pm 26.5 \times 10^3$ μm^2, $47.1 \pm 12.9 \times 10^3$ μm^2, $117.8 \pm 14.3 \times 10^3$ μm^2, and $62.0 \pm 16.4 \times 10^3$ μm^2, respectively (Fig. 4B and Supplementary Fig. 2B). In the CG-injected group, increased expression of collagen III was observed, and the positive areas in CG-injected GATA-3 Tg mice were significantly larger than in the other CG-injected groups.

Increased numbers of F4/80-positive macrophage cells in the submesothelial zone were observed in CG-injected GATA-3 Tg mice

We examined expression of the mouse monocyte/macrophage surface marker F4/80 in the peritoneum (Supplementary Fig. 3A). The mean numbers of F4/80-
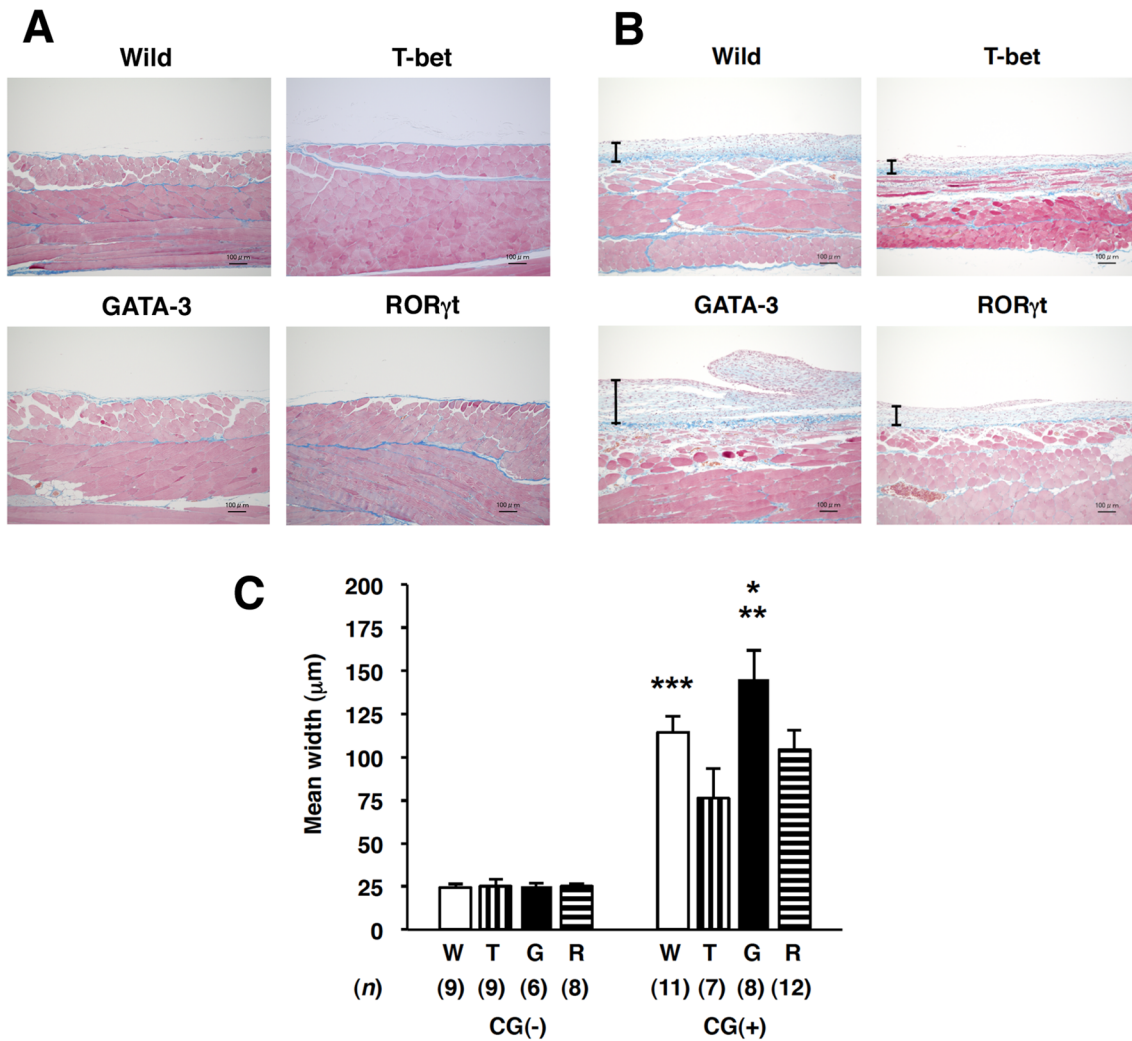
Fig. 3. Representative light microscopic features of peritoneal tissue on days 21 in control mice (A) and CG-injected wild-type (W), T-bet Tg (T), GATA-3 Tg (G), and RORγt Tg (R) mice (B) (Masson-trichrome stain, original magnification ×100, scale bar 100 μm). Bars indicate thickness of the submesothelium. (C) Bar graph showing the thickness of the submesothelial zone. Data represent means ± SEM. *$P<0.01$ vs. CG-injected T-bet Tg and RORγt Tg mice; **$P<0.05$ vs. CG-injected wild-type mice; ***$P<0.05$ vs. CG-injected T-bet Tg mice.

positive cells in control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 3.0 ± 1.1, 1.7 ± 1.7, 3.0 ± 2.0, and 1.7 ± 1.7 in the observation field, respectively (Supplementary Fig. 3B). The mean numbers of F4/80-positive cells in CG-injected wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 19.0 ± 3.9, 7.0 ± 3.0, 44.3 ± 12.1, and 9.7 ± 1.9 in the observation field, respectively (Fig. 4C and Supplementary Fig. 3B). There were no significant differences between the control groups. In the CG-injected groups, the number of F4/80-positive cells was significantly increased in the CG-injected GATA-3 Tg mice compared with the other CG-injected groups.

Increased IFN-γ level in peritoneal fluid of CG-injected T-bet Tg mice and increased IL-13 level in CG-injected GATA-3 Tg mice

CG-injected GATA-3 Tg mice developed a marked severe PF compared with the other CG-injected groups. Th2 cytokines play important roles in fibrosis [39]. Because CG-injected GATA-3 Tg mice developed severe peritonitis and adhesion on day 21, we could not directly examine the peritoneal fluid. Next we examined the expression of cytokines in the peritoneal fluid on day 8 to analyze the development of PF. In this analysis, we examined the levels of IFN-γ, IL-4, IL-5, and IL-13 (Fig. 5 and Supplementary Fig. 4). There were no significant
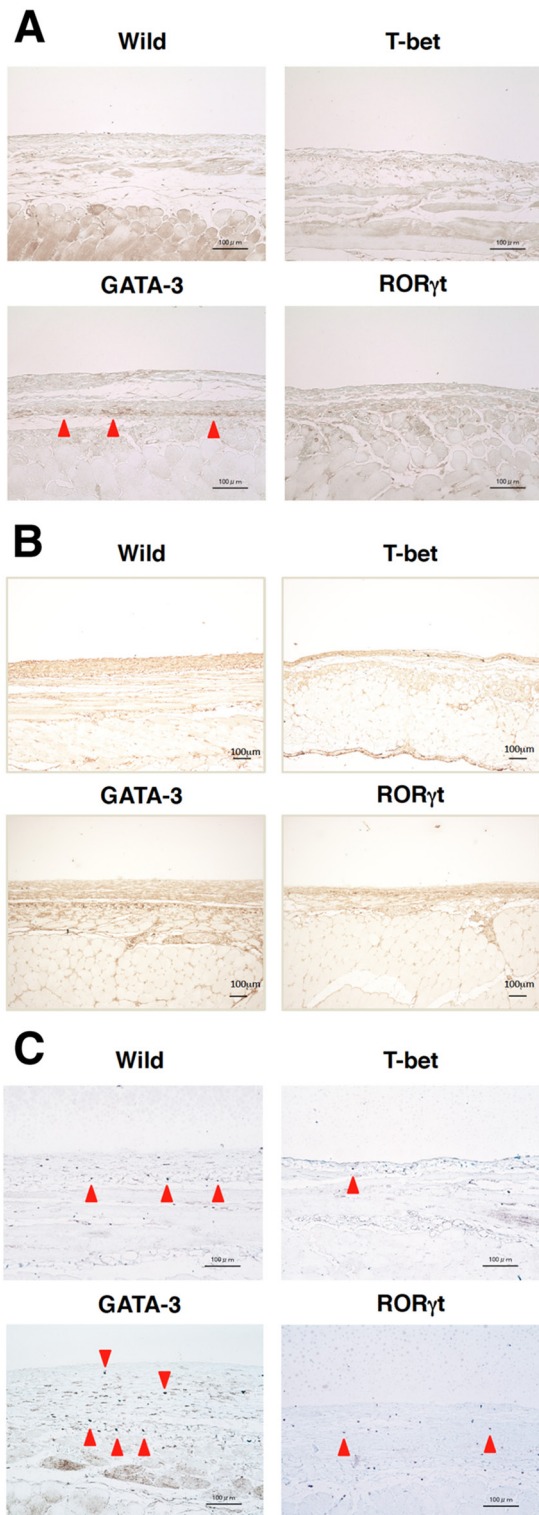
Fig. 4. Immunohistochemistry of α-SMA expression (arrow-heads) (A), collagen III expression (B), and F4/80-positive cells (arrowheads) (C) in peritoneal tissue on days 21 in CG-injected wild-type (W), T-bet Tg (T), GATA-3 Tg (G), and RORγt Tg (R) mice (original magnification ×200 (A and C), ×100 (B); scale bar 100 μm).
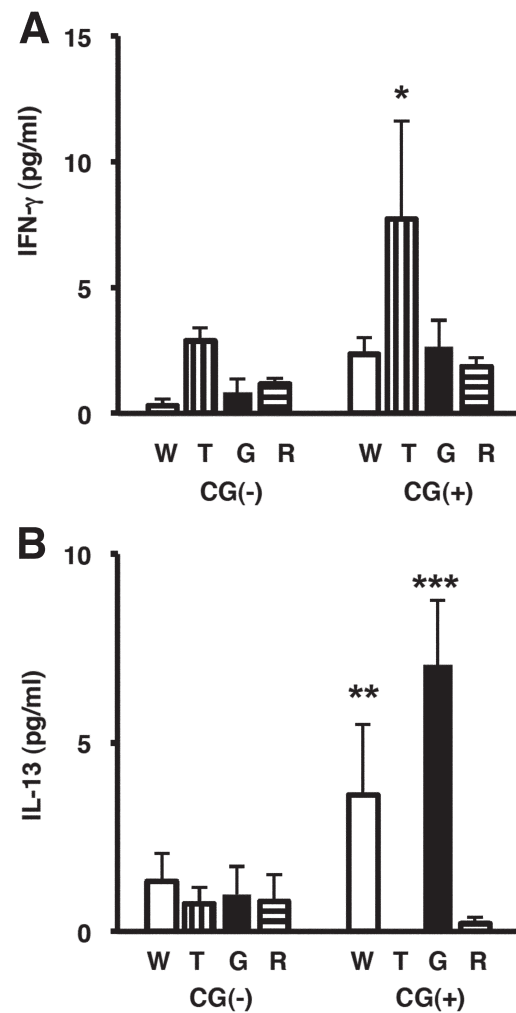

Fig. 5. Expression of cytokines, IFN-γ (A) and IL-13 (B), in peritoneal fluid of control and CG-injected mice. W, wild-type mice; T, T-bet Tg mice; G, GATA-3 Tg mice; R, RORγt Tg mice. Data represent means ± SEM. *$P<0.01$ vs. CG-injected wild-type mice, GATA-3 Tg mice, and RORγt Tg mice; **$P<0.05$ vs. CG-injected T-bet Tg mice and RORγt Tg mice; ***$P<0.01$ vs. CG-injected T-bet Tg mice and RORγt Tg mice; $P<0.05$ vs. CG-injected wild-type mice; $n=5$ in each group.

differences in the levels of IL-4, and IL-5 between wild-type mice and Tg mice (Supplementary Figs. 4A and B). IL-13 levels in CG-injected GATA-3 Tg mice were significantly higher than in CG-injected wild-type mice (Fig. 5B). The mean IL-13 levels in control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 1.3 ± 0.8 pg/ml, 0.7 ± 0.5 pg/ml, 1.0 ± 1.1 pg/ml, and 0.8 ± 0.7 pg/ml, respectively. The mean IL-13 levels in CG-injected wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 3.6 ± 1.9 pg/ml, 0.0 ± 0.0 pg/ml, 7.0 ± 1.7
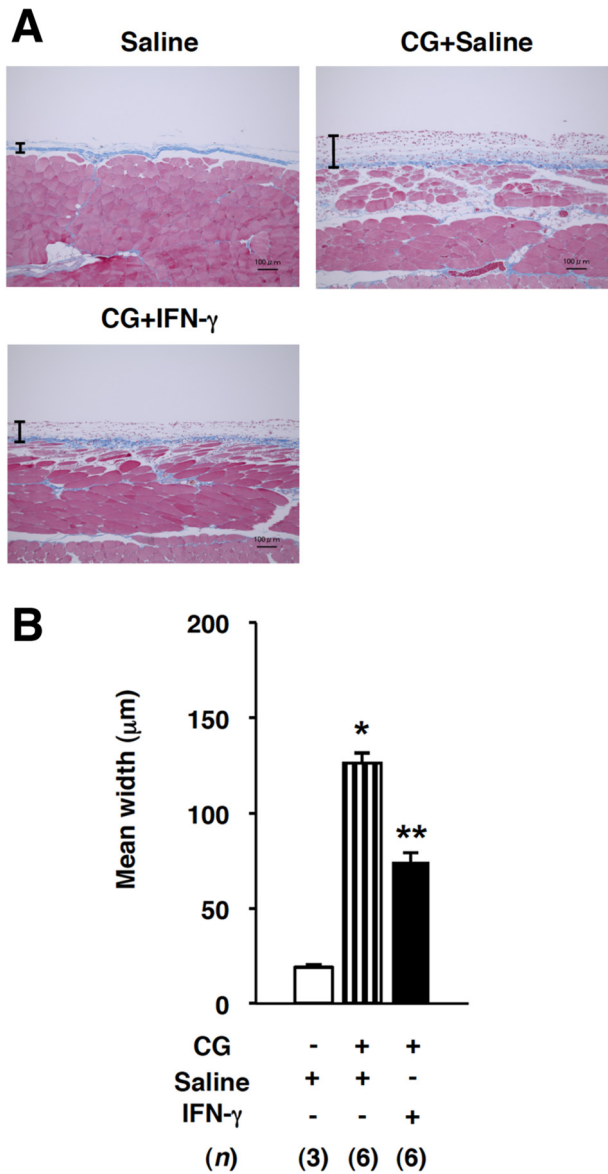
A

Saline   CG+Saline

CG+IFN-γ

B

Fig. 6. Intraperitoneal administration of IFN-γ in CG-injected wild-type mice. Representative light microscopic features of peritoneal tissue on days 21 in saline-injected, CG with saline-injected, and CG with IFN-γ-injected wild-type mice (A) (Masson-trichrome stain, original magnification ×100, scale bar 100 μm). Bars indicate thickness of the submesothelium. (B) Bar graph showing the thickness of the submesothelial zone. Data represent means ± SEM. *$P<0.01$ vs. saline-injected and CG with IFN-γ injected wilt-type mice; **$P<0.01$ vs. saline-injected wild-type mice.

pg/ml, and 0.2 ± 0.2 pg/ml, respectively. Furthermore, IFN-γ levels in CG-injected T-bet Tg mice were significantly higher than in the other CG-injected mice (Fig. 5A). The mean IFN-γ levels in control wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 0.3 ± 0.2 pg/

ml, 2.9 ± 0.5 pg/ml, 0.8 ± 0.5 pg/ml, and 1.2 ± 0.2 pg/ml, respectively. The mean IFN-γ levels in CG-injected wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were 2.4 ± 0.6 pg/ml, 7.7 ± 3.9 pg/ml, 2.6 ± 1.0 pg/ml, and 1.8 ± 0.4 pg/ml, respectively.

Intraperitoneal administration of IFN-γ improved PF

We next assessed the effects of exogenously administered IFN-γ on the development of PF using wild-type mice. Exogenous administration of IFN-γ significantly improved PF (Fig. 6). The mean peritoneal thicknesses of the control (saline only), CG-injected, and CG with IFN-γ administration wild-type mice were 18.9 ± 1.4 μm, 126.5 ± 5.7 μm, and 74.3 ± 4.9 μm, respectively. The thickness of the submesothelial compact zone in mice administered IFN-γ was significantly decreased compared with CG-injected only mice.

Discussion

It is well known that patients with end-stage renal disease present with disturbances of the immune system [5]. Immune system dysfunction in uremia is mainly the result of altered function of various types of immune cells, including polymorphonuclear leukocytes, monocytes, natural killer cells, and T lymphocytes [21, 22]. T cells play an important role in the acquired immune response and a target specific antigen by T cell receptor recognition. The peritoneum is a special environment within which T cells react [7]. Most peritoneal lymphocytes are composed of T cells divided into several subpopulations, including Th cells, cytotoxic T lymphocytes, γδ T cells, memory T cells, and regulatory T cells. Th cells are one of the most important cell populations in the peritoneum and are composed of three major subpopulations, defined as Th1, Th2, and Th17 [8, 25, 29]. Yokoyama *et al.* also reported that Th cells from PD patients manifest a dysregulated differentiation profile characterized by a marked increase in the percentage of Th2 cell with a normal percentage of Th1 cells [45]. In PD patients with peritonitis, an inflammatory process occurs, but the patterns of Th cells and the cytokine secretion in the inflammatory process have not yet been clarified. Previous reports described that peritonitis in PD patients is predominantly a type 1 immune response characterized by the early induction of TNF-α, IL-1β, IL-12, and IL-18, followed by elevated levels of IFN-γ [2, 38]. The presence of a Th1 phenotype in peritoneal

effluents seems to lower the risk of peritonitis [4, 20]. These findings suggested that peritoneal Th1 cells may improve cooperation among T cells, peritoneal macrophages, and peritoneal mesothelial cells. In our study; of CG-injected groups, Th1-biased mice showed the most reduced PF. Our results agree with those of previous reports. Th1 cells produce a high amount of IFN-γ, which can increase the killing activity of peritoneal macrophages and the antigen-presenting activity of peritoneal mesothelial cells. A previous study by Wang and Lin described this possibility in PD patients with peritonitis, showing that early high IL-12, IL-18, and IFN-γ levels correlated with rapid response to antibiotic treatment [7, 38].

EPS is characterized by progressive and excessive fibrotic thickening of the peritoneum. Tissue fibrosis in many disorders, such as idiopathic pulmonary fibrosis, hepatic fibrosis, and systemic sclerosis, is a leading cause of mortality [41]. Although the pathogenetic principles of fibrosis are still unknown, the early stages of fibrotic conditions are characterized by immunologic-inflammatory hallmarks [39]. Fibrotic tissue is always infiltrated by immune cells. Th cells and their secreted cytokines play a prominent role in the initiation and progression of fibrosis. Th1 cells are thought to mediate tissue damage and collagen degradation, whereas Th2 cells and their corresponding cytokines are linked with fibrogenesis. Thus, Th1 and Th2 cytokines play opposing roles in fibrosis: the Th2 cytokines IL-4 and IL-13 are strongly pro-fibrotic, whereas the Th1 cytokines IFN-γ and IL-12 suppress the development of tissue fibrosis [42]. In this study, we showed that IFN-γ administration reduced the deterioration of PF in GC-injected wild-type mice. Many reports have linked IL-13 with fibrosis diseases, but whether IL-13 is associated with EPS is unclear. Indeed, IL-13 was found to be directly fibrotic in a hepatic fibrosis model, and deletion of IL-13 was associated with reduced fibrosis and collagen deposition. A study using double IL-4 and IL-13 knockout mice demonstrated IL-13 was the predominant pro-fibrotic mediator in a natural model of hepatic fibrosis [6, 27]. Furthermore, targeted pulmonary overexpression of IL-13 using genetic approaches in mice resulted lung fibrosis [48]. In this study, we showed that Th2-biased mice developed severe PF and that high levels of IL-13 expression were detected in peritoneal fluid. We also administrated an anti-IL-13 antibody to GC-injected wild-type mice in our study (Supplementary Fig. 5).

However, we could not clearly show the effect of anti-IL-13 therapy. Further experiments would be required to examine the IL-13 antibody volume and the schedule of administration in the GC-injected model.

IL-17 produced by Th17 also has been implicated in some fibrotic disorders [19, 28, 33]. Wang *et al.* reported that manipulation of IL-17 cytokine expression in patients with peritonitis might modulate peritoneal immune responses and affect peritonitis outcomes [37]. In our study, Th17-biased mice, RORγt Tg mice, did not develop a severe form of PF. Transgenic mice overexpressing RORγt under the control of the CD2 promoter induced a Th17-biased background that might have affected Th2 cells or the expression of related factors, which might have contributed to the development of PF. Further studies to define the other Th2-related factors may clarify the mechanisms responsible for the development of PF.

In conclusion, we observed that Th2-biased mice developed severe experimental PF but that Th1-biased mice did not. Our results suggest that Th1 and Th2 cells, and their corresponding cytokines may play important roles in the development of experimental PF. In future studies, the regulation of Th cell subsets, such as Th2 background suppression or Th1 background acceleration, might be effective for EPS treatment.

Acknowledgments

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