

Full Paper

Renal Fibrosis in Murine Obstructive Nephropathy Is Attenuated by Depletion of Monocyte Lineage, Not Dendritic CellsYuichi Machida^{1,2}, Koichiro Kitamoto^{1,2}, Yasukatsu Izumi³, Masayuki Shiota³, Junji Uchida¹, Yukimi Kira⁴, Tatsuya Nakatani¹, and Katsuyuki Miura^{2,*}*Departments of ¹Urology, ²Applied Pharmacology and Therapeutics, ³Pharmacology, and ⁴Central Laboratory, Osaka City University Medical School, Asahimachi, Abeno-ku, Osaka 545-8585, Japan**Received September 22, 2010; Accepted October 25, 2010*

Abstract. The role of renal dendritic cells (DCs) in renal fibrosis is unknown. The present study was conducted to examine the relative role of renal DCs and macrophages in the development of renal fibrosis in murine obstructive nephropathy. CD11c-diphtheria toxin receptor (DTR) transgenic mice and CD11b-DTR transgenic mice were subjected to unilateral ureteral obstruction. To conditionally and selectively deplete DCs or macrophages, DT was given to these mice and kidneys were harvested on day 5. Ureteral obstruction elicited renal fibrosis characterized by tubulointerstitial collagen III deposition and accumulation of α -smooth muscle actin-positive cells. Flow cytometric analysis revealed a marked increase in cell counts of F4/80⁺ macrophages, F4/80⁺ DCs, as well as neutrophils and T cells in the obstructed kidney. DT administration to CD11c-DTR mice led to selective depletion of renal CD11c⁺ DCs, but did not affect renal fibrosis. In contrast, administration of DT to CD11b-DTR mice resulted in ablation of all monocyte lineages including macrophages and DCs and attenuated renal fibrosis. Our results do not support the role of renal DCs, but confirm the importance of monocyte lineage cells other than DCs in the development of the early phase of renal fibrosis following ureteral obstruction in mice.

Keywords: renal fibrosis, inflammation, monocyte, macrophage, dendritic cell

Introduction

Tubulointerstitial fibrosis is the final common pathway of chronic progressive renal disease and is characterized by early inflammatory cell infiltration (1). Since infiltration of the monocyte lineages often correlates with the degree of renal fibrosis, these cells have been considered contributors to the development of fibrosis (2).

Under inflammatory conditions, bone marrow-derived monocytes exit into the systemic circulation, enter tissues, give rise to subsets of macrophages and dendritic cells (DCs) within tissues, and constitute a peripheral mononuclear phagocyte system (3, 4). Recent advances in the study of both functional characteristics and surface markers of cells of the mononuclear phagocyte system have led to an increasing overlap between what is considered a 'macrophage' and a 'DC' (5). The overlap in-

cludes antigen presentation, T cell stimulation, phagocytosis, cytotoxicity, and cell surface expression such as F4/80, CD11b, CD68, and MHC class II, whereas CD11c is unique to DCs. Recent findings have extended further understanding of the origin, anatomy, and function of the renal DC network in a steady state and during periods of renal injury (6, 7). While accumulated evidence has suggested the importance of macrophages in the pathogenesis of renal fibrosis (8), the individual roles of macrophages and DCs in renal fibrosis is unclear. It was reported that DCs not only facilitated accumulation of interleukin-17 (IL-17)-secreting T cells but they also constituted a potent source of proinflammatory mediators including TNF, IL-6, and MCP-1 in the kidney following acute renal obstruction (9). Although the unilateral ureteral obstruction (UUO) model is valuable for understanding of the pathogenesis of inflammation and fibrosis (10), it remains to be clarified whether or not DCs actively contribute to renal fibrosis.

To elucidate the relative contribution of renal macrophages and DCs to the development of renal fibrosis,

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we used transgenic mice with restricted expression of simian or human diphtheria toxin receptor (DTR) under the control of either CD11c promoter (11) or CD11b promoter. By using these mice, CD11c-positive DCs or CD11b-positive macrophages (12, 13) could be selectively and conditionally ablated after DT administration. A number of investigators have utilized these animal models and successfully demonstrated the role of DCs or macrophages in various pathophysiological conditions (4, 11–19). In the present study, these transgenic mice were subjected to UUO and the effects of selective ablation of DCs or macrophages on renal fibrosis were examined.

Materials and Methods

Animals

CD11c-DTR mice [B6.FVB-Tg(Itagx-DTR/eGFP)57Lan/J] on the C57BL/6J background and CD11b-DTR mice [B6.FVB-Tg(ITGAM-DTR/eGFP)34Lan/J] on the C57BL/6J background were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed in a specific pathogen free facility. We produced homozygote mice in both transgenic mice and used them in the present study. The strategy of conditional and selective ablation of murine cells expressing primate DTR is based on the facts that murine DTR binds to DT poorly but murine cells can be rendered sensitive to DT through transgenic expression of primate DTR (20). Administration of DT to CD11c-DTR mice selectively depletes CD11c-positive DCs (11), while it selectively ablates CD11b⁺ macrophages without affecting CD11b⁺ neutrophils in the CD11b-DTR mice (12, 13). However, repeated DT injection is lethal (11, 15) or results in extrahematopoietic toxicity (13, 16). Thus, the time window of DT-induced cell ablation is limited to only one or two days. In order to ablate transgene lineages for longer periods without adverse effects, we used bone marrow chimeric mice through reconstitution of lethally irradiated (950 rad) recipient wild-type mice (male C57BL/6J mice, 6–8-week-old; Charles River, Yokohama) with CD11c-DTR or CD11b-DTR bone marrow cells ($3–5 \times 10^6$ bone marrow cells) (14, 15). These mice were used for experiments at least 8 weeks after bone marrow transplantation. All animal experiments adhered to approved institutional guidelines.

UUO model

UUO was performed aseptically as previously described (21). At 5 days after UUO, kidneys were perfused with ice-cold heparinized saline via heart puncture under anesthesia. Kidneys were excised and renal slices were fixed with methyl-Carnoy's solution or 4% paraformal-

dehyde and embedded in paraffin for immunohistochemical examination. The remaining tissue was used for flow cytometric analysis while a small portion of the renal tissue that contained both cortex and medulla was immediately frozen in liquid nitrogen and stored at -80°C for further analysis.

Selective target cell ablation by administration of DT

Diphtheria toxin (Sigma-Aldrich, St. Louis, MO, USA) was diluted with phosphate-buffered saline (PBS). Either DT (10 ng per g of body weight) or PBS was given intraperitoneally to the bone marrow chimeric mice the day before and 1 and 3 days after UUO.

Immunohistochemistry

Sections (4- μm -thick) of the paraffin-embedded renal tissue were used for immunohistochemistry of α -smooth muscle actin (α -SMA), collagen III, and F4/80-positive mononuclear phagocytes as previously described (21). In CD11c-DTR transgenic mice, the GFP protein is fused to the DTR, resulting in relatively poor fluorescence intensity. Using conventional fluorescence microscopy, CD11c-DTR transgenic DCs can not be reliably detected in histology without using an anti-GFP amplification (22). To stain DCs in CD11c-DTR bone marrow chimeric mice, goat anti-GFP antibody (ab6658; Abcam, Tokyo) was used after antigen exposure with 0.002% proteinase K for paraformaldehyde-fixed sections. Each microphotograph was digitized and the percentages of immunostained area of F4/80, collagen III, α -SMA, and GFP in the renal cortex were analyzed by using ImageJ 1.43 at a magnification of $\times 200$.

Real-time reverse transcriptase-polymerase chain reaction (PCR)

Renal gene expression of TGF- $\beta 1$, collagen type III, and TNF- α was determined by SYBR-green-based real-time reverse transcriptase PCR using a standard curve as previously reported (21). The level of molecules investigated was corrected for glyceraldehydes 3-phosphate dehydrogenase (GAPDH).

Preparation of kidney cell suspension for flow cytometry

Kidney cell suspension was prepared as previously described (21). Kidneys were dissected, placed in Hank's Balanced Salt Solution (HBSS) containing 1.6 mg/ml collagenase IA (Sigma Aldrich, Tokyo) and 200 $\mu\text{g}/\text{ml}$ DNase I (Roche, Indianapolis, IN, USA) for 30 min at 37°C with intermittent agitation and then washed twice in HBSS. Following erythrocyte lysis, cells were resuspended in FACS buffer (PBS / 5% fetal calf serum / 0.05% NaN_2). Kidney cell suspensions to be used for

flow cytometry were allowed to settle for 20 min after which the upper five-sixths were collected for use in flow cytometry.

Flow cytometric analysis

Aliquots of cells were pre-incubated with anti-CD16/CD32 Fc receptor (2.4G2) for 10 min to minimize non-specific antibody binding. Cells were then incubated in FACS buffer at 4°C with various combinations of fluorochrome-labeled and/or biotinylated antibody followed by fluorochrome-labeled streptavidin as previously described (21). The following antibodies were used: anti-CD45-PE-Cy7 (Ly-5), anti-CD11b-PE (Mac-1), anti-F4/80-APC (BM8), anti-CD3e-PE(145-2C11), anti-CD8a-APC (53-6.7), and anti-MHC class II (I-A/I-E)-PE (M5/114.15.2) (eBioscience, San Diego, CA, USA); anti-CD4-APC (GK1.5), anti-CD11c-APC and -FITC (HL3), and anti-Ly-6G-PE (1A8) (BD Biosciences, San Jose, CA, USA). Multi-color flow cytometric analysis for surface marker expression was performed using LSR II and FACSDiva software (BD Biosciences). Determination of CD45⁺ cell absolute counts and individual leukocyte subset number were determined using fluorescent counting beads (TruCOUNT Tubes, BD Biosciences) (21).

Statistical analysis

All data are presented as the mean \pm S.E.M. Data were analyzed using analysis of variance and individual comparisons were made using Tukey's post hoc analysis. As some data showed heteroscedasticity, logarithmic transformation was made before analysis. Statistical significance was defined as $P < 0.05$.

Results

CD11c⁺ DCs increased remarkably in the obstructed kidney and were selectively depleted by DT

Figure 1A is an example of flow cytometry dot plots from the obstructed kidney of CD11c-DTR mouse. As anticipated, CD11c (DC marker)-positive cells were also positive for GFP fluorescence (upper middle). DT effectively eliminated CD11c⁺ GFP-positive cells (upper right), henceforth designated DCs. As shown in the lower panel of Fig. 1A, F4/80⁺ cells could clearly be subdivided into F4/80⁺ macrophages and F4/80⁺ DCs by GFP expression and DT selectively depleted DCs. Cell counts of individual leukocyte populations are summarized in Fig. 1B. In the non-obstructed kidney, FACS analysis revealed that approximately 70% of F4/80⁺ cells were DCs and the remaining 30% were F4/80⁺ macrophages. DT selectively and almost completely ablated renal DCs without affecting F4/80⁺ macrophages in the non-

obstructed kidney (Fig. 1: A and B). Renal F4/80⁺ cells were increased by ureteral obstruction approximately 5-fold (Fig. 1B). In the obstructed kidney, more than 50% of F4/80⁺ cells were DCs and the remaining F4/80⁺ cells were macrophages. Although CD4⁺ T cells, CD8⁺ T cells, and neutrophils also increased in obstructed kidneys, their proportions were relatively small. DT selectively eliminated DCs but had no effect on the numbers of F4/80⁺ macrophages, T cells, or neutrophils (Fig. 1B). Cell number of CD11b that is highly expressed by monocytes, macrophages, DCs, and granulocytes was comparable to that of F4/80⁺ cells irrespective of the presence or absence of ureteral obstruction or DT treatment. MHC class II expression on both F4/80⁺ macrophages and F4/80⁺ DCs were remarkably higher than that on F4/80⁻ / CD11c⁻ cells (including T cells and neutrophils) (Fig. 1C). Furthermore, MHC class II expression on F4/80⁺ DCs was significantly higher than that on F4/80⁺ macrophages, which is consistent with the understanding that DCs have higher potency as antigen presentation cells than macrophages. Ureteral obstruction for 5 days did not affect the cell surface expression of MHC class II on either DCs or macrophages. Immunohistochemical examination revealed that GFP⁺ DCs were located in the tubulointerstitium of the renal cortex but not within the glomeruli in a non-obstructed kidney. There was a marked accumulation of GFP⁺ DCs in the tubulointerstitium of the obstructed kidney that were effectively ablated by DT (Fig. 2A). Similarly, F4/80⁺ cells were accumulated in the tubulointerstitium of obstructed kidneys and were partially diminished by DT (Fig. 2B). F4/80⁺ cells remaining following exposure to DT in the obstructed kidney section are most likely macrophages since FACS analysis revealed that DT had no effects on F4/80⁺ macrophages (Fig. 1B).

Selective ablation of DCs did not affect renal fibrosis of obstructed kidneys

Figure 3, A and B, shows the immunohistochemistry of collagen III and α -SMA (a marker of myofibroblasts and vascular smooth muscle) and the effects of DT. Although the collagen III-positive area was scanty and α -SMA was localized to vascular wall in the non-obstructed kidney, marked accumulation of collagen III and α -SMA were observed in the tubulointerstitial space of the obstructed kidney. Administration of DT did not affect the positively stained area of either collagen III or α -SMA. Furthermore, although ureteral obstruction stimulated mRNA expression of collagen III, TGF- β , and TNF- α , depletion of DCs in CD11c-DTR mice did not affect these up-regulated gene expressions (Fig. 3C).

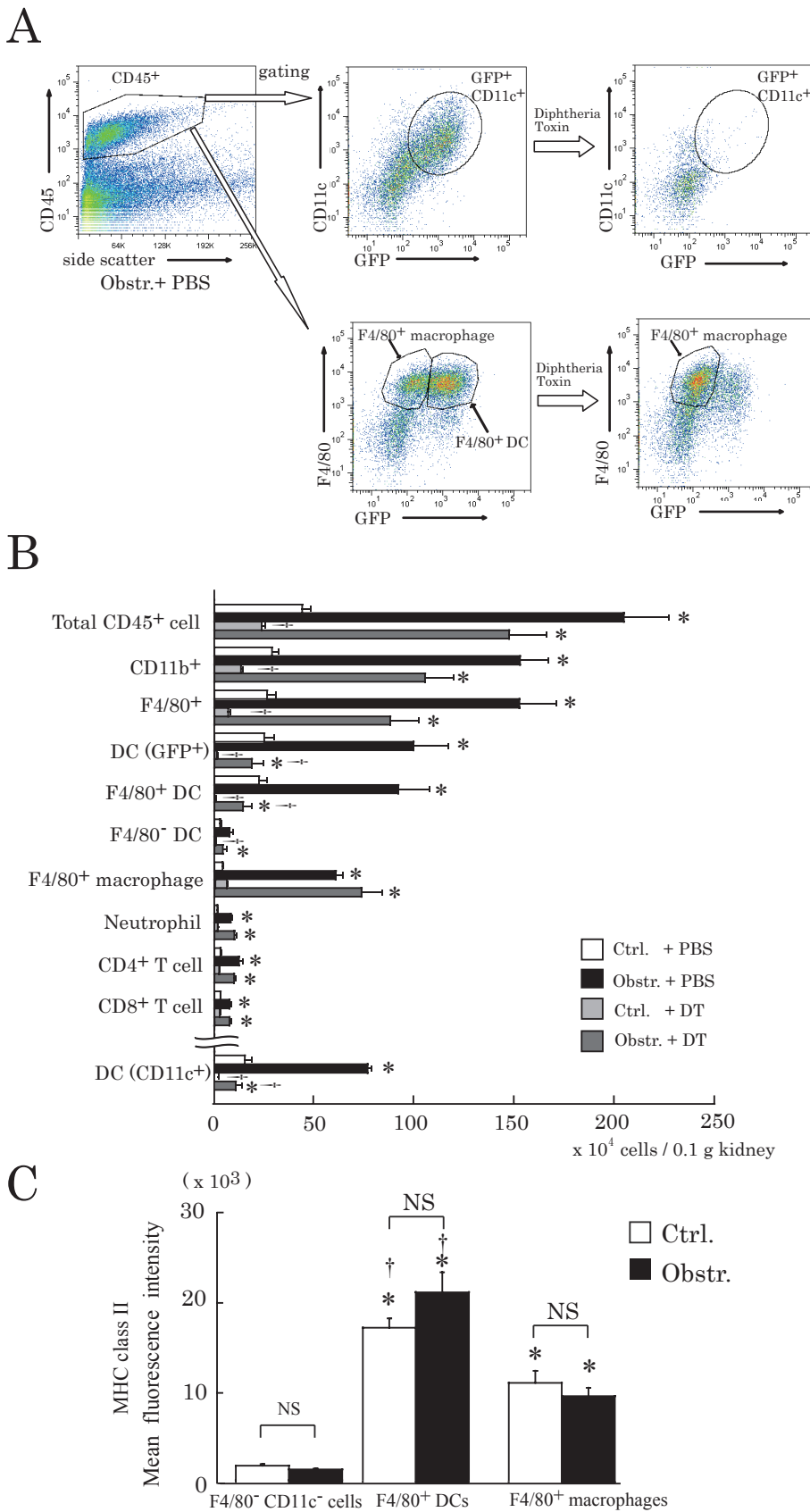


Fig. 1. Flow cytometric characterization of renal infiltrating leukocyte populations of CD11c-diphtheria toxin receptor (DTR) bone marrow chimeric mice at 5 days after unilateral ureteral obstruction (UUO). **A)** Example of multicolor flow cytometry dot plots and gating used to analyze proportions of individual CD45⁺ cell populations within total cell suspensions from an obstructed kidney (left). CD11c⁺ dendritic cells (DCs) were GFP⁺ (upper middle) and were effectively depleted by DT (upper right). FACS plots identifying two populations of F4/80⁺ monocyte lineages that consist of F4/80⁺ / GFP⁺ macrophages and F4/80⁺ / GFP⁺ DCs (lower middle). DT selectively depleted F4/80⁺ DCs, but had no effect on F4/80⁺ macrophages (lower right). **B)** Effects of DT on the cell counts of renal infiltrating leukocyte populations following UUO. Cell suspensions from kidneys were stained and then gated for the CD45⁺ leukocyte population. Staining definitions were F4/80⁺ macrophage: CD45⁺ / F4/80⁺ / GFP⁻ (CD11c⁻), DC (GFP): dendritic cells determined as CD45⁺ / GFP⁺, F4/80⁺ dendritic cell: CD45⁺ / F4/80⁺ / GFP⁺, F4/80⁻ dendritic cell: CD45⁺ / F4/80⁻ / GFP⁺, neutrophil: CD45⁺ / Ly-6G⁺, CD4⁺ T cell: CD45⁺ / CD3⁺ / CD4⁺, CD8⁺ T cell: CD45⁺ / CD3⁺ / CD8⁺. In a separate set of experiments, DCs stained with anti-CD11c-APC (n = 3 in each group) were shown as DC (CD11c⁺) and data were included at the bottom of the figure. It is of note that the pattern of obstruction-induced increase and DT-induced ablation of DCs were quite similar irrespective of DC marker (GFP or anti-CD11c-APC). Ctrl. + PBS: PBS-treated non-obstructed kidney, Obstr. + PBS: PBS-treated obstructed kidney, Ctrl. + DT: DT-treated non-obstructed kidney, Obstr. + DT: DT-treated obstructed kidney. **P* < 0.05, compared to non-obstructed kidneys; †*P* < 0.05, compared to those receiving PBS (n = 4 for each group). **C)** Cell surface expression of MHC class II on F4/80⁺ DCs and F4/80⁺ macrophages in obstructed and non-obstructed kidneys at 5 days following UUO (n = 4 in each group). **P* < 0.05, compared to F4/80⁺ CD11c⁻ cell; †*P* < 0.05, compared to F4/80⁺ macrophage. NS, no significant difference.

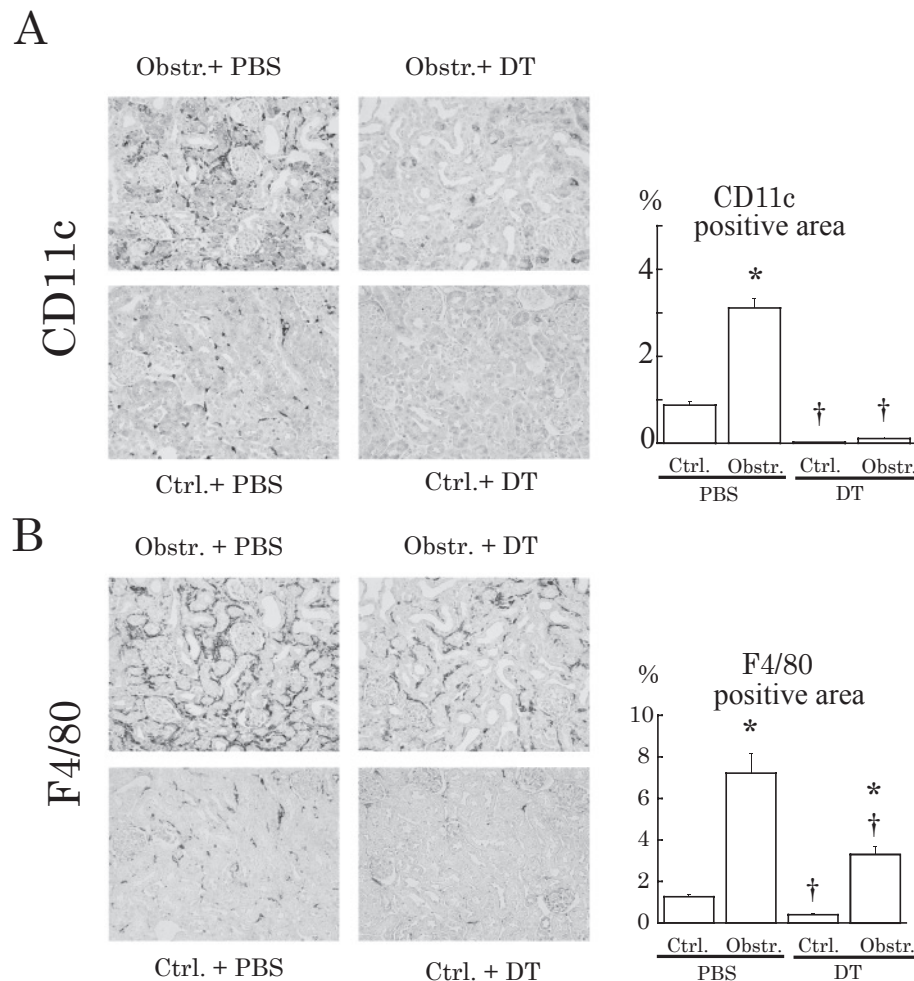


Fig. 2. Immunohistochemistry of GFP-positive DCs and F4/80-positive cells in the renal cortex following ureteral obstruction of CD11c-DTR bone marrow chimeric mice. Serial sections of the same specimen from CD11c-DTR bone marrow chimeric mice were used to stain GFP-positive DCs and F4/80-positive cells. A) DCs of CD11c-DTR bone marrow chimeric mice were immunostained by anti-GFP antibody (original magnification $\times 400$). GFP⁺ DCs were located in the tubulointerstitial space of the non-obstructed kidney. In the obstructed kidney, there was marked accumulation of DCs in the tubulointerstitium but not within the glomeruli. Following DT administration, almost all of the DCs disappeared from the non-obstructed or the obstructed kidney. Quantitative evaluation of GFP⁺ DCs was summarized in the right panel ($n = 3$ in each group). B) Representative photographs of immunohistochemistry of F4/80-positive cells (original magnification $\times 200$). Localization patterns of F4/80⁺ cells are similar to those of GFP⁺ DCs. These cells were localized in the tubulointerstitial space in the non-obstructed kidney and were markedly increased following UUO. DT partially attenuated this increase. Quantitative evaluation of the F4/80-positive area in the renal cortex was shown in the right panel ($n = 7$ in each group). * $P < 0.05$, compared to non-obstructed kidneys; † $P < 0.05$, compared to those receiving PBS. Abbreviations are same as those in Fig. 1B.

Administration of DT in CD11b-DTR mice depleted both DCs and macrophages and attenuated renal fibrosis in obstructed kidney

Figure 4A shows the effects of DT administration on the cell counts of individual leukocyte populations following UUO in the kidneys of CD11b-DTR bone marrow chimeric mice. As was seen in CD11c-DTR mice (Fig. 1B), similar increases in each leukocyte population were observed in the obstructed kidneys. Administration of DT nearly ablated all F4/80⁺ macrophages, F4/80⁺ DCs,

and F4/80⁻ DCs in either non-obstructed or obstructed kidneys, whereas DT did not significantly affect neutrophils or CD8⁺ T cells in the obstructed kidney. DT significantly eliminated CD4⁺ T cells in the obstructed kidney. Immunohistochemistry revealed that DT almost completely depleted F4/80⁺ cells in both obstructed and non-obstructed kidney (Fig. 4: B and C), which is in marked contrast to the partial elimination of F4/80⁺ cells by DT in CD11c-DTR mice (Fig. 2B). Such differences in susceptibility to DT could be clearly explained by the

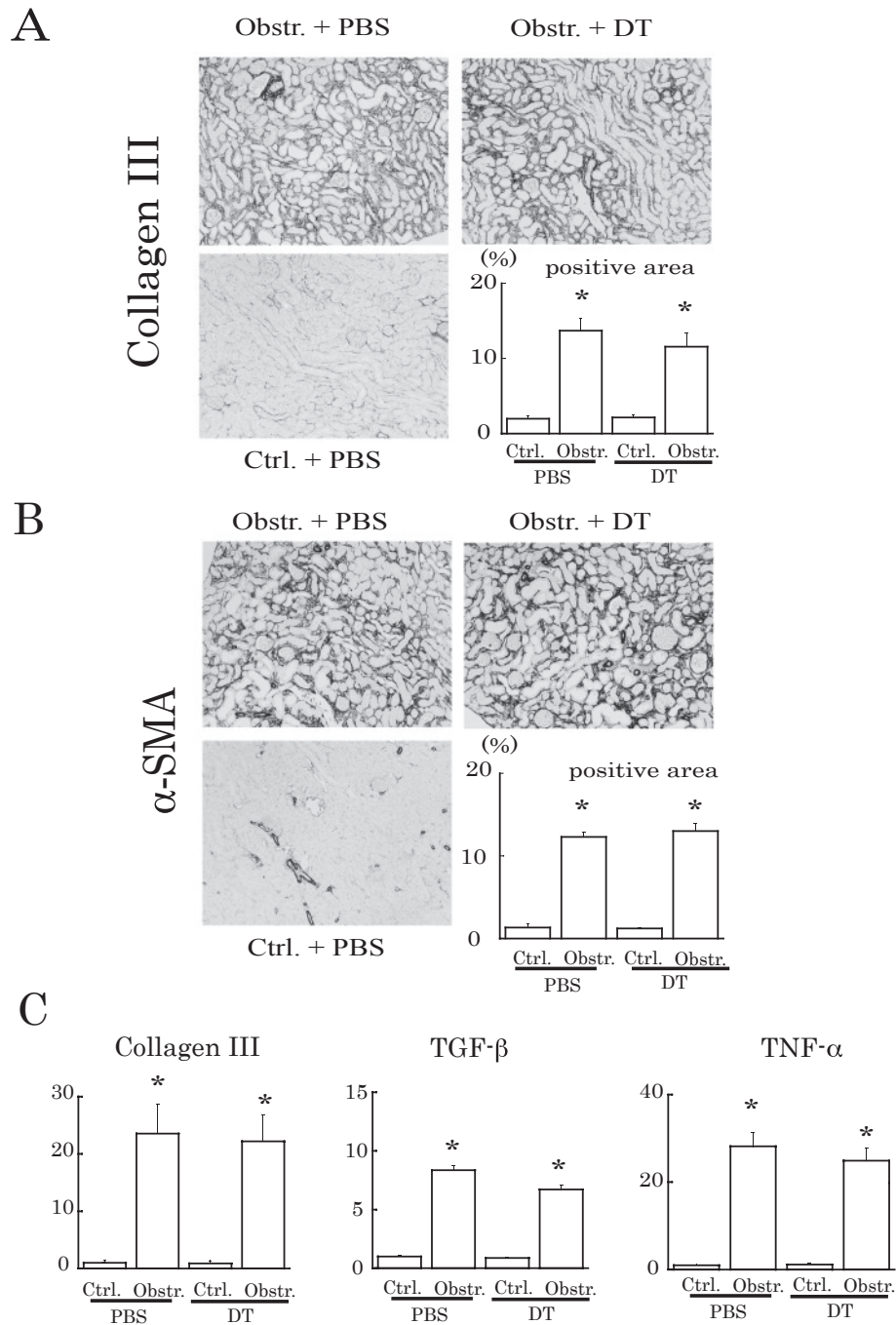


Fig. 3. Immunohistochemistry of collagen III and α -smooth muscle actin (α -SMA) and mRNA expressions of collagen III, TGF- β , and TNF- α following ureteral obstruction of CD11c-DTR mice. Representative photographs of immunohistochemistry of collagen III (A) and α -SMA (B) (original magnification $\times 200$). Percentages of positive area in the renal cortex were summarized (lower right, $n = 7$ for each group). * $P < 0.05$, compared to non-obstructed kidneys. Although ureteral obstruction markedly increased the collagen III- and α -SMA-positive area, DT did not affect these increases. C) mRNA expressions of collagen III, TGF- β , and TNF- α in the kidney tissue. Average mRNA expression in non-obstructed kidney (Ctrl. + PBS) was assigned to a unit ($n = 7$ for each group). * $P < 0.05$, compared to non-obstructed kidneys. Abbreviations are the same as those in Fig. 1B.

facts that DT completely ablated all monocyte lineage including macrophages and DCs in CD11b-DTR mice, whereas DT selectively depleted DTs without affecting

other monocyte lineages in CD11c-DTR mice. DT significantly attenuated renal interstitial accumulation of collagen III and α -SMA in obstructed kidneys (Fig. 5: A

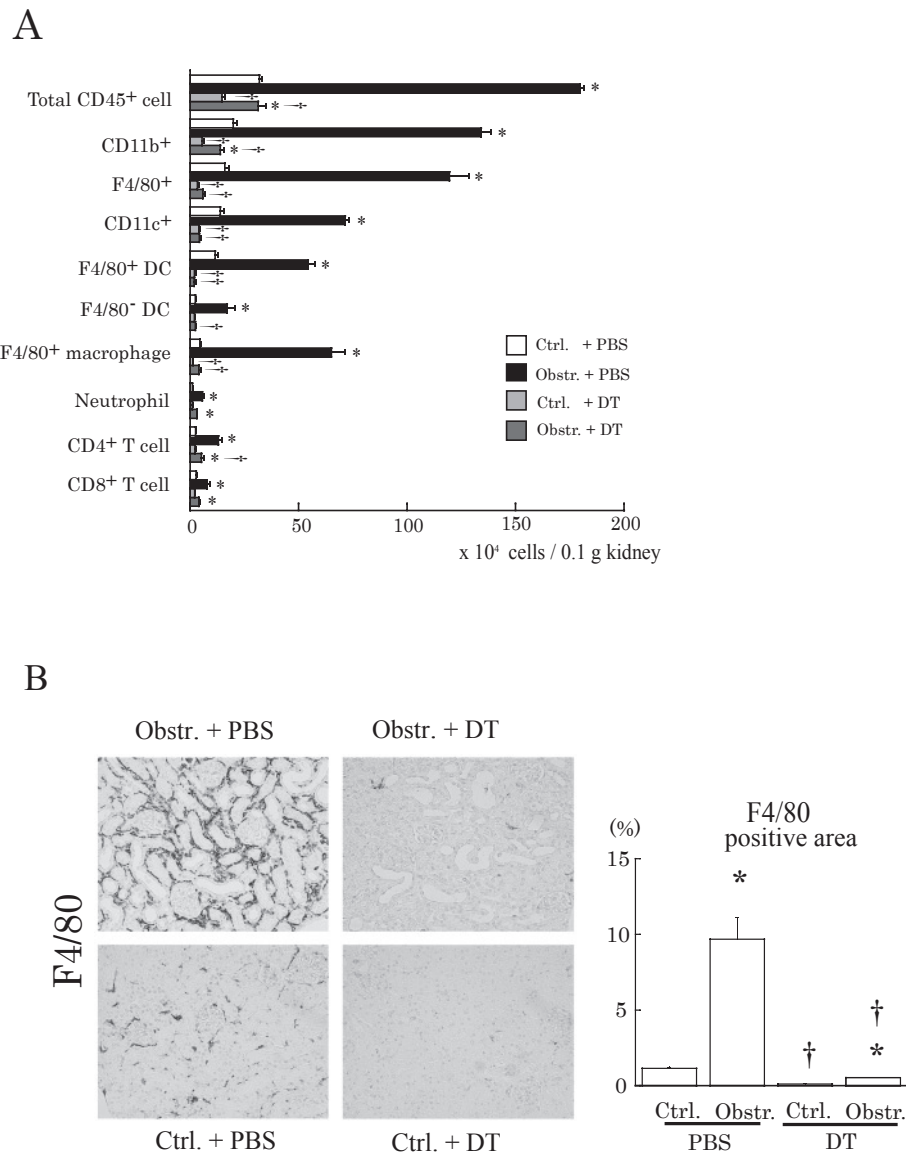


Fig. 4. Effects of DT on flow cytometric analysis of renal infiltrating leukocyte populations and immunohistochemistry of F4/80-positive cells of CD11b-DTR bone marrow chimeric mice at 5 days after UUO. A) Cell suspensions from kidneys of CD11b-DTR mice were stained and then gated for the CD45⁺ leukocyte population. Staining definitions were F4/80⁺ macrophage: CD45⁺ / F4/80⁺ / CD11c⁻, F4/80⁺ dendritic cell: CD45⁺ / F4/80⁺ / CD11c⁺, F4/80⁻ dendritic cell: CD45⁺ / F4/80⁻ / CD11c⁺, neutrophil: CD45⁺ / Ly-6G⁺, CD4⁺ T cell: CD45⁺/CD3⁺/CD4⁺, CD8⁺ T cell: CD45⁺/CD3⁺/CD8⁺. F4/80⁺ macrophages, F4/80⁺ DCs, and F4/80⁻ DCs that accumulated in the obstructed kidney were almost completely depleted by DT (n = 4). **P* < 0.05, compared to non-obstructed kidneys; †*P* < 0.05, compared to those receiving PBS. B) Representative photographs of immunohistochemistry (left) and quantitative evaluation (right) of F4/80-positive cells (n = 6 in each group). There was a marked accumulation of F4/80⁺ cells in obstructed kidney that were almost entirely removed by DT treatment.

and B) concomitantly with the inhibition of mRNA expression of collagen III and TGF- β (Fig. 5C).

Discussion

This study provides two main findings for pathogenesis of obstruction-induced inflammation: I) Conditional and selective ablation of CD11c⁺ DCs in the CD11c-DTR

mice did not affect the early phase of renal fibrosis following UUO. II) Depletion of monocyte lineage including macrophages and DCs in the CD11b-DTR mice resulted in attenuation of renal fibrosis in this model. Although DCs accumulate in the tubulointerstitium of the obstructed kidney, our data do not support the role of DCs in the development of at least the early phase of renal fibrosis. Our findings also provide evidence that

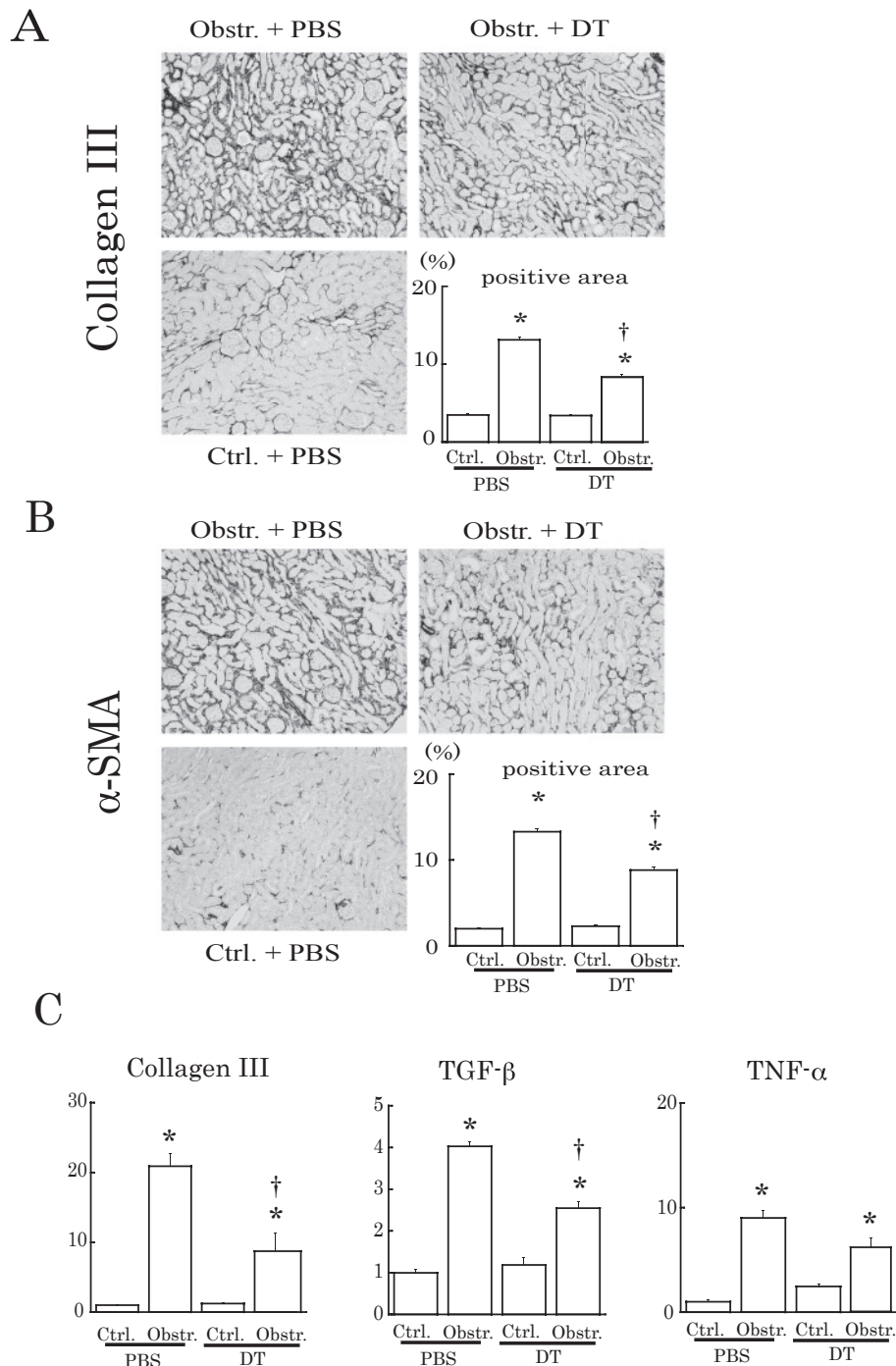


Fig. 5. Immunohistochemistry of collagen III and α -SMA and mRNA expression of collagen III, TGF- β , and TNF- α following ureteral obstruction of CD11b-DTR bone marrow chimeric mice. Representative photographs of immunohistochemistry of collagen III (A) and α -SMA (B) in CD11b-DTR mice (original magnification $\times 200$). Percentages of positive area were summarized (lower right, $n = 7$ for each group). Ureteral obstruction markedly increased the collagen III- and α -SMA-positive area. DT significantly decreased accumulation of both collagen III and α -SMA in CD11b-DTR mice. C) Summary of mRNA expressions of collagen III, TGF- β , and TNF- α in the kidney of CD11b-DTR mice ($n = 7$ for each group). Average mRNA expression in non-obstructed kidney (Ctrl. + PBS) was assigned to a unity. There was a marked up-regulation of collagen III, TGF- β , and TNF- α in obstructed kidneys. DT significantly attenuated the enhanced mRNA expressions of collagen III and TGF- β . * $P < 0.05$, compared to non-obstructed kidneys; † $P < 0.05$, compared to those receiving PBS. Abbreviations are the same as in Fig. 1B.

monocyte lineages other than DCs are involved in the development of renal fibrosis.

In the present study, selective depletion of DCs did not affect the enhanced mRNA expression of TNF- α and renal fibrosis in UUO kidneys. This result is in marked contrast with the previous report by Dong et al. (9) that depletion of DCs by liposome clodronate showed these cells to be the most potent source of TNF- α in the obstructed kidney and play a specific role in recruitment of IL-17-secreting CD4 T cells. Most of the TNF-positive cells were within the leukocyte fraction and magnetic sorting of the CD11c-enriched fraction of the kidney cell suspension contained more TNF- α than the CD11c-depleted leukocyte fraction of the obstructed kidney. TNF- α plays a pivotal role in the development of renal fibrosis following UUO (23–25). Differences between their study and our results may come from the difference in the timing of TNF- α determination. We examined mRNA expression of TNF- α at 5 days, whereas they examined it at 24 and 72 h after ureteral obstruction. TNF- α overexpression at earlier time may have decreased towards the control level. Alternatively, the discrepancy may arise from the specificity of the methods for depletion of DCs. In fact, liposome clodronate eliminates both DCs and macrophages from the obstructed kidney concomitantly with the reduction in renal mRNA expression of TNF- α (21). In contrast, ablation of DCs by DT from CD11c-DTR mice is indeed specific. DT selectively ablated the increased DCs observed in the obstructed kidneys, whereas DT had no effects on the accumulated macrophages. Taken together, although the potential role of renal DCs in the obstructed kidney remains unknown, recruited DCs are not a potent source of TNF- α and do not play a prominent role at least in the early phase of renal fibrosis following ureteral obstruction in mice.

In contrast to the selective susceptibility of DCs to DT in CD11c-DTR mice, DT administration to CD11b-DTR mice led to nearly complete ablation of all CD11b⁺ leukocytes (except neutrophils). Although CD11b-DTR mice have been used to conditionally deplete macrophages (19, 26, 27), our results clearly showed that DT ablated all monocyte lineages and depleted both macrophages and DCs from the kidney of CD11b-DTR mice. In contrast, DT administration to CD11b-DTR mice depleted F4/80⁺ macrophages but did not affect DCs in the spleen and lymph nodes (28). Qi et al. noted similar findings using a murine kidney transplantation model that DT treatment in CD11b-DTR mice significantly reduced circulating monocytes, eliminated renal F4/80⁺ cells, and CD11c⁺ cells, but had no effects on CD11c⁺ DCs in the spleen (29). Thus, susceptibility of CD11c DCs to DT is different among the various organs in CD11b-DTR mice. Although our data obtained by using CD11b-DTR mice

supports a causal relationship between monocyte lineages and renal fibrosis, studies using CD11b-DTR mice could not distinguish the individual roles of renal macrophages and DCs separately. Nevertheless, when the data combined with those obtained from CD11c-DTR mice, the present study strongly suggests that monocytes/macrophages but not DCs play pivotal roles in the development of the early phase of renal fibrosis, in part via TGF- β -dependent mechanisms. Although monocyte-derived fibrocytes may also be involved since collagen-I-producing hematopoietic cells called fibrocytes develop from CD11b⁺ monocytes (30, 31), it is unlikely since fibrocytes made no significant contribution to interstitial fibrosis in the mouse UUO model (32).

Our present study revealed that DT not only depleted monocyte lineages but significantly reduced CD4⁺ T cells in the obstructed kidney of CD11b-DTR mice. However, it should be pointed out that the numbers of neutrophils and CD8⁺ T cells also tended to decline, although not significantly. Such decrease in their cell numbers is possibly due to the attenuation of renal fibrosis since T cells do not bear CD11b and neutrophils are not susceptible to DT in the transgenic mice (13).

In conclusion, our results do not support the role of renal DCs but suggest the importance of cells of monocyte lineage other than DCs in the development of the early phase of renal fibrosis following UUO in mice. Therapeutic strategies targeting these monocyte lineages may have potential to prevent renal fibrosis.

Acknowledgments

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