学位論文の関連論文の研究背景及び要旨

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［題名］

Anti-TNF-α Agent Infliximab and Splenectomy Are Protective Against Renal Ischemia-Reperfusion Injury

（抗 TNF-α 製剤であるインフリキシマブと脾臓摘出は腎虚血再灌流障害から腎臓を保護する）

［研究背景及び要旨］

【研究の背景・目的】腎臓の虚血再灌流障害は、急性尿細管壊死を伴う急性腎障害を引き起こす。腎臓移植において、虚血時間の延長は移植腎機能の低下や移植腎の生着率の低下の原因となる。そのため虚血再灌流障害を防ぐことは非常に重要であるが、虚血再灌流障害を減弱させるための確立された治療法は存在しない。腎臓の虚血再灌流障害における急性尿細管壊死は、髄質外層の低酸素状態および同部位への炎症性物質や血管作動性物質の誘導により引き起こされる。それらのうち、炎症性サイトカインである TNF-α、IL-1β、IL-6 は虚血再灌流障害の進行に重要な役割を持ち、中でも TNF-α は NF-κB などの活性化を介して尿細管細胞のアポトーシスを引き起こす。これらの炎症性サイトカインは、主に腎の髄質外層に動員された単球/マクロファージから生成される。

熱ショック蛋白質（Heat Shock Protein, HSP）は、他の蛋白質が正しいフォールディングをすることを促進する分子シャペロンとして作用し、虚血再灌流障害などの蛋白質毒性ストレスから細胞を保護する。実際、HSP70 の発現は腎虚血再灌流障害で上昇することが知られており、障害のマーカーとして考えられている。

腎臓移植では、ドナー特異抗原を有するレシピエントにおいて抗ドナー抗体が産生されることを抑制する目的で、脾臓摘出術が行われることがある。我々は以前、脾臓摘出術により血清の TNF-α を低下させることで、腎臓の虚血再灌流障害を軽減できることを報告した。しかし、脾臓摘出術を行った際の炎症反応と組織障害との関係については十分に理解されていない。今回、我々は腎組織中の炎症性サイトカインの発現および障害マーカーとしての HSP70 の発現、そして、腎組織中への単球/マクロファージの動員を調べることで、脾臓摘出による炎症反応と組織障害との関係について検討した。さらに、抗 TNF-α 製剤である infliximab（IFX）が脾臓摘出と同様の効果を示すか否かを検討した。

【方法】Wistar ラット（7 週齢、雄）を用いた。虚血時間と腎機能障害、炎症性サイトカインおよび HSP70 との関係を調べるため、左腎動静脈をクランプ（30 分、45 分、60 分）した後に開放し、直後に右腎を摘出した。24 時間後に血液検

体および左腎を採取した。脾臓摘出およびIFX投与モデルにおける虚血時間は45分とした。脾臓摘出は右腎を摘出した直後に施行し、IFXは手術開始1時間前に腹腔内に投与した（10mg/kg）。腎機能障害は血清クレアチニンおよび尿素窒素を測定することで評価し、炎症性サイトカイン（TNF-α、IL-1β、IL-6）およびHSP70の発現量はウエスタンブロット法で解析した。腎組織中への単球/マクロファージの動員の定量化のために、ED-1抗体を用いて免疫染色を行い、陽性細胞の数をカウントした。

【結果】虚血時間が長くなるにつれ、血清のクレアチニンおよび尿素窒素が著明に上昇し、各種炎症性サイトカインおよびHSP70の発現量も著明に上昇した。脾臓摘出を行うことにより、血清クレアチニンと尿素窒素の上昇は抑制され、腎組織中の炎症性サイトカインおよびHSP70の発現量の上昇も抑制された。経時的変化を検討したところ、再灌流直後に炎症性サイトカインの発現量が増加し、その後3時間を経過した頃より腎機能が増悪し、HSP70の発現量も増加した。IFXを投与した場合も脾臓摘出と同様に、炎症性サイトカインおよびHSP70の発現量の低下とともに、腎機能障害の軽減を認めた。ED-1抗体を用いて免疫染色を行ったところ、障害を加えた腎臓ではED1抗体陽性の単球/マクロファージの細胞数の増加を認めたが、脾臓摘出およびIFX投与群ではその増加は顕著に抑制された。

【考察、結論】今回の研究で、脾臓摘出とIFXの投与はともに虚血再還流後の腎臓における炎症性サイトカインの産生を抑制することが分かった。この効果は、ともに腎臓における単球/マクロファージの細胞数増加の抑制を伴っていた。脾臓は単球/マクロファージの貯蔵臓器であること、IFXはMCP-1などのケモカインの産生を抑制する効果が報告されていることから、それぞれの作用により単球/マクロファージの細胞数の増加が抑制されたものと考えられた。一連の結果より、虚血再灌流初期のサイトカインの発現を抑制するか単球/マクロファージの動員を抑制することで腎機能障害を軽減できることが示唆された。脾臓摘出は侵襲が大きいこと、IFXが関節リウマチやクローン病などを有する患者に安全に使用されていることから、腎移植における虚血再灌流障害を予防するためにはIFXの投与が有益であると考えられる。今後は腎移植モデルを用いた検討が必要である。

Anti-TNF-α Agent Infliximab and Splenectomy Are Protective Against Renal Ischemia-Reperfusion Injury

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Background. Renal ischemia-reperfusion (I/R) injury is associated with delayed graft function and results in poor long-term graft survival. We previously showed that splenectomy (SPLN) protects the kidney from I/R injury and reduces serum TNF-α levels. Herein, we further investigated the effects of SPLN on inflammatory responses and tissue injury in renal I/R by examining the expression of major inflammatory cytokines and heat shock protein 70 (HSP70). Because it was shown previously that the anti–TNF-α agent infliximab (IFX) attenuated renal I/R injury, we also investigated whether IFX administration mimics the effects of SPLN. **Methods.** The left renal pedicles of adult male Wistar rats were clamped for 45 minutes and then reperfused for 24 hours; right nephrectomy and SPLN were performed immediately. A separate cohort was administered IFX 1 hour before surgery in lieu of SPLN. **Results.** Serum creatinine and blood urea nitrogen levels were markedly elevated by I/R injury; these increases were significantly reversed by IFX. Furthermore, IFX inhibited the induction of inflammatory cytokines and HSP70 during renal I/R injury. Time-dependent profiles revealed that the expression of inflammatory cytokines was elevated immediately after I/R, whereas levels of HSP70, serum creatinine, and blood urea nitrogen began to rise 3 hours postreperfusion. Macrophages/monocytes were significantly increased in I/R-injured kidneys, but not in those administered IFX. The outcomes of SPLN mirrored those of IFX administration. **Conclusions.** Splenectomy and TNF-α inhibition both protect the kidney from I/R injury by reducing the accumulation of renal macrophages/monocytes and induction of major inflammatory cytokines.

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Renal ischemia-reperfusion (I/R) injury after surgery or trauma causes acute renal failure, which is accompanied by acute tubular necrosis.[1] In renal transplantation, prolonged ischemia is associated with delayed graft function, and has a negative impact on long-term graft survival.[2,3] Therefore, avoiding renal I/R injury is critical. No therapeutic strategies to ameliorate I/R injury have yet been established.

Acute tubular necrosis during renal I/R injury is caused by hypoxia of the outer medulla and the induction of inflammatory and vasoactive mediators, which impair endothelial and tubular cell function.[1] Among them, major inflammatory cytokines including TNF-α, IL-1β, and IL-6 play important roles in I/R injury progression.[4–7] TNF-α is one of the most important inflammatory cytokines that induce apoptotic cell death by activating NF-κB, c-Jun N-terminal kinase, and p38-mitogen-activated protein kinase pathways.[5] These cytokines are mainly produced by activated monocytes/macrophages, which infiltrate the outer stripe of the kidney medulla.[8,9] Heat shock proteins are molecular chaperones that facilitate protein folding, and protect cells from proteotoxic stress such as I/R injury. The expression of heat shock protein 70 (HSP70) is elevated in renal I/R injury and is considered a marker of such injury.[10]

Splenectomy (SPLN) is sometimes performed in conjunction with renal transplantation in donor specific antigen-positive patients to suppress the production of antidonor antibodies. We previously showed that SPLN ameliorates renal I/R injury by reducing serum TNF-α levels in rats[11] and also reduces mortality rates.[12] However, the relationship between inflammatory response and tissue injury in splenectomized animals is not well understood. In this study, we evaluated the effects of SPLN on renal I/R injury outcome by examining the expression of major

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Y.N. designed and performed most experiments and analyzed the data. M.F. and K. U. performed some experiments and supervised the project. N.I., A.N., and H.M. designed and supervised the project. K.N., M.M., K.F., E.T., R.T. supervised the project. Y.N. and A.N. wrote the article. All authors contributed and commented on the article.

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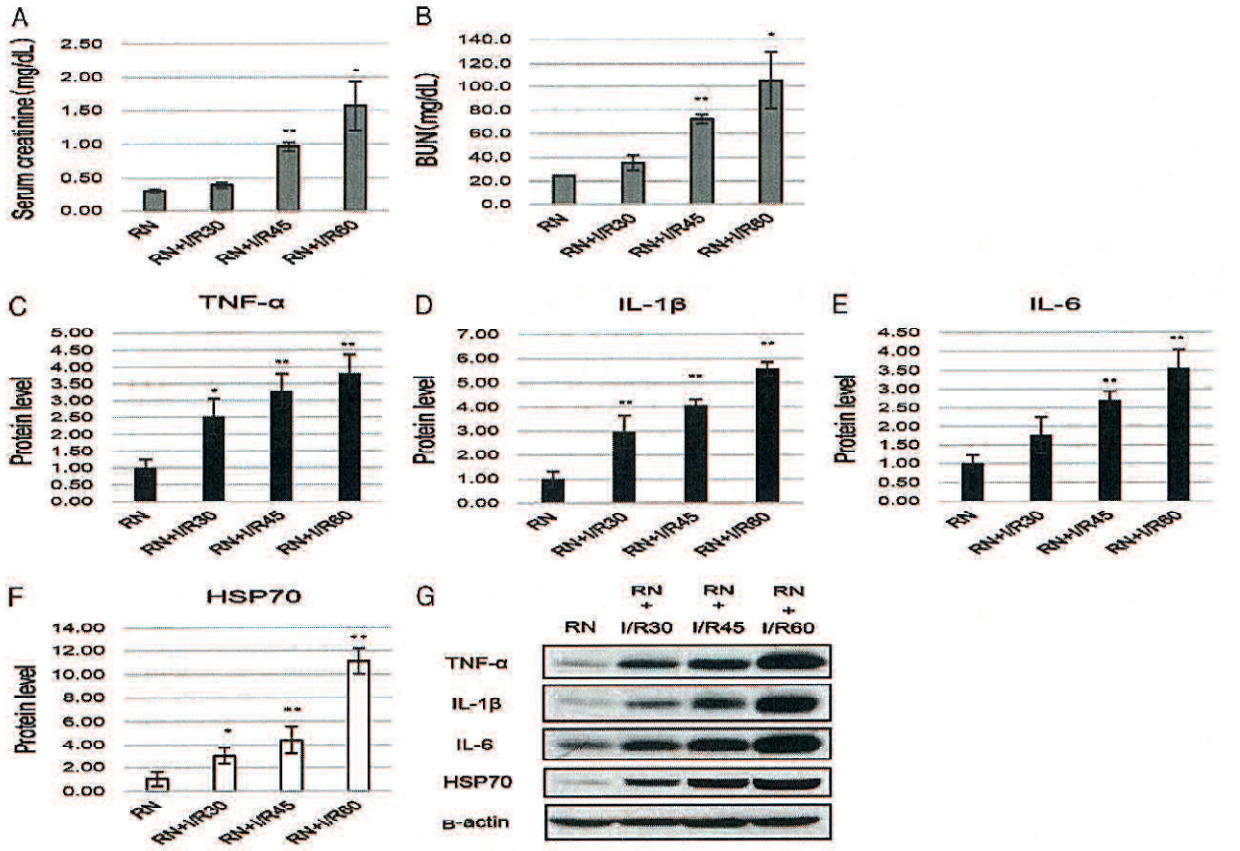

FIGURE 1. Expression of inflammatory cytokines and HSP70 in renal I/R injury. A and B, serum creatinine and BUN levels for each I/R injury condition. The left renal pedicle was clamped for 30 (I/R30), 45 (I/R45), or 60 (I/R60) minutes, and then unclamped for 24 hours. RN was performed immediately after unclamping (RN + I/R30, RN + I/R45, and RN + I/R60, respectively). Serum creatinine and BUN levels were measured. C-F, protein levels of inflammatory cytokines and HSP70 in the injured kidney. Kidneys were treated as described above. The untreated left kidney was used as a control after RN. Kidney extracts were subjected to Western blotting (representative blots are shown in G), and intensities of the bands were estimated using the ImageJ software (National Institutes of Health) (n = 3 for each group). $^{*}P < 0.05$, $^{**}P < 0.01$.

inflammatory cytokines and HSP70, as well as the infiltration of monocytes/macrophages into the kidney. Furthermore, we compared the effects of SPLN with those of an anti-TNF-α antibody, infliximab (IFX), after it was discovered that its administration reduced oxidative stress markers in a rat kidney I/R model.[13]

MATERIALS AND METHODS

Rats and the Renal I/R Injury Model

All experiments using rats were strictly performed according to standards for care and keeping. Adult male Wistar rats (Kyudo, Saga, Japan) weighing 258 to 269 g were individually housed in cages with standard chow and water under a 12-hour light-dark cycle. Rats were anesthetized by intraperitoneal injection with pentobarbital (50 mg/kg). After the skin was shaved and sterilized with 10% povidone-iodine, a midline skin incision was made. To induce renal I/R injury, the left renal pedicle was clamped for 30 (I/R30), 45 (I/R45), or 60 (I/R60) minutes, and then unclamped for 24 hours. Reperfusion was confirmed visually by a change in the color of the kidney. The right kidney was removed immediately after the left renal pedicle was unclamped. Three rats were included per I/R injury group except for experiments involving SPLN and IFX, where the I/R45 groups comprised 4 or 5 rats.

Splenectomy

Splenectomy was performed immediately after right nephrectomy (RN).[11] For this set of experiments, animals were divided into 4 groups. The first group of rats was subjected to RN only (n = 3). The second group was subjected to RN and SPLN (RN + SPLN) (n = 3). In the third group, the left renal pedicle was clamped for 45 minutes and reperfused, and RN was immediately performed (RN + I/R45) (n = 4). In the fourth group, the left renal pedicle was clamped for 45 minutes and reperfused, and both RN and SPLN were immediately performed (RN + I/R45 + SPLN) (n = 4). After 24 hours, the left kidney was dissected.

Administration of IFX

Infliximab (Remicade, an anti-human TNF-α antibody; Janssen Biotech, Inc, Horsham, PA) was administered intraperitoneally at a dose of 10 mg/kg 1 hour before surgery. Rats were divided into 3 groups. The first group of rats underwent only RN (n = 3), the second group underwent RN and I/R45 (RN + I/R45) (n = 5), and the third group underwent RN and I/R45, and were also administered IFX (RN + I/R45 + IFX)

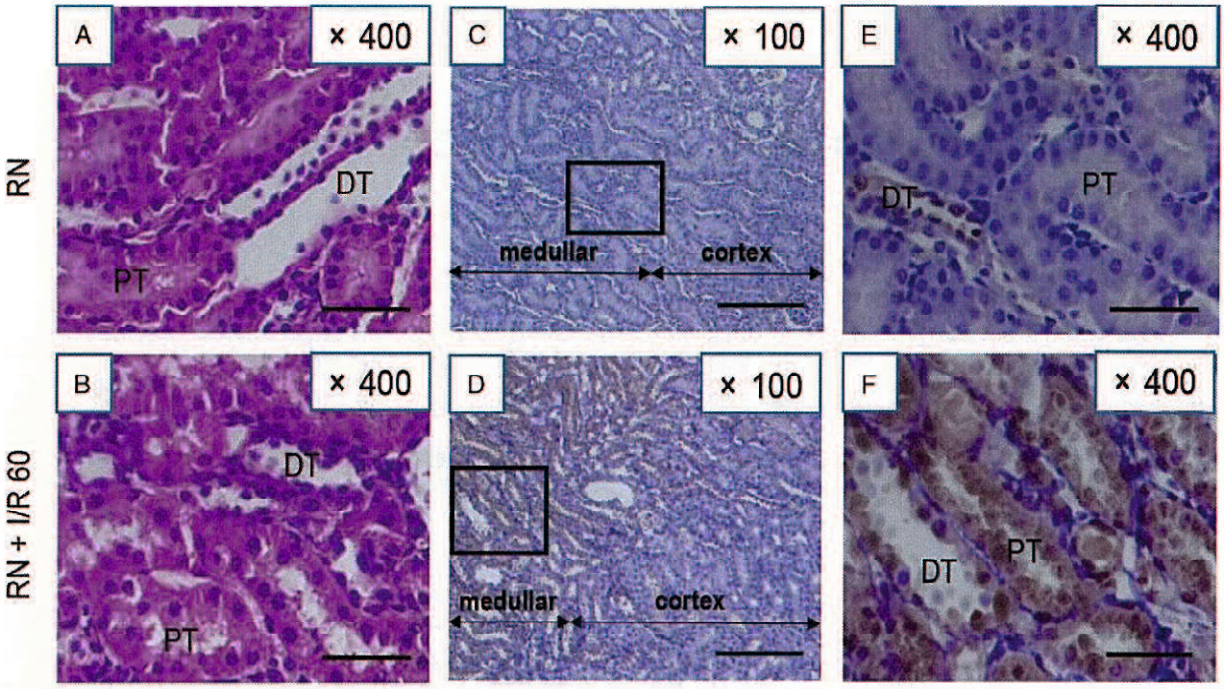

FIGURE 2. Histological examination of the injured kidney. Sections (5 μm thick) from the RN group (A) and the RN + injury (clamping) for 60 minutes followed by reperfusion for 24 hours (ischemia/reperfusion [I/R60]) group (B) were stained with hematoxylin-eosin; a section of the outer stripe of the outer medulla of the rat kidney is shown. The same sections from the RN group (C, E) and RN + I/R60 group (D, F) were also immunostained with anti-HSP70 antibody (W27). Boxed areas in the outer stripe of the outer medulla in C and D are enlarged in E and F, respectively. Magnifications are indicated on each picture. Distal and proximal nephron tubules are indicated by DT and PT, respectively. Bar, 50 μm at ×400 and 200 μm at ×100. DT, distal nephron; PT, proximal nephron.

(n = 5). After reperfusion, the left kidney was harvested at 24 hours.

Renal Function

To evaluate renal function, blood samples were obtained before sacrificing and centrifuged at 2500*g* for 10 minutes. The supernatants were stored at −80°C. Serum creatinine and blood urea nitrogen (BUN) were measured using a 7180 Clinical Analyzer (Hitachi, Tokyo, Japan).

Western Blot Analysis

The left kidney was homogenized in lysis buffer containing 1.0% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 1 μg/mL leupeptin, 1 μg/mL pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. Proteins were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 30 minutes at room temperature, and then incubated with a primary antibody (1:500 dilution in TBST) overnight at 4°C. Antibodies used were mouse monoclonal IgG against TNF-α (MAB510; R&D Systems), IL-1β (MAB5011; R&D Systems), and IL-6 (MAB5061; R&D Systems). The blots were washed 3 times in TBST for 5 minutes and then incubated with secondary antibody for 45 minutes at room temperature. Signals were detected by the Amersham ECL system (GE Healthcare). To detect HSP70, the nitrocellulose membranes were blocked with 5% skim milk in phosphate-buffered saline (PBS) for 30 minutes at room temperature, and then incubated with a mouse monoclonal IgG against HSP70 (W27; Santa Cruz) (1:1000 dilution in PBS) overnight at 4°C.

Histopathology and Immunohistochemistry

The left kidney was fixed in 10% formalin/PBS and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin-eosin to evaluate glomerular and tubular damage. The section was also stained with a mouse monoclonal IgG for HSP70 (W27) (ab3148; Abcam) or for CD68 (ED1) (MCA341; Serotec). ED1-positive cells were counted under ×100 magnification at 3 fields per section using the Image J software (National Institutes of Health) (n = 3).

Statistical Analysis

Significant values were determined via Student *t* test using StatMateIV, and *P* less than 0.05 was considered significant. A significant change of serum creatinine was defined as a greater than 1.5-fold increase over control levels, per the Kidney Disease | Improving Global Outcomes Guidelines.[14] All values were reported as median and standard deviation.

RESULTS

Induction of inflammatory cytokines and HSP70 during renal I/R injury

Serum creatinine and BUN levels were elevated only slightly in the RN + I/R30 group compared with those in the RN group, but were significantly elevated in the RN + I/R45 and RN + I/R60 groups (Figures 1A, B); this indicated that we had successfully produced I/R injury in rat kidneys. Because damage to the kidney is associated with an increase

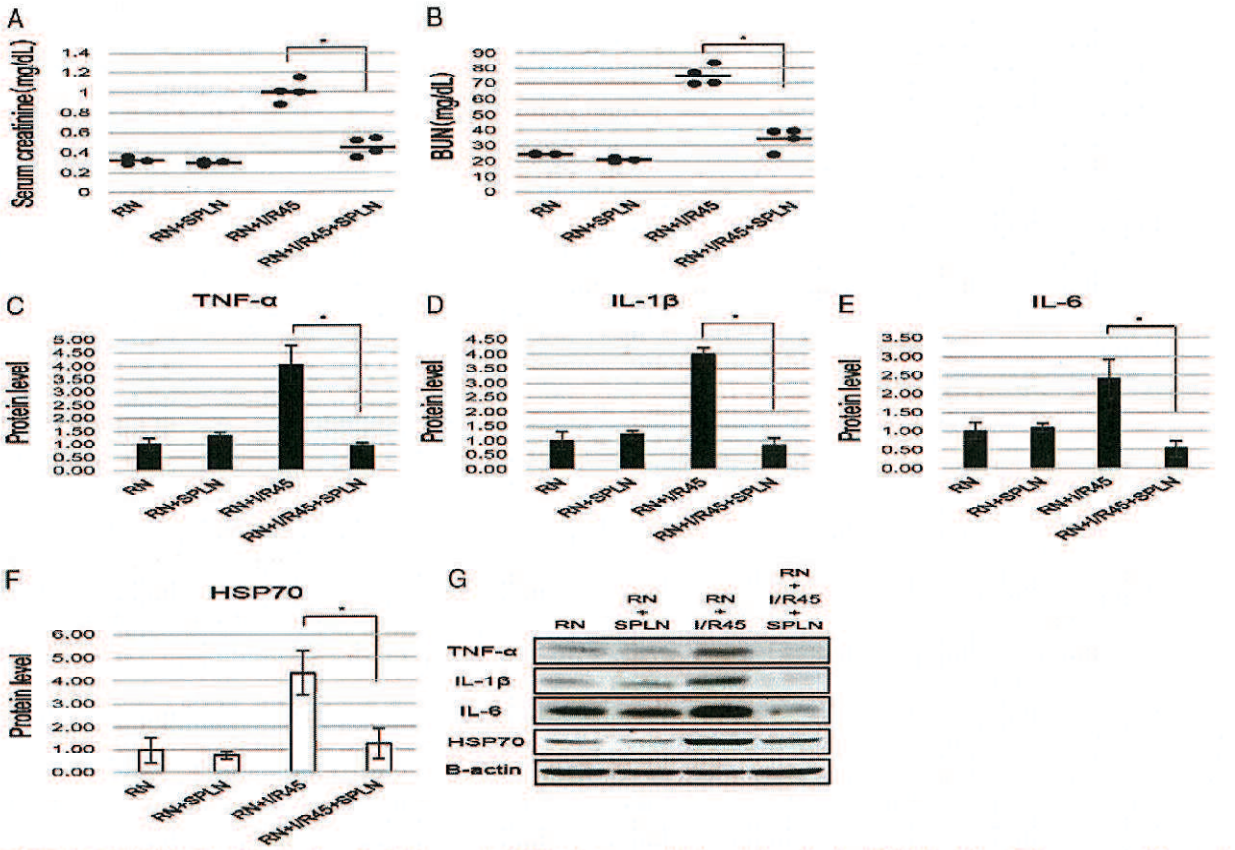

FIGURE 3. SPLN inhibits the induction of cytokines and HSP70 during renal ischemia/reperfusion (I/R) injury. A and B, serum creatinine and BUN levels. The left renal pedicle was clamped for 45 minutes and then unclamped for 24 hours (I/R45). SPLN and RN were performed immediately after unclamping, and serum creatinine and BUN levels were measured (Bar: median). C-F, protein levels of inflammatory cytokines and HSP70 in kidneys treated as described above. The unclamped left kidney was used as a control after RN. Kidney extracts were subjected to western blotting (representative blots are shown in G), and intensities of the bands were estimated using the ImageJ software (National Institutes of Health) (RN: n = 3; RN + SPLN: n = 3; RN + I/R45: n = 4; RN + I/R45 + SPLN: n = 4). *$P < 0.01$.

in serum TNF-α level,[11,15] we examined the expression of major inflammatory cytokines in the kidney after I/R injury. Protein levels of TNF-α, IL-1β, and IL-6 were higher after 30 minutes of ischemia (RN + I/R30, RN + I/R45, and RN + I/R60) compared with those in the control (RN) (Figures 1C-E). Furthermore, the expression of HSP70, which is a marker of renal I/R injury,[10] was induced moderately at 30 and 45 minutes of ischemia and highly at 60 minutes (Figures 1F, G). These results demonstrate that renal dysfunction in I/R injury is associated with the elevated production of major inflammatory cytokines and induction of HSP70 in the kidney.

Histological examination confirmed that tubular damage in our renal I/R model occurred mainly in the outer stripe of the outer medulla of the kidney after I/R injury.[11] Tubular dilatation, swelling, and necrosis of tubular epithelial cells, as well as the loss of brush borders, were observed (Figure 2A, B). Heat shock protein 70 was constitutively expressed at distal nephron tubules under normal conditions and was induced markedly at the proximal tubules in the outer stripe of the outer medulla after I/R injury (Figures 2C-F).[10]

SPLN Inhibits the Induction of Cytokines and HSP70 During Renal I/R injury

Because we previously showed that SPLN protected the kidney from I/R injury and reduced serum TNF-α levels,[11] we evaluated the expression of inflammatory cytokines and HSP70 in the kidney. The left kidney underwent ischemia for 45 minutes followed by 24 hours of reperfusion; SPLN plus RN were immediately performed after initiating reperfusion. Serum creatinine and BUN levels were markedly elevated by I/R injury, but these increases were significantly suppressed when SPLN was performed (Figures 3A, B).[11] We found that protein levels of IL-1β and IL-6 as well as of TNF-α in the kidney were markedly elevated in the RN + I/R45 group but not when SPLN was performed (Figures 3C-E). Furthermore, HSP70 was upregulated in RN + I/R45 group kidneys but not in the RN + I/R45 + SPLN group (Figures 3F, G). These results indicate that SPLN inhibits the expression of inflammatory cytokines and HSP70 in the kidney during I/R injury.

Heat shock protein-70 can prevent renal I/R injury, whereas inflammatory cytokines accelerate it.[16] Therefore, we examined time-dependent expression of inflammatory cytokines and HSP70 during renal I/R injury. Serum creatinine and BUN levels began to rise at 3 hours of reperfusion after 45 minutes of ischemia and were significantly higher at 3 hours and higher still at 24 hours of reperfusion (Figures 4A, B). HSP70 protein levels in the kidney were relatively unchanged at 1, 10, 30, and 60 minutes of reperfusion after 45 minutes of ischemia, but were significantly elevated

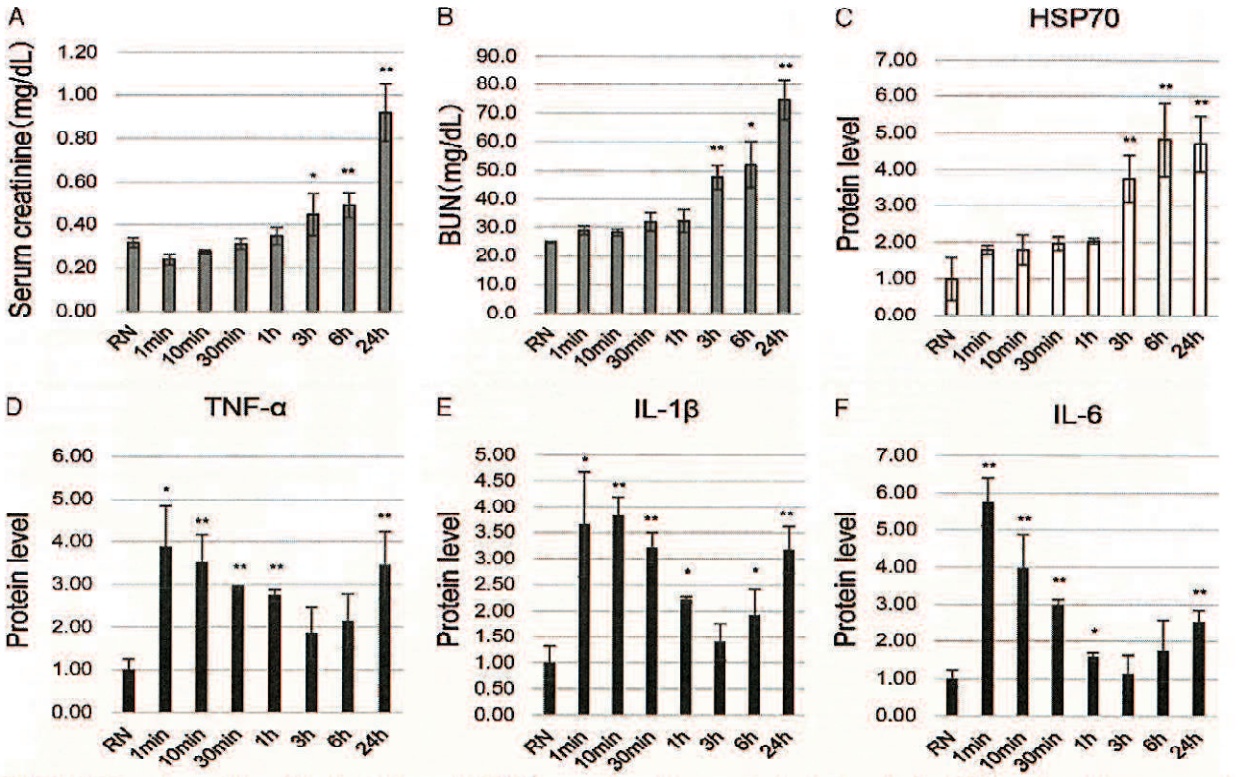

FIGURE 4. Time-dependent profiles of cytokine and HSP70 expression during renal ischemia/reperfusion (I/R) injury. A and B, serum creatinine and BUN levels. The left renal pedicle was clamped for 45 minutes, and then unclamped for 1, 10, 30 minutes, 1, 3, 6, and 24 hours. RN was immediately performed after unclamping. Serum creatinine and BUN levels were measured. The *P* values were calculated by comparing serum creatinine and BUN levels in rats whose left renal pedicles were unclamped for 1 minutes. C-F, protein levels of inflammatory cytokines and HSP70 in the injured kidney. The kidneys were treated as described above, and untreated left kidneys were used as controls after RN. Kidney extracts were subjected to Western blotting, and intensities of the bands were estimated using the ImageJ software (National Institutes of Health) (n = 3 for each group). The *P* values in relation to the RN group. $^{*}P < 0.05$, $^{**}P < 0.01$.

at 3, 6, and 24 hours of reperfusion (Figure 4C). In contrast, protein levels of TNF-α, IL-1β, and IL-6 in the kidney were significantly elevated at 1 minute of reperfusion after 45 minutes of ischemia, and gradually decreased to baseline by 3 hours (Figures 4D-F). IL-6 levels at 3 hours of reperfusion after ischemia were similar to that in the control kidney (RN), and levels of TNF-α and IL-1β at the same time point were only slightly higher than those in the control kidney (RN). Remarkably, levels of these inflammatory cytokines were again significantly elevated at 24 hours of reperfusion after ischemia. These results demonstrate that the induction of inflammatory cytokines at an early stage in the kidney precedes renal I/R injury, which is evidenced by the elevated levels of renal HSP70 protein as well as serum creatinine and BUN.

SPLN as Well as a TNF-α Inhibitor Block Accumulation of Macrophages/Monocytes in the I/R-Injured Kidney

TNF-α is one of the major inflammatory cytokines induced during renal I/R injury,[5,17] and its inhibition is known to suppress the production of other inflammatory cytokines in uveitis patients.[18] We examined whether administration of an anti–TNF-α antibody, IFX, mimicked the effects of SPLN with respect to renal I/R injury. Serum creatinine and BUN levels in IFX-treated rats undergoing I/R for 45 minutes (RN + I/R45 + IFX) were significantly lower than those in RN + I/R45 rats (Figures 5A, B). Protein levels of TNF-α, IL-1β, IL-6, and HSP70 at 24 hours after reperfusion were significantly and consistently lower in the kidneys of the RN + I/R45 + IFX group than in the RN + I/R45 group (Figures 5C-G). These results indicate that IFX, like SPLN, inhibits the expression of major inflammatory cytokines and HSP70 during renal I/R injury.

It has been suggested that monocytes and macrophages contribute to the late production of inflammatory cytokines during renal I/R injury.[8] We investigated infiltration of these cells by immunohistochemistry using an antibody against ED1, a marker of rat macrophages/monocytes. We found that macrophage/monocyte levels were significantly higher in the kidneys of RN + I/R45 group rats at 24 hours after reperfusion, whereas there was low macrophage/monocyte accumulation in the kidneys of the RN + I/R45 + SPLN and RN + I/R45 + IFX groups (Figures 6A-E). These results demonstrate that IFX mimics the effect of SPLN during renal I/R injury; specifically, it inhibits the accumulation of macrophages/monocytes in the injured kidney.

DISCUSSION

In this study, we demonstrated that SPLN inhibited the expression of inflammatory cytokines and HSP70 in the I/R-injured kidney, which was accompanied by reduced accumulation of macrophages/monocytes. Moreover, administration of IFX mimicked the effect of SPLN during renal I/R injury.

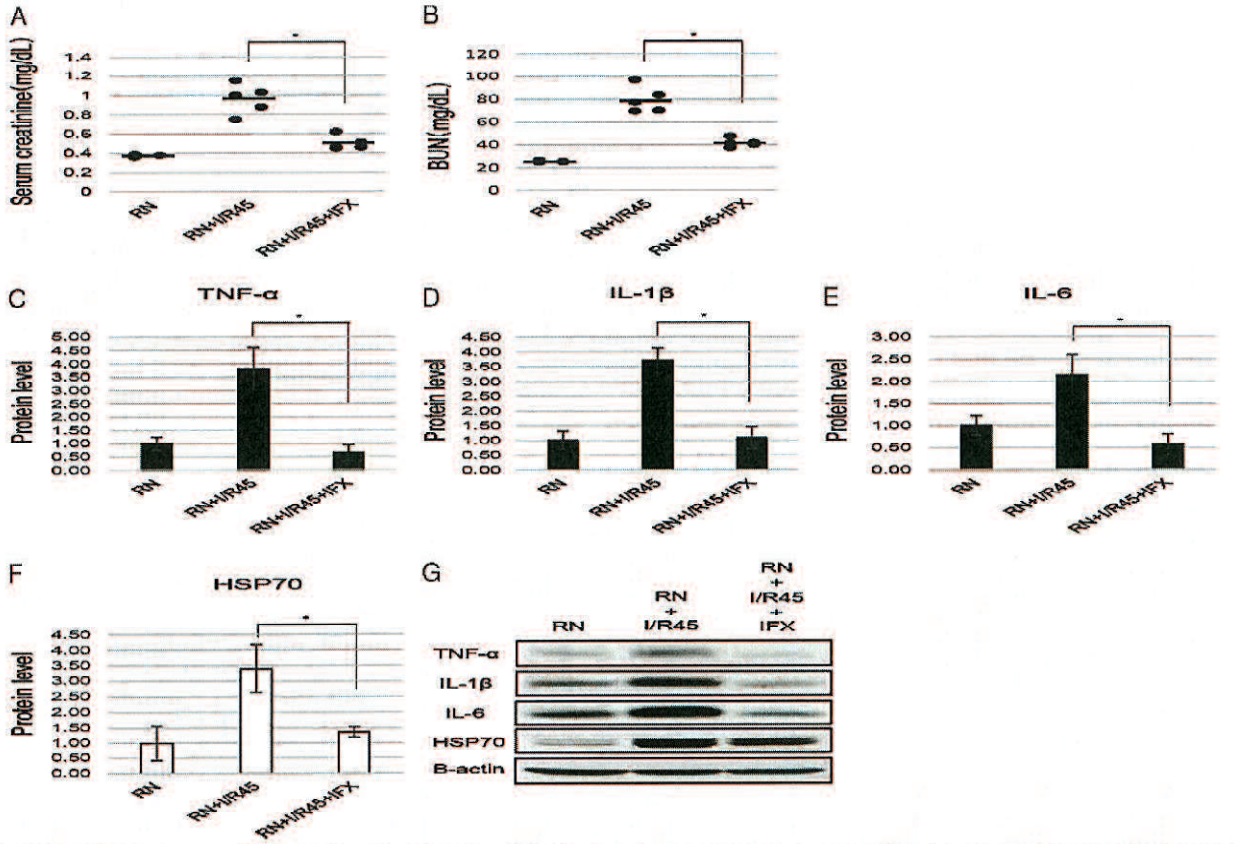

FIGURE 5. IFX suppresses the induction of cytokines and HSP70 during renal ischemia/reperfusion (I/R) injury. A and B, serum creatinine and BUN levels. The left renal pedicle was clamped for 45 minutes, and then unclamped for 24 hours (I/R45). IFX was administered intraperitoneally 1 hour before surgery. Serum creatinine and BUN levels were measured (Bar: median). C-F, protein levels of inflammatory cytokines and HSP70 in the injured kidney. Untreated left kidneys were used as a control after RN. Kidney extracts were subjected to western blotting (representative blots are shown in G), and intensities of the bands were estimated using the ImageJ software (National Institutes of Health) (RN: n = 3; RN + I/R45: n = 5; RN + I/R45 + IFX: n = 5). *$P < 0.01$.

In postischemic tissues, splenic macrophages/monocytes are suggested to play an important role during I/R injury.[19] It was reported that splenic monocytes moved to injured sites during myocardial ischemic injury.[20] Furthermore, SPLN protected from hepatic I/R injury by reducing levels of TNF-α, which was produced from monocytes/macrophages.[21,22] We showed that ED1-stained macrophages/monocytes had significantly increased in the I/R-injured kidney 24 hours after reperfusion, whereas accumulation of these cells was low in rats that underwent SPLN (Figure 6). Levels of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, were elevated after 45 minutes of ischemia and attenuated at 1 or 3 hours after the reperfusion (Figure 4); they were then reelevated in the injured kidney at 24 hours. However, cytokine levels were constant or even reduced in kidneys of splenectomized rats (Figure 3). The reduced production of these cytokines at 24 hours after reperfusion was associated with the inhibition of the elevation of renal HSP70 expression and serum creatinine and BUN levels (Figure 3). We postulate that an initial elevation of inflammatory cytokines in the I/R-injured kidney may induce renal infiltration of macrophages/monocytes from the spleen, which themselves produce more cytokines that subsequently injure the renal tissue.

Macrophages/monocytes in the I/R-injured kidney produce TNF-α, IL-1β, and IL-6[8]; these cytokines reinforce the secretion of each other. For example, IL-1 promotes production of TNF-α and IL-6, and IL-6 in turn does the same to TNF-α and IL-1β during renal I/R injury.[4,23] We showed that protein levels of TNF-α, IL-1β, and IL-6 were significantly elevated after 30 minutes of ischemia (RN + I/R30, I/R45, and I/R60) compared with controls (RN) (Figure 1), and histological examination confirmed that tubular damage occurred in our renal I/R model (Figure 2). TNF-α is a major inflammatory cytokine that induces apoptotic cell death by activating signaling pathways such as NF-κB, c-Jun N-terminal kinase, and p38-mitogen-activated protein kinase pathways.[5] IL-1β is associated with the programmed cell death pathway known as pyroptosis and is considered a predictor of progression of acute kidney injury.[6] Furthermore, increased expression of IL-6 is associated with interstitial fibrosis and glomerulosclerosis.[7] Therefore, inhibiting inflammatory cytokines is important for recovery from renal I/R injury. Inhibition of TNF-α can suppress the production of other inflammatory cytokines[18]; in fact, we demonstrated that administration of the anti-TNF-α inhibitor IFX suppressed renal infiltration of macrophages/monocytes, as well as the production of major inflammatory cytokines and the elevation of renal HSP70 expression (Figures 5, 6), as did SPLN. It was reported that TNF-α also promotes the expression of monocyte chemotactic protein-1/chemokine (C-C motif)

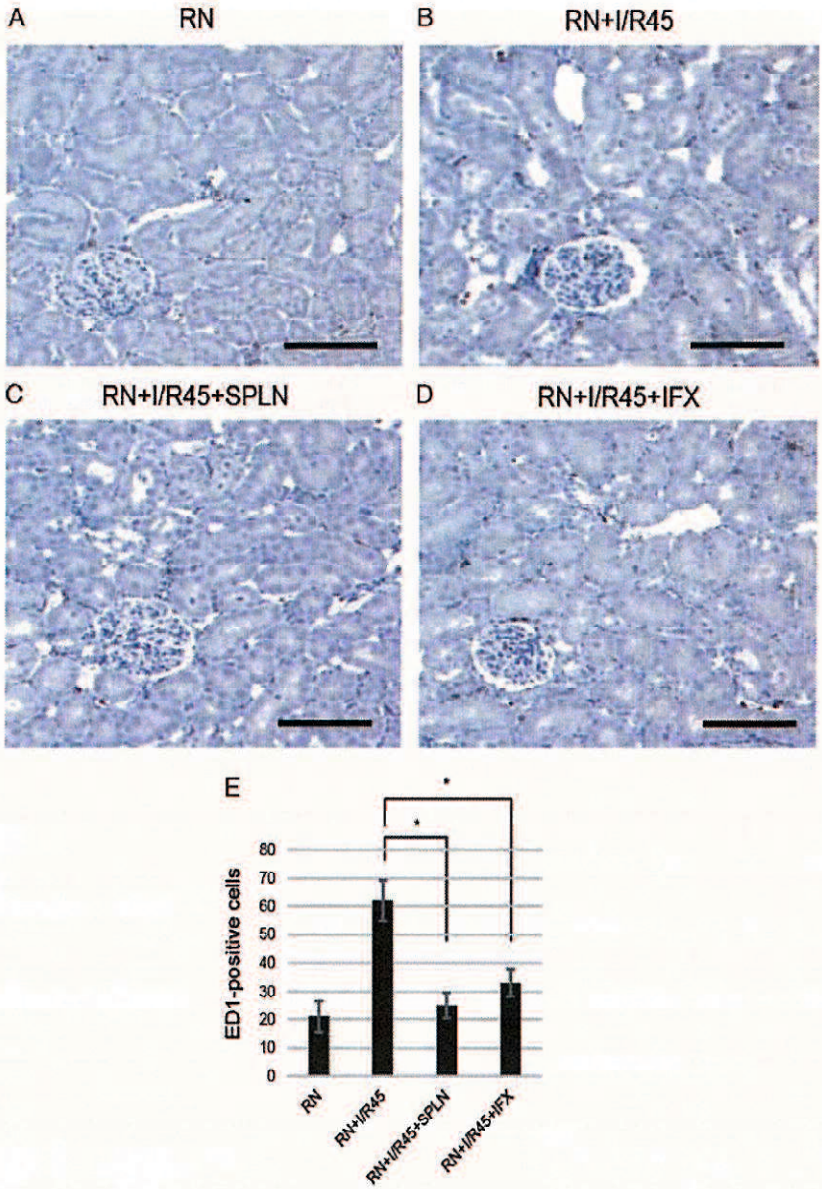

FIGURE 6. Infiltration of macrophages/monocytes into the injured kidney. The left renal pedicle was clamped for 45 minutes, and then unclamped for 24 hours (I/R45). SPLN and RN were immediately performed after unclamping. IFX was administered intraperitoneally 1 hour before surgery. The kidneys were dissected and subjected to immunohistochemistry using an antibody against ED1, a marker of macrophages/monocytes. A, RN group. B, RN + I/R45 group. C, RN + I/R45 + SPLN group. D, RN + I/R45 + IFX group. E, numbers of ED1-positive cells were counted using the ImageJ software (National Institutes of Health) (n = 3). Bar, 200 μm. *$P < 0.01$.

ligand-2, which induced monocyte infiltration during renal I/R injury,[24] and that IFX inhibits its expression.[25] Therefore, the reduced infiltration of macrophages/monocytes by IFX may be due to diminished production of monocyte chemotactic protein-1/chemokine (C-C motif) ligand-2. In any case, our results support the notion that TNF-α is a pivotal cytokine that induces renal dysfunction during I/R injury.

Nowadays, renal transplantation for donor-specific antigen-positive patients is mainly undertaken after treatment with rituximab, a monoclonal anti-CD20 antibody that targets B lymphocytes, to prevent rejection.[26] Rituximab has an effect that is equivalent to that of SPLN,[26] which is accompanied by surgical stress. Likewise, we showed that administration of IFX had the same effect on renal I/R injury as that of SPLN. The protective effect of IFX on I/R injury was reported not only in the kidney but also the liver, intestine, and spinal cord in animals.[13,27–29] Tasdemir et al[13] reported that IFX may protect against renal I/R injury by countering oxidative stress in rat; however, they did not investigate the relationship between proinflammatory cytokines and renal function. Di Paola et al[30] showed that mouse renal function after I/R injury is protected by IFX administration. In our study, we showed that IFX reduced the production of cytokines and improved renal function after renal I/R injury, and IFX also reduced the accumulation of macrophages/monocytes. IFX is clinically effective and safe for the treatment of patients afflicted with rheumatoid arthritis and Crohn disease.[31,32] Therefore, our data suggest that IFX may also be useful for

the prevention of I/R injury in human renal transplantation. Further experiments using renal transplantation models are required to confirm our findings.

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