Role of $\alpha/\beta$ and $\gamma/\delta$ T cells in renal ischemia-reperfusion injury

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Hochegger K, Schätz T, Eller P, Tagwerker A, Heinginer D, Mayer G, Rosenkranz AR. Role of $\alpha/\beta$ and $\gamma/\delta$ T cells in renal ischemia reperfusion injury. Am J Physiol Renal Physiol 293: F741–F747, 2007. First published June 13, 2007; doi:10.1152/ajprenal.00486.2006.—T cells have been implicated in the pathogenesis of renal ischemia-reperfusion injury (IRI). To date, data about the role of the T cell receptor (Tcr) are contradictory. We hypothesize that the Tcr plays a prominent role in the late phase of renal IRI. Therefore, renal IRI was induced in Tcr-deficient and wild-type mice by clamping renal pedicles for 30 min and reperfusion for 24, 48, and 72 h. Serum creatinine increased equally in all three groups 24 h after ischemia but significantly improved in Tcr-deficient animals compared with wild-type controls after 72 h. A significant reduction in renal tubular injury and infiltration of CD4$^+$ T cells in both Tcr-deficient mice compared with wild-type controls was detected. Infiltration of $\alpha/\beta$ T cells into the kidney was reduced in $\gamma/\delta$ T cell-deficient mice until 72 h after ischemia. In contrast, $\gamma/\delta$ T cell infiltration was equal in wild-type and $\alpha/\beta$ T cell-deficient mice, suggesting an interaction between $\alpha/\beta$ and $\gamma/\delta$ T cells. Data from $\gamma/\delta$ T cell-deficient mice were confirmed by in vivo depletion of $\gamma/\delta$ T cells in C57BL/6 mice. Whereas $\alpha/\beta$ T cell-deficient mice were still protected after 120 h, $\gamma/\delta$ T cell-deficient mice showed a “delayed wild-type phenotype” with a dramatic increase in kidney-infiltrating $\alpha/\beta$, Tcr-expressing CD4$^+$ T cells. This report provides further evidence that $\alpha/\beta$ T cells are important mediators in renal IRI, whereas $\gamma/\delta$ T cells play a role as mediator cells in the first 72 h of renal IRI.

Acute renal failure

Acute renal failure is a common clinical event that is followed by decreased allograft survival in the setting of kidney transplantation and is associated with mortality rates of 30–40% in patients with native kidneys (6, 12, 25). Evidence from several studies suggests that T cells could be important mediators in renal ischemia-reperfusion injury (IRI). Lymphocytes are found in postischemic human and rat kidneys, particularly in the outer medulla (24). The use of genetically modified T cell-deficient mice, including CD4$^{-/-}$, CD4/CD8$^{-/-}$, nu/nu, and Rag1$^{-/-}$ mice, the Rabb group provided strong evidence that T cells play a crucial role in the pathogenesis of renal IRI (3, 19, 27). They found CD4$^+$ T cells to be the major T cells involved in renal IRI (3) and Th1 phenotype to be deleterious (27).

There exist two distinct T cell populations, expressing either the $\alpha/\beta$ T cell receptor (Tcr) or the $\gamma/\delta$ Tcr, and to date the exact role of these two populations in renal IRI is still unknown. The population of $\alpha/\beta$ T cells expresses CD4 and/or CD8 and recognizes MHC-associated peptides. The vast majority of peripheral blood $\gamma/\delta$ T cells, which represent only 5% of the circulating T cell population in humans and mice, simultaneously lack CD4 and CD8 expression (reviewed in Refs. 8 and 9). It was speculated that this cell population might bridge innate and adaptive immunity, and more recently it was shown that $\gamma/\delta$ T cells can act as antigen-presenting cells (2).

Recently, Savransky and coworkers (23) provided first evidence that $\alpha/\beta$ as well as $\gamma/\delta$ T cells could play a role in renal IRI. They performed renal IRI in wild-type, $\alpha/\beta$, and $\gamma/\delta$ T cell-deficient mice by clamping both renal pedicles for 30 min and evaluating all mice after 24 h only. Interestingly, they found no significant differences in serum creatinine but significant protection of renal histology in the $\alpha/\beta$ and $\gamma/\delta$ T cell-deficient mice compared with wild-type mice 24 h after ischemia (23). Interestingly, an earlier study by Faubel and coworkers (4) described CD4$^+$ T cell-depleted as well as $\alpha/\beta$ T cell-deficient mice not to be protected from renal IRI 24 h after ischemia.

In the present study, we provide evidence that $\alpha/\beta$ T cells act as effector cells in renal IRI, whereas $\gamma/\delta$ T cells are important within the first 72 h of renal IRI and are intermediators between innate immunity and the CD4$^+$, $\alpha/\beta$ Tcr-expressing effector T cells.

Materials and Methods

Animals and renal ischemia model. Experiments were performed in male C57BL/6, Tcr$^{b+\delta+}$/Mom, and Tcr$^{b+\delta+}$/Mom mice (weight 18–22 g, 8–12 wk old, Jackson Laboratory, Bar Harbor, ME). Mice were maintained on a standard diet, and water was freely available. Mice were anesthetized, and an incision was made on the central abdomen. Kidney tissue was fixed in buffered 4% formalin overnight and then embedded in paraffin wax. The kidneys were sectioned at 5-μm thickness and stained with periodic acid–schiff and Masson’s trichrome.

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acid-Schiff (PAS) or hematoxylin-eosin using a standard protocol. Tubular injury was assessed in PAS-stained sections using a semiquantitative scale as recently described (20, 21). Briefly, the percentage of cortical tubules showing epithelial necrosis was scored: 0 = no tubular necrosis; 1 = <10%; 2 = 10–25%; 3 = 26–75%; and 4 = >75% tubular necrosis. The scoring was performed by one pathologist in a blinded manner.

The three-layer immunoperoxidase technique was used for the detection of macrophages and T cells in the kidney sections as described previously (22). Macrophages were stained using clone ER-HR3 rat anti-mouse macrophage antibody (Abcam, Cambridge, UK), and a semiquantitative scoring system was performed as follows: 0 = no cells stained positive, 1+ = 5 to 10 cells, 2+ = 10–50 cells, 3+ = 50–200 cells, and 4+ = over 200 cells stained positive per low-power field. For the detection of CD4+ T cells a rat anti-mouse CD4 monoclonal antibody (mAb; clone YTS191.1, Serotec) was used. In all cases, an IgG2a isotype antibody (clone G155–178, mouse CD4 monoclonal antibody (mAb; clone YTS191.1, Serotec) was used. In all cases, an IgG2a isotype antibody (clone G155–178, Pharmingen, San Diego, CA) served as a negative control. Biotin-conjugated goat anti-rat IgG antibody (Jackson ImmunoResearch Laboratories, Cambridge, UK) was used as a secondary antibody, followed by incubation with an avidin-biotin complex (Vector Laboratories, Burlingame, CA) and subsequent development with 0.4% 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) for 10 min and counterstaining with Gill’s Hematoxylin No. 3 (Polysciences, Warrington, PA). For the detection of α/β T cells, the same protocol with the following antibodies was performed: Armenian hamster anti-mouse Tcr β-chain antibody (clone H57–597, Pharmingen), Armenian hamster IgG2A isotype antibody (Pharmingen), and biotin-conjugated goat anti-Armenian hamster IgG (Jackson ImmunoResearch). Quantitation of T cells was done by counting the number of cells in six adjacent high-power fields of renal cortex and medulla, as has been recently described (26).

Quantitation of mRNA by real-time PCR. Total RNA was isolated from kidneys using TRizol reagent (Sigma) according to a standard protocol. Thereafter, 1 µg of total RNA was reversely transcribed using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and random primers (Invitrogen). Real-time PCR was performed on an ABI Prism 7700 (Applied Biosystems, Foster City, CA) using the TaqMan gene-expression assay (Applied Biosystems) for detection of MPO (Mm00447886_m1) and IFN-γ (Mm00801778_m1) according to the manufacturer’s protocol. For detection of TNF-α, the reference gene β-actin as well as the Tcr α- and δ-chain by real-time PCR, the SYBR Green Mastermix (Invitrogen), and the following primers were used: TNF-α forward 5‘-GAA CTG GCA GAA GAG CTA CT-3‘, TNF-α reverse 5‘-AGG GTC TGG GCC ATA GAA CT-3‘; β-actin forward 5‘-GAA GTG TGA CGT TGA CAT CCG-3‘; β-actin reverse 5‘-TGC TGA TCC ACA TCT GCT GGA-3‘; and Tcrδ forward 5‘-TAG TCA CAC AGG AGA GTT TTC-3‘, Tcrδ reverse 5‘-CTT TAT TGT CTG CTT GGA GAG C-3‘. For evaluation of data we used the 2−ΔΔCt method (14) and evaluated the fold-increase compared with sham-operated controls.

In vivo depletion of γ/δ T cells. Three days before inducing of renal IRI, γ/δ T cells were depleted by intraperitoneal injection of 500 µg of an anti-γ/δ Tcr antibody (clone UC7–13D5 NA/LE, Pharmingen) into C57BL/6 mice. As a control, C57BL/6 mice received 500 µg of hamster isotype control antibody (Jackson ImmunoResearch Laboratories). Forty-eight hours after ischemia, the mice were killed and single-cell suspensions from inguinal lymph nodes were stained with an FITC-labeled anti-γ/δ Tcr antibody (clone GL3, Pharmingen). Cells were analyzed by using the Epics XL-MCL Flow Cytometry System (Beckman Coulter). Data were collected from at least 10,000 events. Additionally, RNA was isolated from lymph nodes as well as kidneys of depleted mice and analyzed by real-time PCR for the detection of the Tcr δ-chain as described above.

Statistical analysis. The data are reported as means ± SE. In the case of comparing three groups, we performed the Kruskal-Wallis test. When significances were detected, groups were compared by using the Mann-Whitney U-test. The level of significance was corrected to the number of groups, and P < 0.025 was considered as significant. In the case of comparing only two groups, the Mann-Whitney U-test was performed, and P < 0.05 was considered as significant. Significances regarding survival were evaluated by using the log-rank test.

RESULTS

Renal injury in α/β and γ/δ T cell-deficient and wild-type mice. CD4+ T cells have been shown to play a major role in renal IRI (3). Therefore, we evaluated the role of α/β and γ/δ T cells in renal IRI by clamping the renal pedicles of α/β and γ/δ T cell-deficient mice and wild-type mice for 30 min. Serum creatinine was equally increased in all three groups 24 h after ischemia compared with sham-operated controls, as has been described before (23). However, after 72 h significantly decreased serum creatinine levels were found in α/β and γ/δ T cell-deficient mice compared with wild-type mice (Fig. 1). In line with this, survival was significantly reduced in wild-type mice after 72 h of reperfusion, whereas none of the Tcr-deficient mice died from acute renal failure (Fig. 2). The tubular injury was decreased in kidneys of α/β and γ/δ T cell-deficient mice 24, 48, and 72 h after ischemia compared with wild-type mice. Sham-operated mice showed normal kidney architecture (Fig. 3A). Wild-type mice showed severe tubular injury in the majority of the tubules as reflected in cast formation, loss of brush-border membranes, sloughing of tubular epithelial cells, and dilatation of tubules (Fig. 3B). In contrast, α/β and γ/δ T cell-deficient mice showed dilatation of tubules and loss of the brush border in some tubular cells only (Fig. 3C and D). Quantitation of tubular necrosis revealed significantly decreased tubular necrosis in kidneys of α/β and γ/δ T cell-deficient mice compared with wild-type mice 24, 48, and 72 h after ischemia (Fig. 3E).

Polymorphonuclear neutrophil granulocyte and macrophage infiltration. Polymorphonuclear neutrophil granulocytes as well as macrophages have been shown to be critical mediators in the development of acute tubular necrosis. In our study, we were able to quantitate the number of polymorphonuclear neutrophil granulocytes and macrophages in renal cortex and medulla. Polymorphonuclear neutrophil granulocytes were detected in all three groups, but macrophages were significantly reduced in kidneys of α/β and γ/δ T cell-deficient mice compared with wild-type mice 24, 48, and 72 h after ischemia (Fig. 3E).

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regulate immune responses (11, 17). Therefore, we evaluated the mRNA expression of the Th1 cytokines TNF-α and IFN-γ in kidneys of wild-type, α/β, and γδ T cell-deficient mice 24, 48, and 72 h after ischemia. TNF-α mRNA levels were significantly increased in all three groups compared with sham-operated controls, but there were no significant differences found between all groups 24, 48, and 72 h after ischemia (Fig. 6A). As has been shown earlier (23), decreased mRNA IFN-γ levels were detected in kidneys of mice that underwent renal IRI compared with sham-operated controls. However, again no significant differences were found among wild-type, α/β, and γδ T cell-deficient mice (Fig. 6B).

Renal injury in α/β and γδ T cell-deficient and wild-type mice 120 h after renal IRI. Since we were able to demonstrate that α/β as well as γδ T cells play an important role after reperfusion intervals of 48 and 72 h, we were still interested in the outcome of α/β and γδ T cell-deficient mice compared with wild-type controls after longer reperfusion intervals. Therefore, α/β and γδ T cell-deficient and wild-type mice were subjected to 30 min of ischemia and 120 h of reperfusion. Not surprisingly, 75% of wild-type mice did not survive the 120-h reperfusion interval, whereas all α/β T cell-deficient mice survived (Fig. 7A). Interestingly, 50% of γδ T cell-
deficient mice died between 72 and 120 h after induction of renal IRI (Fig. 7A). Analysis of cellular inflammation revealed no difference in the infiltration of macrophages, neutrophil granulocytes, and the cytokines TNF-α and IFN-γ (data not shown). However, a significant increase in the effector T cell population, expressing the Tcr and CD4, was found in kidneys of γδ T cell-deficient mice 120 h after ischemia compared with γδ T cell-deficient animal after 72 h of reperfusion (Fig. 7B).

In vivo depletion of γδ T cells. To support the finding on the role of γδ T cells in renal IRI in genetically modified mice, we performed in vivo depletion of γδ T cells in C57BL/6 mice using the anti-Tcr γδ antibody UC7–13D5 (15). Three days after injection of 0.5 mg γδ T cell-depleting antibody or the respective isotype control antibody, mice were subjected to 30 min of renal ischemia and followed for 48 h. At the end of experiment, the mice were analyzed for their γδ T cell content in lymph nodes and kidneys. Significantly decreased numbers of γδ T cells were detected by flow cytometry (Fig. 8A) as well as quantitative PCR (Fig. 8B) in inguinal lymph nodes of mice that received the γδ T cell-depleting antibody. Interestingly, local γδ T cells in the kidney were not depleted by the antibody (Fig. 8B). The γδ T cell-depleted mice displayed significantly decreased serum creatinine and a tubular necrosis score compared with respective control animals (Fig. 8C), thus supporting the data obtained from genetically modified γδ T cell-deficient animals.

Fig. 5. Infiltration of T cells in kidneys. Infiltration of αβ (A) and CD4+ (B) T cells was analyzed by immunohistochemistry. Staining was performed on kidneys of wild-type (filled bars), γδ (grey bars), and αβ T cell-deficient mice (open bars) 24 (n = 5/group), 48 (n = 5/group), and 72 h (n = 8/group) after ischemia. Values are means ± SE. C: quantitative PCR for detection of the Tcr δ-chain was performed in kidneys of wild-type mice (filled bars) and αβ T cell-deficient mice (open bars). Values are means ± SE. *P < 0.025 compared with wild-type mice.
Renal IRI is still associated with increased mortality and morbidity in the clinical setting of acute renal failure or decreased allograft survival in kidney transplantation (6, 25). Recently, T cells gained interest as key players in renal IRI. The Rabb group (3) presented convincing evidence that T cells are involved in the pathogenesis of renal IRI and lately described a possible direct pathophysiological role for the Tcr in renal IRI (23). We now extend those findings in the early phase of renal IRI by showing significant protection in γδ T cell-deficient mice compared with wild-type mice after longer intervals of reperfusion. α/β and γδ T cell-deficient mice displayed significantly decreased serum creatinine levels 72 h after ischemia, increased survival rates, and decreased tubular necrosis at all tested time points. We additionally confirmed these data from γδ T cell-deficient mice in an independent experimental setting by depleting γδ T cells in vivo. However, when we looked at longer reperfusion intervals, γδ T cell-deficient mice showed decreased survival.

There are contradictory data about a possible role of Tcr α/β- and Tcr γδ-expressing T cells in the pathogenesis of renal IRI. First, Faubel and coworkers (4) induced renal IRI in α/β T cell-deficient mice and found no differences in serum creatinine as well as histological indices between knockout mice and respective wild-type mice after 24 h of reperfusion. In contrast, Savransky and coworkers (23) presented first data about a possible involvement of α/β as well γδ T cells in renal IRI. They detected no significant differences in serum creatinine levels after 24 h of reperfusion but significantly improved histology in α/β as well γδ T cell-deficient mice compared with wild-type mice. They concluded that the Tcr plays a direct but modest pathophysiological role and that alloantigen-independent activation in renal IRI can lead to engagement of antigen-specific molecules on T cells. In line with this, we detected no significant differences between the three groups of mice in serum creatinine after 24 h but significant histological improvements at all tested time points in α/β and γδ T cell-deficient mice. Interestingly, the serum creatinine levels decreased dramatically with ongoing reperfusion time in the α/β as well as γδ T cell-deficient mice, while remaining high in wild-type mice. Importantly, all T cell-deficient mice sur-

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vived the 72-h reperfusion interval, but >20% of the wild-type mice died because of acute renal failure.

We think that the data presented by Savransky and coworkers (23) and our group can be explained by equal numbers of infiltrating neutrophil granulocytes and macrophages in kidneys of α/β and γ/δ T cell-deficient and wild-type mice as demonstrated by equal MPO levels, leading to a transient increase in serum creatinine 24 h after ischemia, as described before (10, 13, 28). This fact might explain the findings of various groups that Tcr-deficient mice have equal levels of serum creatinine in the early phase of reperfusion (4, 23). However, importantly, in light of our findings α/β and γ/δ T cells seem to be necessary to prolong inflammation and kidney damage, since serum creatinine and the tubular injury score were significantly diminished in α/β as well as γ/δ T cell-deficient mice compared with wild-type controls 72 h after ischemia.

As already described by others, small numbers of CD4+ infiltrating T cells, but not CD8+ T cells, were already found in kidneys after 24 h of renal IRI (3, 23). Interestingly, significantly decreased numbers of α/β Tcr-expressing CD4+ T cells were detected in γ/δ T cell-deficient mice compared with wild-type controls, whereas, not surprisingly, no α/β Tcr-expressing CD4+ T cells were found in α/β T cell-deficient mice. Therefore, γ/δ T cells seem to be necessary to mediate the influx of CD4+ α/β T cells at least within the first 72 h after ischemia. The infiltration of CD4+ T cells into the respective mice as well as the protection of α/β T cell-deficient mice from renal IRI at later time points of reperfusion support previous reports about a key role of T cells in renal IRI (3, 18, 19, 23). Additionally, these data led us hypothesize that CD4+ α/β T cells are effector cells and lead to prolonged inflammation in renal IRI. The population of γ/δ T cells has been described to bridge innate and adaptive immunity (reviewed in Refs. 5, 16, and 18). Here, we detected equal numbers of γ/δ T cells in α/β T cell-deficient mice and wild-type mice after renal IRI. Together with the finding of equal infiltration of neutrophil granulocytes and macrophages in kidneys of all three groups of mice, we suggest that γ/δ T cells bridge innate, namely, neutrophil granulocytes and macrophages, and CD4+, α/β Tcr-expressing, effector T cells representing adaptive immunity. In light of the fact that γ/δ T cells represent only 5% of the circulating T cell population (8, 9), this population would be an attractive therapeutic target in renal IRI. However, these data are put into perspective by the fact that γ/δ T cell-deficient mice were found to be more susceptible to renal IRI after longer reperfusion intervals such as 120 h. In the γ/δ T cell-deficient mice, the inflammatory response seems to be dramatically delayed, since significantly increased infiltrating CD4+, α/β Tcr-expressing, effector T cells were found in these kidneys. We speculate that the massive influx of macrophages and their cytokine production can, after reperfusion intervals of 120 h, finally overcome the lack of γ/δ T cells and lead to the influx of effector T cells.

Th1-dependent cytokines, such as TNF-α, have been shown to be regulated in renal IRI (11, 17). In accordance with Savransky and coworkers (23), we detected an increase in TNF-α concentration in kidneys of mice that underwent renal IRI compared with sham-operated mice. However, we were not able to detect significant differences in TNF-α and IFN-γ mRNA expression between α/β and γ/δ T cell-deficient mice and wild-type mice at any tested time point, which might be explained by the different techniques (quantitative PCR vs. protein expression assay) used. Thus we speculate that the massive renal infiltration of macrophages, which was equal in all three groups, and their subsequent production of cytokines may overwhelm the cytokine production of T cells.

Finally, to confirm our results from genetically modified animals and underline a possible central role of γ/δ T cells in
renal IRI, we depleted this population in vivo in C57BL/6 mice by using an antibody directed against the γδ TcR. γδ T cell-depleted animals were found to have significantly decreased indices of acute renal failure. Interestingly, γδ T cell-depleted animals were not as protected from renal IRI as γδ T cell-deficient mice. This difference could, on the one hand, be explained by the chosen shorter reperfusion time of 48 h since serum creatinine in γδ T cell-deficient mice was found to be even more decreased after 72 h of reperfusion. On the other hand, resident γδ T cells in the kidney, in contrast to γδ T cells in lymph nodes, were obviously not depleted, suggesting a pathophysiological role for resident γδ T cells in the kidney.

Taken together, we found α/β T cells to act as effector cells since α/β T cell-deficient mice were found to be protected from renal IRI at all tested time points. In contrast, γδ T cells have the potential to mediate between the innate and adaptive immune response in a restricted time span of reperfusion. The absence of γδ T cells delays the inflammatory response in renal IRI for nearly 72 h, but later on effector T cells find their way into the kidney and exert their destructive effects without the presence of γδ T cells. Further studies are necessary to explore why the absence of γδ T cells can only postpone the inflammatory response.

GRANTS

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