Double-Negative αβ T Cells Are Early Responders to AKI and Are Found in Human Kidney

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ABSTRACT
Ischemia-reperfusion injury (IRI) is a major cause of AKI, and previous studies established important roles for conventional CD4+ T cells, natural killer T cells, and CD4+CD25+FoxP3+ Tregs in AKI pathogenesis. We recently identified CD4-CD8- (double-negative; DN) T cells as an important subset of αβ T cell receptor-positive cells residing in mouse kidney. However, little is known about the pathophysiologic functions of kidney DN T cells. In this study, we phenotypically and functionally characterized murine kidney DN T cells in the steady state and in response to IRI. Unlike CD4+ and CD8+ T cells, DN T cells in the steady state expressed high levels of CD69, CD28, and CD40L; differentially expressed IL-27 and IL-10 anti-inflammatory cytokines; spontaneously proliferated at a very high rate; and suppressed in vitro proliferation of activated CD4+ T cells. Within the first 3–24 hours after IRI, kidney DN T cells expanded significantly and upregulated expression of IL-10. In adoptive transfer experiments, DN T cells significantly protected recipients from AKI by an IL-10–dependent mechanism. DN T cells also made up a large fraction of the T cell compartment in human kidneys. Our results indicate that DN T cells are an important subset of the resident αβ+ T cell population in the mammalian kidney and are early responders to AKI that have anti-inflammatory properties.


AKI is associated with high mortality in native kidneys, increased morbidity in allografts, and very high health care costs. Among the most common causes of AKI in both native and transplanted kidneys is ischemia-reperfusion injury (IRI). Both innate and adaptive immune cells contribute to the disease pathogenesis.1 Dendritic cells have also been implicated in AKI.2,3

During the last two decades, the focus has been on conventional T cells, leading to important advances in understanding contributions of specific subsets in promoting and suppressing AKI.4–6 Efforts to understand the role of nonconventional T cells in IRI7 have been growing. As a consequence of such efforts, we identified CD4-CD8- (double-negative; DN) cells as an unconventional and unexpected component of αβ+ T cell receptor (TCR) cells in the normal mouse kidney.8 DN αβ+ T cells are rare in secondary lymphoid organs and are among the least studied subset of αβ+ T cells.9

Hence, very little is known about their roles in normal and ischemic kidneys.

In the present study, we phenotypically and functionally characterized murine kidney DN T cells both in the steady state and in response to IRI. DN T cells also made up a large fraction of the T cell compartment in human kidneys. Our results indicate that DN T cells are an important subset of the resident αβ+ T cell population in the mammalian kidney and are early responders to AKI with anti-inflammatory properties that can improve AKI

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outcome. Our findings are of potentially clinical relevance because we found DN T cells in the “normal” portion of kidneys removed due to renal masses in humans. We conclude that DN T cells are a prominent T cell subset in mouse and human kidneys with potentially important immunoregulatory functions that can be harnessed to develop therapies for kidney diseases.

RESULTS

DN T Cells Are a Dominant Subset in Murine Kidneys with a Unique Phenotype

Using a modified protocol, we isolated and investigated the phenotypic and functional characteristics of DN T cells. In extensively flushed kidneys, DN T cells constituted 20%–38% of αβ+ T cells of normal mice (Figure 1A). In contrast, DN T cells made up only 5%–11% of αβ+ T cells in lymph node and spleen, respectively (Figure 1A) and were rare in peripheral blood (1.0±0.2) (data not shown). To distinguish our DN T cell population from NKT cells, we excluded CD1d/PBS57 tetramer+ natural killer T (NKT) cells (5% of the kidney T cells) from our analysis. Phenotypically, kidney DN T cells expressed significantly less CD62L and higher levels of CD44 and CD69 compared with their CD4+ and CD8+ counterparts. In addition, DN T cells expressed high levels of CD40L and CD28 co-stimulatory molecules (Figure 1, B and C). These results confirm and extend our previous findings that DN T cells make up a large component of αβ+ T cells in the kidney and display an activated phenotype in the steady state.

Kidney Resident DN T Cells Actively Proliferate during the Steady State

The activated phenotype of DN T cells prompted us to assess whether they are actively proliferating in the steady state. We used two independent approaches to assess proliferation of kidney DN T cells and compare it to that of their CD4+ and CD8+ counterparts. In the first set of experiments, we assessed proliferation by analyzing intracellular expression of the Ki67 antigen. This nuclear protein is present in all phases of proliferating cells but is absent in G0 phase and widely used as a proliferation marker. There were more Ki67+ DN T cells (36%) in comparison to CD4 (5.6%) and CD8 (1%) subsets (Figure 2A), indicating steady-state proliferation of kidney DN, but not CD4 or CD8 T cells. In the second sets of experiments, we used the bromodeoxyuridine (BrdU) incorporation assay as an alternative approach to confirm this observation. The activated phenotype of Ki67+, DN T cells incorporated BrdU by flow cytometry. Consistent with their high expression of Ki67, DN T cells incorporated BrdU at significantly higher rate than did the CD4 and CD8 T cells, all isolated from same kidneys (P<0.0001) (Figure 2B). The high proliferative property of kidney DN T cells was not a property shared by DN T cells isolated from lymph node, which were cycling at rates similar to those of CD4 and CD8 T cells (Figure 2C). Thus, using two independent approaches, we found that kidney DN T cells were dividing at an exceptionally high rate in the steady state; this is a unique property not shared by kidney CD4 and CD8 cells.

Kidney DN T Cells Suppress CD4 T Cell Proliferation

We and others have previously shown that DN T cells from nonrenal sources, such as the periphery of gld mice, have T cell–suppressive functions. To determine whether kidney DN T cells have suppressive functions, we assessed their ability to inhibit T cell proliferation using a standard T cell suppression assay. We purified CD4 T cells, labeled them with carboxyfluorescein succinimidyl ester (CFSE), and cultured them alone or in the presence of kidney DN T cells. The cocultures were activated with CD3/CD28 beads, and proliferation was determined after 7 days by measuring CFSE dilution (Figure 3A) as previously described. The proliferation of CD4 T cells was significantly inhibited in the presence of kidney DN T cells, but not by the addition of a similar number of unlabeled CD4 T cells (Figure 3B). These results demonstrate that kidney DN T cells have suppressor function.

DN T Cells Are Early Responders to IRI-Induced AKI

Given the highly proliferative nature of kidney DN T cells in the steady state, we sought to understand how they would respond to alterations in their microenvironment caused by IRI. We subjected mice to bilateral IRI and assessed the effect on DN cell homeostasis compared with that of CD4 and CD8 T cells at three time points (3, 24, and 72 hours) (Figure 4A). DN T cells expanded significantly and became the dominant subpopulation 3h after IRI. DN T cells maintained dominance until 24 hours after IRI, as indicated by their high frequency and absolute numbers (Figure 4B). DN T cells then decreased significantly to below their steady state in both frequency and absolute numbers by 72 hours after IRI. This kinetics was distinct from those of CD4 and CD8 T cells, neither of which displayed significant alterations in their absolute numbers in the current studies. However, their frequencies significantly decreased because of the significant increases of the absolute number of DN T cells. Thus, the significant increases in the frequencies of DN T cells at 3 and 24 hours after IRI was not at the expenses of CD4 or CD8 T cells. Moreover, 3-hour post-schemia DN T cells incorporated more BrdU than DN T cells at the steady state (Figure 4 C). These results show that DN T cells are highly sensitive to alterations in the renal microenvironment. In addition, they reveal that DNT cells respond to IRI in a potent innate-like manner, resulting in their predominance during the first 24 hours.

Cytokine Production by Kidney Resident DN T Cells in the Steady State and in Response to IRI

Cytokines are essential mediators of T cell effector functions. Whereas the proinflammatory cytokines such as IFN-γ and
TNF-α can cause tissue damage, the anti-inflammatory cytokines such as IL-10 and IL-27 can prevent tissue damage and maintain immune homeostasis. To determine the major cytokines produced by kidney DN T cells and their modulation by IRI, we performed global gene expression analysis of DN, CD4+ and CD8+ T cells in the steady state and after ischemia-reperfusion (Martina et al. Manuscript in preparation). IL-27 and IL-10 were significantly expressed in DN T cells at the steady state, both at the mRNA and protein levels (Figure 5, A and B). In addition, DN T cells expressed lower levels of IL-17A at the protein level (Supplemental Figure 1).

To further evaluate the modulation of IL-10 and IL-27 expression by DN T cells in response to IRI, we subjected mice to IRI. Three hours later we sorted kidney CD4, CD8, and DN T cells and analyzed each subset for IFN-γ, IL-27, and IL-10, using normal kidney DN T cells as control. Global gene expression analysis of kidney DN T cells showed that gene encoding IL-10 increased 16-fold, whereas the one encoding IL-27 decreased by about 2-fold (Figure 5C). The microarray data were validated using quantitative PCR, which showed that expression of IL-10 was significantly increased in DN T cells after IRI (6-fold), whereas that of IL-27 decreased by about 0.3-fold (Figure 5D). The results were further confirmed at the protein expression level expression; IL-10 increased (P=0.02) and IL-27 decreased (P=0.003) after 3 hours of IRI (Figure 5E and Supplemental Figure 2). We measured intracellular cytokines after phorbol 12-myristate 13-acetate/ionomycin stimulation because unstimulated cells...
induced little or no detectable intracellular cytokines (Supplemental Figure 3).

**Adoptive Transfer of DN T Cells Prevents AKI**

Given the in vitro ability of kidney DN T cells to suppress CD4 T cell proliferation (Figure 3) and their production of IL-10 (Figure 5), we next determined whether DN T cells could inhibit IRI-induced AKI and whether any effect is IL-10 dependent. For these experiments, we used DN T cells isolated from lymph nodes of gld donors because large numbers of cells were needed for successful adoptive transfer studies. Gld DN T cells, similar to kidney DN T cells, can suppress T cell proliferation in vitro.10 In addition, adoptively transferred gld DN T cells migrated to the kidney (Supplemental Figure 4). We adoptively transferred DN T cells into two groups of wild-type (WT) B6 mice, 24 hours before IRI. To assess the role of IL-10, we treated mice in one group with anti–IL-10R mAb; mice in the control group received isotype control antibody. As additional controls, we infused total T cells or no cells (PBS alone) in two groups of recipients and subjected all mice to the same experimental conditions as the mice injected with DN T cells. Mice were monitored for survival and kidney function for 72 hours after IRI with serum creatinine (SCr) measured every 24 hours. Mice that received DN T cells were significantly protected (P≤0.01) from AKI in comparison to those that received only PBS or total T cells (Figure 6). There was no mortality in the 10 mice that received DN T cells before AKI. However, 2 of 10 mice died in the PBS group and 2 of 9 mice died in total T cells group. Furthermore, the protective effect of DN T cell transfer was significantly abrogated by the co-injection of anti–IL-10R neutralizing mAb as indicated by SCr (±SEM) levels measured in mice that received DN T cells alone or with anti–IL-10R at 24 hours (0.7±0.2 versus 2.0±0.2; P≤0.01), 48 hours (0.4±0.1 versus 2.4±0.4; P≤0.001) and 72 hours (0.5±0.1 versus 2.0±0.6; P≤0.001). These results show that DN T cells are protective during AKI and this protection is IL-10 dependent.

**Kidney DN T Cell in Humans**

To evaluate whether DN T cells are also present in the human kidney, we isolated αβ T cells from “normal” tissue of patients with renal cell carcinoma undergoing total or partial nephrectomy, using our modified protocol for isolation of DN T cells from nonlymphoid tissues.10 Flow cytometric analysis revealed that DN T cells were present in each of the examined biopsy specimens and their frequency varied from 18.3% to 61% of αβ T cells (Figure 7) similar to mouse kidneys. Demographic information for these cases (age, sex, diagnosis) and αβ TCR levels are shown in Supplemental Figures 5 and 6, respectively.

**DISCUSSION**

Whereas most efforts to understand the roles of αβ T cells in normal and ischemic kidneys have focused on the CD4, CD8, CD4CD25FoxP3, and NKT subsets, this study...
demonstrates for the first time that kidney DN αβ T cells are major modulators of renal immune responses. We found that DN T cells made up third of all αβ+ T cells in the mouse kidney and that they expressed high levels of the CD69, CD28, and CD40L co-stimulatory molecules. Furthermore, unlike CD4 and CD8 T cells, DN T cells divided at elevated rates in the steady state, uniquely expressed IL-27 and IL-10 and suppressed CD4 T cell proliferation in vitro. The frequency and absolute numbers of DN T cells rapidly increased within 3 hours after IRI, and expansion of DN T cells was associated with significant upregulation of IL-10 expression. The frequency and absolute numbers of DN T cells returned to levels below normal by 72 hours after IRI. Consistent with their upregulation of IL-10 after IRI, DN T cells were protective during AKI by an IL-10-dependent pathway. These findings are likely to have clinical relevance because DN T cells were also found in high numbers in human kidney samples.

DN T cells constitute 20%–38% of αβ T cells in the normal mouse kidney, which is similar to the frequency of CD8 T cells. However, their physiologic role appears to differ from that of CD8 and CD4 T cells. In support of this notion, DN T cells are constitutively dividing in the steady state, suggesting active interactions with their microenvironment in a manner that is different from that of CD4 and CD8 T cells. They could be involved in recognition and sensing self-antigens that are not recognized by the conventional T cell subsets. DN T cells also express elevated levels of IL-27 and IL-10. Whereas IL-10 is one of the most potent anti-inflammatory cytokines known, both pro- and anti-inflammatory functions have been ascribed to IL-27. In addition, IL-27 can promote IL-10 production. Future studies should determine how DN T cells use IL-27 and IL-10 to maintain immune homeostasis in the kidney. The high expression levels of CD28 and CD40L, two of the major co-stimulatory molecules, could give DN T cells advantages in outcompeting conventional T cells for interactions with local antigen presenting cells (APCs) and substantially influencing both cellular and humoral immune responses in the kidney. For example, CD40L/CD40 can influence the ability of CD4 T cells to regulate activation, B cell class switching, and licensing of DCs to activate CD8 T cells. Furthermore, it will be particularly important in the future to determine whether the lack of CD4 and CD8 co-receptors confers a unique ability that allows DN T cells to interact with nonprofessional APCs, including renal tubular...

**Figure 3.** Kidney DN T cells suppress CD4+ T cell proliferation. (A) Histograms show CFSE dilution under the indicated culture conditions. Numbers indicate percentages of CD4+ that had proliferated, as indicated by CFSE dilution. (B) Graph shows cumulative results. Each symbol represents a single co-culture. Unst, unstimulated. ***P<0.001.
epithelia. Our previous results show that CD4+2, but not CD4+, T cells are readily activated by staphylococcal superantigen immobilized on the surface of specific mAbs23 and by the intestinal epithelial cell line Caco2.24 Expression of CD4+ in cells also contributes to the termination of expansion of chronically CD4+ T cells by predisposing them to activation-induced cell death.25,26 Hence, the lack of co-receptors could potentially be vital in allowing continuous proliferation of kidney DN T cells. Future studies should examine and distinguish between these possibilities. In addition, it will be important to determine the MHC restriction elements of kidney DN T cells and whether their proliferation is driven by cytokines or in response to autoantigens.

The observation that DN T cells are the predominant early αβ+ T cell responders to IRI by virtue of their rapid and innate-like expansion in numbers within hours after the injurious insult is another key finding of this study. Although we cannot formally rule out infiltration, the low frequency of DN T cells in peripheral blood make this possibility unlikely. In addition, during IRI, the reason underlying their loss is unknown but needs to be investigated in the future. Our results also identify kidney DN T cells as an unexpected and significant contributor of IL-10 in the steady state and at the early phase of IRI. IL-10 has been implicated in ameliorating renal tissue injury under different pathophysiological conditions.27 Consistent with these reports, our results show that the ability of DN T cells to improve the course of ischemia-mediated AKI was IL-10 dependent. Thus, while kidney DN T cells can suppress CD4 T cell proliferation, peripheral gld DN T cells can suppress IRI-induced AKI. Unfortunately, difficulty in obtaining sufficient kidney DN T cells hampered our efforts to directly assess their suppressive function in vivo. However, gld mouse–derived DN T cells, produced in large numbers, also have in vitro suppressive function and readily migrated into the kidney after adoptive transfer (Supplemental Figure 4). Furthermore, IL-10 and FasL genes are closely localized on chromosome 1, precluding the generation of IL-1027/27/27 double knockout to determine whether the role of IL-10 in regulating the suppressive ability of DN T cells is cell autonomous. Moreover, DN T cell–derived IL-10 could contribute to the induction of other IL-10–producing cell types,19 including type 1 regulatory cells and Breg cells, that could subsequently become major sources during the repair phase after contraction of DN T cells. Kidney DN T cells are also a major source of IL-27, a member of the IL-10 family that has been implicated in IL-10 induction,17 and IL-27–deficient mice develop exacerbated experimental crescentic GN.28 Interestingly, IRI did not enhance but rather decreased expression of IL-27 by DN T cells. This finding

![Figure 4](https://example.com/finalimage.png)

**Figure 4.** Rapid expansion of DN T cells in response to IRI. (A) Dot plots show frequencies of DN, CD4+, and CD8+ subsets of gated kidney αβ T cells at baseline (untreated mice) and indicated time points after IRI from one of three independent experiments with similar results. (B) Graph shows pooled absolute numbers of DN, CD4+, and CD8+ T cells from the three experiments (n=10 per group) ***P<0.001. Error bars represent SD. (C) Histogram shows BrdU incorporation by kidney DN T cells from mice at baseline compared with 3 hours after IRI (n=3 mice). FMO, or fluorescence minus one.
should be further explored in the future to evaluate its significance given that IL-27 can exert proinflammatory functions.29

These findings have relevance to humans, as we found a large fraction of DN T cells in “normal” sections of human kidneys surgically excised because of adjacent renal carcinomas. However, we cannot exclude the possibility that the seemingly normal sections may have abnormalities not viewed histologically. Thus, given their high frequency and unique features.

Figure 5. Kidney DN T cells express distinct cytokines. (A) Relative mRNA level of indicated cytokines by different T cell subsets measured in the steady state by real-time PCR compared with CD4 T cells (*P<0.05; **P<0.001). (B) Protein level using intracellular staining. Kidney DN T cells expressed significantly higher levels of IL-27, intermediate expression of IL-10, and low expression of IFN-γ in the steady state. (C) Heat map shows changes in cytokines and cytokine receptors in kidney DN T cells in response to ischemia-reperfusion. Arrows show IL-10 and IL-27 cytokines. (D) Relative mRNA level of indicated cytokines by purified DN T cells isolated 3 hours after IRI. The expression of IL-10 by DN T cells was significantly enhanced at 3 hours after IRI (6-fold) but was reduced for IL-27 (0.44-fold) and for IFN-γ (0.003-fold). The real-time data were normalized with actin values and compared with DN T samples from normal (no-IRI) mice. (E) Intracellular protein level expression showed a significant increase for IL-10 (*P=0.02) and a decrease in IL-27 (**P=0.003) expression after 3 hours of IRI. Error bars indicate SEM.
response to IRI in mice, studying mouse and human kidney DN T cells will likely improve understanding of IRI, with possible relevance to other organs. This study paves the way for future studies that will elucidate DN T cell functions and their role in autoimmunity and perhaps harness them as a novel therapeutic target.

**CONCISE METHODS**

**Mice**

Male C57BL/6 WT mice were bred under specific pathogen-free conditions at the central animal facility of the Johns Hopkins University. For all experiments, male mice 8–10 weeks of age were used in accordance with the Animal Care and Use Committee guidelines.

**Human Samples**

Kidney samples were obtained from partial nephrectomies performed because of renal cell carcinoma. Samples were obtained and analyzed at different time points. The kidney tissue was digested according to our protocol for isolation of lymphocytes; stained with mAb anti-CD45 (HI30) APC-Cy7, CD8α (53–6.7) FITC/PerCP, CD4 (RM4–5) APC/PerCP, CD28 (37.51) PE, and CD44 (IM7) PE (BD Pharmingen); and analyzed by flow cytometry. The institutional review board approved the experiments.

**Isolation of Kidney T Cells**

Our protocol for kidney and peripheral T cell isolation was described before. Briefly, mice were anesthetized with intraperitoneal pentobarbital (75 mg/kg), underwent midline abdominal incisions, were exsanguinated, and had their renal pedicles dissected and kidneys removed. Spleen, kidney draining lymph nodes, and inguinal lymph nodes were removed to use as control. Spleen and lymph node cells were treated with erythrolysis buffer (ammonium-chloride-potassium) to remove red blood cells. Cells then were re-suspended and counted; purity of cells was usually >94% of viable lymphocytes. The absolute number of lymphocytes was calculated by multiplying the total number of CD45+ cells by the percentage of each subset (determined by flow cytometry).

**Antibodies and Reagents**

All reagents used for media, PBS, and Percoll were obtained from Sigma-Aldrich and BD Bioscience. The fluorochrome-conjugated mAb to mouse antigens used for flow cytometry analysis were: CD45 (30-F11) APC-Cy7, αβTCR (H57–597) Pacific blue, CD8α (53–6.7) FITC/PerCP, CD4 (RM4–5) APC/PerCP, CD28 (37.51) PE, CD69 (H1.2F3) FITC, CD62L (MEL-14) PECy7 (Biolegend), and CD44 (IM7) PE (BD Pharmingen). For intracellular staining mAb, the following were used: IL27 (MM27–7B1) PE (eBioscience), IL10 (JES5–16E3) PE, IL-17 (TC11–18H10) BV421, and IFN-γ (XMG1.2) PE (BD Biosciences).
Intracellular Cytokine Staining
Single-cell suspensions were prepared and stimulated with 5 ng/ml phorbol 12-myristate 13-acetate and 500 ng/ml ionomycin (Sigma-Aldrich) for 4 hours at 37°C in a 5% CO2 humidified atmosphere incubator in the presence of Golgi Plug (BD Biosciences). Surface staining of stimulated cells was performed with mAb anti-CD45, anti-CD8, anti-CD4, and anti-ab+TCR Pacific blue for 30 minutes at 4°C. Cells were then permeabilized with perm/wash solution followed by an additional 30 minutes of incubation with fluorochrome-conjugated monoclonal antibodies IL-27, IL-10, IL-17A, IFN-γ, or fluorescence minus one–matched control antibodies and analyzed by an LSR II flow cytometer.

Flow Cytometry Analysis
Lymphocytes were preincubated with anti-CD16/CD32 Fc-R for 10 minutes to minimize nonspecific antibody binding. Cells were then incubated with various combinations of mAb for 25 minutes at 4°C, washed twice with FACS buffer, and subsequently analyzed. Six-color immunofluorescence staining was analyzed with LSR II using FACS Diva software (BD Biosciences) and analyzed using FlowJo V10 software (Tristar Software). The lymphocytes were gated using forward- and side-scatter to exclude debris and dead cells, and at least 100,000 events acquired.

BrdU Proliferation Assay and Ki-67 Expression
To assess proliferation in kidney purified T cells, we performed a BrdU assay. Mice were injected intraperitoneally with 1 mg of BrdU in PBS twice within a 24-hour period, followed by analysis of different T cell subsets for BrdU incorporation using the BrdU flow kit protocol (BD Pharmingen) following manufacturer instructions. Cells were analyzed using LSR-II cytometry. We also analyzed different T cell subsets for Ki-67 expression using anti–Ki-67 mAb (SolA15) PerCP-eFluor 710 by flow cytometry as described earlier.

CFSE Proliferation Assay
We used standard functional suppression assay to analyze the ability of purified kidney DN T cells to suppress CD4 T cell proliferation as previously described.13 We cultured freshly isolated, CFSE-labeled splenic CD4 T cells alone or in the presence of unlabeled CD4+CD25− T cells as a control, kidney DN T cells at ratios of 1:1 and 1:0.5, stimulated with anti-CD3/CD28 beads (Dynabeads Mouse T activator; Life Technologies). Proliferation was measured after 7 days by flow cytometry.

Mouse Renal Ischemia Model
An established model of renal ischemia-reperfusion in mice was used. Briefly, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (75 mg/kg). Following an abdominal medial incision, the renal pedicle was dissected, and a microvascular clamp (Roboz Surgical Instrument, Gaithersburg, MD) was placed on each renal pedicle for 30 minutes. During the procedure, animals were kept well hydrated with warm saline and at a constant temperature (37°C). After 30 minutes of ischemia, the clamps were removed and the wounds were sutured. The animals were allowed to recover with free access to food and water.

Figure 7. Detection of DN T cells in human kidneys. Plots show percentages of T cell subsets from four different individuals who underwent partial or total nephrectomies secondary to renal cell carcinoma. CD45+ T cells were gated, and frequencies of DN, CD4+ and CD8+ subsets among gated αβ+ TCR cells were determined. Numbers indicate frequency in each quadrant.
Adoptive Transfer and Assessment of Kidney Injury

Highly purified (>97%) total T cells were isolated from spleens using the Pan T cell isolation Kit (Miltenyi Biotec), whereas DN T cells (>95% pure) were isolated from lymph nodes of gld mice as recently described.30 We transferred (2.5 × 10⁶) DN T cells or conventional αβ T cell into separate groups of 8-week-old C57BL/6 male mice (n = 10 per group), 24 hours before ischemia-reperfusion. Mice in the DN T group were divided into two subgroups treated with 100 μg of anti-IL-10R neutralizing mAb intraperitoneally (BioXcell) or isotype control antibody. The first dose was given 72 hours before surgery, with subsequent injections given 24 hours before and immediately after the surgery. Blood samples were collected at 0, 24, 48, and 72 hours after IRI to measure serum creatinine. Additionally, WT mice that did not receive any cell type (PBS only) were used as control and serve as baseline information on IRI-induced AKI. Mortality rate between multiple groups were performed by a one-way ANOVA test. Comparisons were drawn using a two-tailed Student-Newman-Keuls test where appropriate. Statistical significance was determined as P<0.05.

Statistical Analyses

Data were expressed as mean±SD or mean±SEM using Prism 6 (GraphPad Software); n indicates the number of animals per group. Comparisons were drawn using a two-tailed t test. Comparisons between multiple groups were performed by a one-way ANOVA test followed by the Student-Newman-Keuls test where appropriate. Statistical significance was determined as P<0.05.

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DISCLOSURES

None.


Supplemental Figure 1: IL17A protein level using intracellular staining. Kidney DN T cells show low expression of IL17A in the steady state.
Supplemental Figure 2: Intracellular cytokines expression in post IR kidneys. Representative FACS plots showing intracellular cytokines in CD4, CD8 and DN T cells isolated from post-ischemic kidneys.
Supplemental Figure 3: Effect of PMA treatment on intracellular cytokine levels. Representative FACS plots showing overlaid panels to demonstrate the effect of PMA treatment on intracellular IL10 and IL27 expression in CD4, CD8 and DN T cells.
Supplemental Figure 4: Homing of gld DN T cells into the kidney. B6 mice (n= 5) were injected with CFSE-labeled gld DN T cells or PBS and analyzed 24 h later for presence of CFSE-labeled cells in kidney tissue. Each dot plot represents one recipient.
Supplemental Figure 5: Demographics for human samples. Table shows percentage of human kidney T cell subsets age, gender and diagnosis for each case.
Supplemental Figure 6: TCR $\alpha\beta^+$ expression in human kidney T cells. Plots show the expression level of TCR $\alpha\beta^+$ in lymphocytes from human kidney samples.