T Cells Modulate Neutrophil-Dependent Acute Renal Failure during Endotoxemia: Critical Role for CD28

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Sepsis still represents the leading cause of acute renal failure (ARF), especially in critically ill patients (1,2). However, precise mechanisms that lead to ARF during sepsis remain largely unknown. Both human and animal studies could detect only minimal cellular death, suggesting subcellular damage rather than overall tissue loss as a correlate of organ failure during sepsis (3,4).

Various leukocyte subsets, including lymphocytes and neutrophils (PMN), have been implicated as key factors in the development of systemic inflammation during sepsis; interactions between these cell types have also been considered crucial (3,5). The mechanisms and the functional relevance of these interactions, especially with respect to ARF, are still only poorly understood.

LPS is a major pathogenic factor for the inflammatory response during Gram-negative bacteremia, as it can activate monocytes/macrophages and dendritic cells (6). These cells stimulate PMN and T lymphocytes through secretion of cytokines (PMN and T lymphocytes) or by means of direct cell–cell interactions (T lymphocytes). PMN are thought to cause tissue injury either through release of cytotoxic substances or through impairment of microcirculatory flow (5), whereas T lymphocytes seem to exert their effects via further release of pro- or anti-inflammatory substances (7,8).

CD28, an important co-stimulatory molecule for T cell activation during antigen responses (9,10), is also essential for LPS-induced T lymphocyte activation and proliferation (11,12). Activation and proliferation of T lymphocytes after LPS administration are not MHC restricted but require CD14-dependent interactions with monocytes (12). CD28 is an integral membrane protein homodimer whose extracellular region contains one Ig-like domain; it is constitutively expressed on most murine T cells as well as on 90% of human CD4+ T cells (10). Binding of CD28 to its ligands, CD80 (B7.1) and CD86 (B7.2), which are found on activated antigen-presenting cells, represents the initial step in CD28-mediated co-stimulation (10). CD28-mediated co-stimulation itself leads to activation of various transcription factors (13–15). This results in induction of IL-2 transcription, expression of CD25, and entry into cell cycle (16).

Blockade of CD28 signals by antibodies or by generation of CD28 gene-deficient mice caused attenuated inflammatory responses in several (experimental) diseases, including contact hypersensitivity (17), graft-versus-host disease (18), psoriasis (19), bleomycin-induced lung fibrosis (20), and asthma (21). Moreover, antibody blockade of CD28 signals during murine septic shock drastically improved survival rate. This protection was associated with a significant decrease in serum TNF-α.
levels, attributable to the induction of IL-10 expression (22). In a model of bleomycin-induced lung fibrosis, CD28 gene-deficient mice demonstrated reduced concentrations of chemokines, which were crucial for leukocyte recruitment and activation (20). It is interesting that recent studies have revealed that CD28 blockade also seemed to reduce the infiltration of PMN into chronically inflamed tissues (19,20,23,24). Because these inflammatory diseases were not primarily PMN-dependent, it remains unknown whether T cells can modulate acute, PMN-dependent inflammatory syndromes and subsequent organ failure.

To elucidate further the interactions between T cells and PMN during sepsis as well as their functional consequences with respect to kidney function, we developed a murine PMN-dependent model of LPS-induced ARF. We demonstrate that T cells, via their CD28 pathway, can control renal function and PMN recruitment into the kidney during endotoxemia.

Materials and Methods

Animals
We used adult C57BL/6 wild-type mice (WT) and corresponding mice with a null mutation in the CD28 gene (CD28−/−) (25), both from the Jackson Laboratory (Bar Harbor, ME). Mouse colonies were maintained under specific pathogen-free conditions. All experiments had been approved by local government authorities and were in agreement with the NIH Guide for the Care and Use of Laboratory Animals.

Reagents
If not stated otherwise, reagents were obtained from Sigma-Aldrich (Taufkirchen, Germany).

LPS Injection to Induce ARF
Mice received an intraperitoneal injection of 10 µg/g body wt LPS (Escherichia coli O111:B4). Two, 4, 12, and 24 h after LPS injections, mice were anesthetized to harvest both kidneys and to collect blood samples. Untreated, genotype-matched mice served as controls (“0 h”)

Renal Function
Plasma creatinine and blood urea nitrogen (BUN) concentrations were measured using commercially available kits.

Myeloperoxidase Activity
Renal myeloperoxidase (MPO) activity, indicating PMN infiltration into the kidney, was measured according to our previously published protocol (26,27). Briefly, samples were homogenized in ice-cold 20 mM KPO4 buffer. After removing 17,000 × g supernatants, pellets were resuspended in ice-cold 20 mM KPO4 buffer, followed by two additional spins. Then, 0.5% (wt/vol) hexacyltrimethylammonium bromide-10 mM EDTA in 50 mM KPO4 was added to the pellet. Suspensions were sonicated, freeze-thawed, and incubated for 20 min at 4°C. Supernatants (17,000 × g) were used to measure MPO. An assay buffer that contained 0.2 mg/ml o-dianisidine and 158 µM H2O2 in 50 mM KPO4 was added to the supernatant. Changes in absorbance were determined by bicinchoninic acid assay (Pierce Chemical Co., Rockford, IL). To validate MPO as an indicator of renal PMN infiltration, we used PMN-specific immunostaining (see below) and counted the number of PMN in kidney sections from WT without and 24 h after LPS injection (blinded investigator, 10 high-power fields per section, ×40, n = 6 each).

Immunohistochemistry: PMN
As described previously (28), paraffin-embedded kidney sections (5 µm) were incubated with a rat anti-mouse monoclonal antibody (clone 7/4; Serotec, Dusseldorf, Germany) against a polymorphic 40-kD antigen expressed by PMN. This was followed by a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) and finally by avidin-biotin-peroxidase (Vector Laboratories).

PMN Depletion Experiments
Twenty-four hours before LPS injection, groups of both WT and CD28−/− received an injection of 20 µl/g body wt rabbit anti-mouse PMN serum as recommended by the manufacturer (Accurate Chemical, Westbury, NY). In preliminary experiments, this sufficiently depleted (<170/µl) circulating PMN for at least 24 h but did not affect other leukocyte subsets. Leukocytes were counted using Kimura’s stain.

To exclude complement depletion as confounding factor, we measured circulating complement protein C3 levels in untreated mice and in mice that had received anti-mouse PMN serum. C3 is the most abundant complement protein and plays a pivotal role in all three complement pathways. Circulating C3 levels were measured using a C3 capture ELISA. After determination of optimal antibody concentration and serum dilution, ELISA were carried out as follows. Capture monoclonal antibody against mouse C3 (clone 11H9, 100 ng; Hycult Biotechnology, Uden, The Netherlands) was coated on 96-well plates (Immuno Maxisorb, Nunc, Germany). After washing and blocking, plates were loaded with samples (in triplicate, 1:100 dilution). Later, primary antibody (polyclonal rabbit anti-mouse C3, 100 ng; Hycult Biotechnology) was added to each well. After incubation and repeated washes, 660 ng of goat anti-rabbit IgG peroxidase conjugate in blocking solution was added to each well. OPD (1 mg/ml; Sigma-Aldrich) and 1% (vol/vol) H2O2 in PBS were added to each well afterwards. After 30 min, H2O2 was pipetted into each well, and adsorption was measured at 490/540 nm. Zymosan-stimulated plasma served as positive control.

Surface CD28 Staining and Flow Cytometry
For evaluating CD28 surface expression, peripheral blood leukocytes were stained with PE-conjugated anti-mouse CD28 (clone 2D2.2 antibody and with either FITC-conjugated anti-mouse CD3 (clone 17A2) or FITC-conjugated anti-mouse Ly-6G (clone RB6–8C5). Appropriate Ig isotypes served as controls (all antibodies from Pharmingen-BD Biosciences, Heidelberg, Germany). After red blood cell lysis with 1.5 M NH4Cl, samples were run on a FACScan flow cytometer (BD Biosciences). Data analysis was performed using CellQuest software (BD Biosciences).

Adaptive Transfer
For further exploring the role of T cells and CD28 in our model, CD28−/− received wild-type CD3+ T cells before LPS injection. Briefly, spleens were harvested from WT under sterile conditions. Splenic cells were collected by homogenization and centrifugation. After red blood cell lysis with 1.5 M NH4Cl, T cell enrichment was performed using a commercially available CD3-negative selection/enrichment column (R&D Systems, Wiesbaden, Germany), thereby minimizing the risk of T cell activation during preparation. Flow cytometry after isolation demonstrated that >83% of all vital cells were CD3+, and that CD28 surface expression remained unchanged. Cells were suspended in HBSS that contained 10% (vol/vol) FCS. Two hours before LPS injection, 1.5 × 107 CD3+/CD28−/− cells each were administered to CD28−/− via tail-vein injection. Sham adop-
tive transfer consisted of injecting HBSS and FCS only. To rule out any enhancement or attenuation of renal injury as a result of T cell preparation, CD3⁻/CD28⁻/⁻ cells were injected into WT.

Chemokine Gene Array
To analyze renal chemokine expression in WT and CD28⁻/⁻, we used a commercially available, nonradioactive chemokine gene array (Superarray/Biomol, Hamburg, Germany). Briefly, mouse kidneys were homogenized, and total RNA was isolated using Trizol reagent (Invitrogen/Life Technologies, Karlsruhe, Germany). After isolation, 2.5 μg of mRNA was used as the template for biotin-labeled cDNA probe synthesis; labeling with biotin-16 to 2 deoxyuridine-5'-triphosphate (Roche, Mannheim, Germany) was performed according to the manufacturer’s instruction. Labeled probes were hybridized to GEArray Q series membranes that contained 67 chemokine and chemokine receptor genes each. After incubation and several washes, membranes were blocked and exposed to chemiluminescent detection (alkaline phosphatase-conjugated streptavidin 1:5000 dilution, CDP-star solution). Chemiluminescence was recorded with an appropriate camera. Digital image analysis of the developed blot membranes (NIH/Scion image) was used to quantify chemokine mRNA in relation to house- hold genes, here glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Plasma Chemokine Concentrations
Using a commercially available ELISA kit (R&D Systems), we measured the plasma concentration of keratinocyte-derived chemokine (KC), a growth-related oncogene 1 (Gro-1) gene product.

Immunohistochemistry: CD3⁺ Cells
Two different monoclonal antibodies were used to detect renal T cell infiltration.

Monoclonal Antibody CD3-12
After antigen retrieval with an acid-based formula (Vector Laboratories; protocol according to the manufacturer’s instructions), paraffin-embedded tissue sections (5 μm) were incubated with this rat IgG1 monoclonal antibody (1:500; Serotec), raised against a cytoplasmic epitope shared by both human and murine CD3(ex 29,30). Next, tissue sections were incubated with biotinylated secondary antibody (1:250; Vector Laboratories) in 10% rabbit serum and finally with avidin-biotin peroxidase (Vector Laboratories).

Monoclonal Antibody 48-2B
Paraffin-embedded tissue sections (5 μm) were incubated with this hamster IgG1 monoclonal antibody (1:200; Santa Cruz Biotechnology, Heidelberg, Germany), raised against CD3ε of mouse origin. After- ward, sections were incubated with biotinylated secondary antibody (1:250; Vector Laboratories) in 10% goat serum and subsequently with avidin-biotin peroxidase (Vector Laboratories).

Paraffin-embedded spleen sections were used as positive controls for both antibodies.

Statistical Analyses
Statistical analysis included one-way ANOVA, Student-Newman-Keuls test, and t test where appropriate. All data are presented as mean ± SEM.

Results
LPS Leads to Intrarenal ARF and Renal PMN Recruitment in WT
After LPS injection, WT demonstrated a strong, significant rise in plasma creatinine and BUN concentrations over 24 h (Figure 1, a and b). A nearly unchanged BUN:creatinine ratio (Figure 1c) indicated the development of severe intrarenal ARF in WT. Compared with control mice (Figure 1d), renal histology from LPS-treated mice (Figure 1, e and f) revealed hyperemia and tubular edema but almost no signs of cell death. Renal MPO was significantly increased 24 h after LPS injection (Figure 2a), indicating an almost three-fold increase in tissue PMN content. PMN-specific immunostaining (Figure 2b) and subsequent counting of PMN gave similar results, thereby validating MPO as a measure of renal PMN infiltration.

CD28 Gene-Deficient Mice Are Protected from LPS-Induced ARF and Concomitant PMN Recruitment: Critical Role for PMN
When injected with LPS, CD28⁻/⁻ developed only minor kidney dysfunction as pointed out by a significant but small
increase in plasma creatinine concentrations 24 h later (Figure 3a). This increase in CD28"" was significantly smaller than that observed in WT. Plasma BUN concentrations 24 h after LPS administration were also significantly lower in CD28"" (55.4 ± 8.8 mg/dl). CD28"" further revealed significantly lower renal MPO (Figure 3b) than WT 24 h after LPS injection, indicating reduced renal PMN recruitment.

LPS injection itself caused only moderate leukopenia in both WT and CD28"" over 24 h; total and differential white blood cell (WBC) counts were approximately 60 to 70% lower than those at baseline (control WT: total WBC 11244 ± 423/μl, PMN 1279 ± 84/μl; control CD28"": total WBC 12157 ± 646/μl, PMN 1421 ± 178/μl).

Figure 2. Systemic LPS administration also leads to strong neutrophil (PMN) recruitment into the kidney. Renal myeloperoxidase (MPO) activities indicated an almost three-fold increase in tissue PMN content 24 h after injection of LPS (a). PMN-specific immunostaining (b; kidney section 24 h after LPS) and subsequent counting of PMN gave similar results and thereby validated the use of renal MPO as an indicator of PMN infiltration in our model.

Figure 3. CD28 gene deficiency protects from severe ARF and PMN recruitment into the kidney after LPS injection—a critical role for PMN. Compared with WT, CD28 gene-deficient mice (CD28"") exhibited significantly smaller plasma creatinine concentrations at 24 h after LPS application, indicating only mild renal dysfunction (a). LPS injections also caused a significant renal PMN recruitment in WT, as shown by an almost three-fold increase in renal MPO activity (b, left). CD28"", by contrast, did not display any significant PMN recruitment into the kidney at the same time (b, right). When sufficiently PMN depleted, both WT and CD28"" demonstrated almost identical plasma creatinine concentrations that were comparable to that observed in untreated CD28"".
CD28 Expression on T Lymphocytes and PMN

We used flow cytometry to assess CD28 surface expression on peripheral blood T lymphocytes (CD3+ cells) and PMN (Ly-6G+ cells). As shown in Figure 4a, the vast majority of CD28 expression in untreated control mice can be found on CD3+ cells. Ly-6G+ cells, by contrast, did not stain positive for CD28, neither under baseline conditions (Figure 4b) nor after LPS stimulation in vivo (Figure 4c). Thus, CD28 seemed to be expressed mainly on mature T lymphocytes but not on PMN. Both CD3 and CD28 expression remained unchanged after stimulation with LPS (data not shown).

Mature T Lymphocytes Modulate Kidney Dysfunction and Renal PMN Recruitment after LPS Administration via CD28-Dependent Mechanisms

We used an adoptive transfer model to clarify further the role of T cells and CD28 in LPS-induced renal PMN recruitment and kidney dysfunction. CD28−/− received either 1.5 × 107 CD3+/CD28+/+ cells ("adoptive transfer") or 10% FCS in HBSS ("sham adoptive transfer") 2 h before LPS administration.

Twenty-four hours after LPS, CD3+/CD28+/+ cells had fully restored a wild-type phenotype in CD28−/− with respect to ARF and renal PMN recruitment. Injection of CD3+/CD28+/+ cells into CD28−/− resulted in an almost twice as high plasma creatinine concentration compared with those that had received only FCS in HBSS (Figure 5a), thereby completely abolishing the protection described above. Adoptive transfer of CD3+/CD28+/+ cells into CD28−/− also reversed the attenuation of MPO activities well below that observed in corresponding control mice (WT: 42.4 ± 8.1 mU/mg protein, CD28−/−: 55.9 ± 9.8 mU/mg protein). Because PMN-depleted mice cannot recruit PMN into the kidney, these results further support the interpretation of renal MPO activity as an indicator of renal PMN content.

In agreement with our previous studies (27), we also found

For exploring the functional relevance of PMN in LPS-induced ARF, both WT and CD28−/− mice received anti-mouse PMN serum 24 h before LPS injection. When compared with untreated control mice, injections of heterologous anti-PMN serum into WT and CD28−/− did not cause significant changes in complement C3 protein levels (<1.1- and 1.2-fold increases, respectively). PMN depletion also had no impact on baseline serum creatinine concentrations (WT 0.27 ± 0.02 mg/dl, CD28−/− 0.55 ± 0.05 mg/dl; n = 6 each). PMN-depleted WT and CD28−/− had similar, statistically not different plasma creatinine concentrations 24 h after LPS application (Figure 3c). These were comparable to that seen in untreated CD28−/−. As the blockade of CD28 did not provide any additional relevant protection, PMN appeared as a key mediator in the development of endotoxemic ARF.

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Figure 5. Adoptive transfer (AT) of CD3+ cells from WT into CD28−/− reconstitutes wild-type phenotype in CD28−/−. Compared with CD28−/− that received only buffer media (sham AT), those that were given wild-type CD3+ cells 2 h before LPS administration demonstrated plasma creatinine concentrations (a) as well as renal MPO activities very similar to that found in WT (b). Thus, injections of CD3+ cells from WT into CD28−/− completely abolished the protection from ARF and renal PMN recruitment seen in untreated CD28−/−.
renal PMN recruitment observed in untreated CD28−/− or in CD28−/− after sham adoptive transfer (Figure 5b). “Control adoptive transfer” of CD3+/CD28+/+ into WT and subsequent LPS injection led to a level of ARF (serum creatinine concentration 1.14 ± 0.06 mg/dl) equally severe to that seen in non-pretreated WT. Thus, enhancement or attenuation of renal injury as a result of T cell isolation and subsequent adoptive transfer seemed unlikely.

### Attenuated Renal Chemokine Expression in CD28 Gene-Deficient Mice after LPS Injection

To compare renal chemokine mRNA expression in WT and CD28−/−, we used chemokine/chemokine receptor gene arrays on total renal RNA extracts from WT and CD28−/− before and 2 and 12 h after LPS administration (Figure 6). Renal chemokine mRNA expression seemed to be similar between WT and CD28−/− in untreated control mice. In both WT and CD28−/−, renal Gro-1 mRNA expression at baseline represented only a small fraction of that observed for GAPDH, i.e., 7 and 6% of GAPDH, respectively. LPS injection led to a massive upregulation of Gro-1, IP-10, and monokine induced by IFN-γ (Mig) gene mRNA expression in WT kidneys (Figure 6, top). At 2 h after LPS injection, Gro-1 mRNA expression was 2.3-fold higher than GAPDH mRNA expression; after 12 h, Gro-1 mRNA expression was still 1.3-fold higher than GAPDH mRNA expression. CD28−/−, however, showed only a weak upregulation of these three genes within the kidney (Figure 6, bottom). Gro-1 mRNA expression resembled only 69 and 33% of that seen for GAPDH mRNA expression at 2 and 12 h after LPS injection, respectively.

### CD28 Modulates Systemic Chemokine Response after Endotoxin Injection

LPS administration induced a massive release of the PMN-specific chemokine KC, peaking 4 h after injection in WT (Figure 7a). Whereas baseline plasma KC concentrations were almost identical between CD28−/− and WT, CD28−/− exhibited an enormously attenuated increase in plasma KC at 4 h after LPS (Figure 7b).

### LPS Administration Does not Lead to Lymphocyte Recruitment into the Kidney

Immunostaining for CD3+ cells (Figure 8A) with two different antibodies (CD3-12 and 48-2B) failed to show renal T lym-

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**Figure 6.** Gene array to compare renal chemokine and chemokine receptor gene expression between WT and CD28−/−. Before, 2 h, and 12 h after LPS injection, renal mRNA was extracted from WT and CD28−/−. After labeling, probes were hybridized to membranes that contained 67 chemokine/chemokine receptor genes each and exposed to chemiluminescent detection. Whereas baseline renal chemokine expression seemed identical between WT (top) and CD28−/− (bottom), CD28−/− displayed an attenuated upregulation of growth-related oncogene-1 (Gro-1) and monokine induced by IFN-γ (Mig) after LPS administration. (NC, pUC18 DNA as negative control; PC1, β-actin as positive control 1; PC2, glyceraldehyde-3-phosphate dehydrogenase as positive control 1; PC3, cyclophilin as positive control 3; PC4, ribosomal protein L13a as positive control 4).
Phagocyte recruitment after LPS inoculation (Figure 8, b through d, CD3-12). Only very few, randomly appearing CD3\(^+\) cells could be detected in control WT (Figure 8b) or in WT that received an injection of LPS (Figure 8, c and d). It therefore seems unlikely that T cell–mediated PMN recruitment is a local, cell–cell phenomenon.

**Discussion**

In several studies, CD28 blockade has led to significant and clinically relevant modulation of inflammatory diseases (17–24). Besides reduced T cell infiltration, some of these studies have reported a diminished tissue PMN\(^-\) recruitment (19,20,23,24).

PMN and their infiltration into the kidney have been proposed to cause renal dysfunction during sepsis (31–33). Our data provide additional experimental evidence for this concept of PMN-dependent organ failure during endotoxemia.

In our model of LPS-induced ARF, CD28\(^-\)/CD3\(^+\) exhibited significantly milder kidney dysfunction and lower renal PMN recruitment than corresponding WT. CD28 expression under these circumstances was largely restricted to mature T cells. Reconstitution of CD28\(^-\)/CD3\(^+\) cells from WT before LPS challenge clearly substantiated a critical role for T cells and their CD28 pathway in ARF and concomitant renal PMN recruitment.

Our study also provides further evidence for a nonantigenic but inflammation-modulating role of T cells. However, the impact of T cells on PMN observed here is in striking contrast to two previous studies, in which T cells modulated kidney function after local (34) or whole-body ischemia-reperfusion (35) but did not control renal PMN recruitment, yet the role of T cells in the development of postschematic ARF was also dependent on CD28 (34). With respect to PMN recruitment, one therefore may speculate that the functional consequences of CD28-mediated T cell activation during endotoxemia are different from those during local or global ischemia-reperfusion.

During endotoxemia, T cells seemed to exert their effects systemically rather than locally, as immunostaining could not detect significant renal T cell recruitment. Direct cellular interactions between PMN and T lymphocytes within the kidney therefore did not seem to take place. This concept is supported by the fact that CD28 greatly modulated systemic concentrations and renal mRNA expression of KC, a PMN-specific (CXC-) chemokine. KC is very potent activator of PMN, leading to degranulation, respiratory burst, and adhesion on endothelial cells (36,37). T cells are not known to produce PMN-specific chemokines. They can, however, profoundly alter the homeostasis of various pro- and anti-inflammatory cytokines (7,8), eventually affecting chemokine production in tissues or circulating leukocytes.
As LPS itself has only a very little direct effect on the kidney (38), cytokines such as TNF-α or IL-1β have been implicated in LPS-mediated leukocyte recruitment during sepsis. LPS is the most effective inducer of TNF-α by monocytes and can also lead to TNF-α secretion by lymphocytes (22,39). In a previous study, mice could be protected from lethal septic shock by an antibody that interferes with CD28 signaling (22). This blockade led to a significant decrease in serum TNF-α levels, which in turn was attributed to the induction of IL-10 expression (22). IL-10 is a product of various cells, including T cells and monocytes, and is known as a potent anti-inflammatory mediator (39). It can inhibit the secretion of different proinflammatory cytokines and the expression of PMN-specific chemokines, such as KC and MIP-2, both representing murine Gro-1 gene products (39–41). In addition to increased chemokine serum concentrations, IL-10 gene-deficient mice displayed markedly diminished PMN recruitment into inflamed tissues (40,41).

Because CD28 can control, at least partially, the TNF-α–IL-10 homeostasis, which in turn can influence chemokine expression, our data provide plausible explanations for the observed reduction in PMN-dependent damage and in renal PMN recruitment. One can hypothesize that the reduced Gro-1 mRNA upregulation as well as the attenuated increase in plasma KC seen in CD28−/− after LPS injections might have been caused by an altered TNF-α–IL-10 homeostasis. Because murine Gro-1 is encoding for PMN-specific chemokines, namely KC and MIP-2, decreased renal expression of these chemokines could have been the cause for a diminished PMN recruitment into the kidney. The decrease in Gro-1 expression can be explained by CD28-mediated changes in cytokine homeostasis. Both IP-10 and Mig are secreted by PMN (42–44). Besides attenuated chemokine expression by residual renal cell populations, the decrease in IP-10 and Mig mRNA could be due to diminished renal PMN recruitment. The consequences of reduced plasma KC levels remain more speculative. On the basis of current knowledge, it seems unlikely that circulating KC can get deposited in the renal microcirculation and can subsequently lead to PMN recruitment. Chemokines are known to cause PMN activation (2), resulting in release of cytopathic substances and stiffness of PMN. As a consequence of reduced KC concentrations, PMN might secrete less cytopathic substances and might be more deformable within the renal microcirculation, resulting in less “microcircular trapping.”

In summary, we demonstrated that T cells, via their CD28 pathway, are potent regulators of kidney function and renal PMN recruitment during endotoxemia. As PMN resemble key cellular mediators in our model of LPS-induced ARF, T cells therefore emerge as crucial modulators of LPS-induced ARF. Because T lymphocytes did not seem to infiltrate the kidney but rather to control systemic PMN-specific chemokine homeostasis, their effect on PMN seems to be a remote one, originating outside the kidney.

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