The role of ICAM-1 in endotoxin-induced acute renal failure

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Wu X, Guo R, Wang Y, Cunningham PN. The role of ICAM-1 in endotoxin-induced acute renal failure. Am J Physiol Renal Physiol 293: F1262–F1271, 2007. First published August 1, 2007; doi:10.1152/ajprenal.00445.2006.—The pathogenesis of acute renal failure (ARF) occurring during the course of sepsis is incompletely understood. Intercellular adhesion molecule–1 (ICAM-1) is a key cell adhesion molecule upregulated by LPS, which binds to the integrins CD11a/CD18 and CD11b/CD18 present on the surface of leukocytes. We hypothesized that ICAM-1 facilitates renal injury in LPS-induced ARF. To test this, three groups of mice (n = 8 per group) were injected intraperitoneally with 6 mg/kg LPS: 1) normal C57BL/6 mice, 2) mice with a targeted deficiency of ICAM-1 (ICAM-1−/−), and 3) mice expressing very low levels of CD18 (CD18-def). ICAM-1−/− mice were significantly less responsive to LPS-mediated ARF, as opposed to CD18-def mice, which developed severe ARF, as did wild-type controls (48 h blood urea nitrogen 143 ± 31.5, 70.8 ± 24.4, and 185 ± 16.6 mg/dl in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P < 0.05). At death, ICAM-1−/− mice had significantly less renal neutrophil infiltration than the other two groups, as well as less histological tubular injury. Depletion of neutrophils with mAb Gr-1 led to a profound exaggeration of tumor necrosis factor (TNF) release and high mortality, but neutrophil-depleted mice receiving 10-fold less LPS were protected against ARF despite TNF release similar to what is normally associated with LPS-induced ARF. LPS caused a significant increase in renal expression of chemokines; however, this increase was significantly exaggerated in CD18-def mice, which may account for their lack of protection. In conclusion, these data show that ICAM-1 plays a key role in LPS-induced ARF.

ACUTE RENAL FAILURE (ARF) is a common result of sepsis and septic shock and is responsible for significant morbidity and mortality (52). Despite its prevalence, therapeutic options for treating this condition are limited to supportive interventions such as dialysis. One animal model that has offered insight into the pathogenesis of septic ARF is that of LPS-induced ARF (53). While many bacterial products may contribute to renal injury, LPS, a component of the outer cell membrane of gram-negative bacteria, reproduces most of the clinical sequelae of sepsis when injected into animals, including ARF. LPS-induced ARF is associated with mild and patchy areas of tubular injury similar to what is seen in human ARF (8, 56).

LPS administration is well-known to trigger an influx of neutrophils into various organs, such as lung and liver (1, 20). This process is directed by the systemic release of chemotactic chemokines such as macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) and the local expression of adhesion molecules such as E- and P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) on endothelium within sites of inflammation (1, 20, 29, 34). In previous work, we confirmed that administration of LPS causes an influx of neutrophils into the kidney in parallel with a decline in renal function. Interventions that protected against LPS-induced ARF, such as interruption of TNFRI signaling or use of caspase inhibitors, also led to a decrease in neutrophil infiltration in parallel with a decrease in renal expression of MIP-2 and ICAM-1 (7, 9, 15). These findings suggest that this influx of neutrophils may contribute to the renal functional and structural injury that is seen in response to LPS.

In particular, the adhesion molecule ICAM-1 has been found to be important for the firm adhesion of neutrophils and other leukocytes to the endothelium, allowing their attachment before their potential traffic between adjacent endothelial cells to reach underlying structures. ICAM-1 is expressed mainly on endothelial cell surfaces, interacting with two principal ligands, β2 integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), present on circulating leukocytes (12, 24). ICAM-1 has been shown by others to be acutely upregulated in kidney after LPS administration, primarily on the renal endothelium, but also in renal tubules (28). Mice lacking expression of ICAM-1 have reduced mortality in response to septic shock (59, 64). Furthermore, mice deficient in ICAM-1 experienced less renal leukocyte infiltration and reduced functional and structural injury in response to ischemia-reperfusion injury (IRI) (23), with similar protection observed with the use of ICAM-1 or CD11/CD18 neutralizing Abs (22). Taken together, this background suggests that ICAM-1-mediated leukocytic infiltration may have a pathogenic role in LPS-induced ARF. Therefore, the following study was performed to address this hypothesis.

METHODS

ARF model. Three strains of male mice were studied: mice lacking expression of ICAM-1 (ICAM-1−/−), mice deficient in CD18 (CD18-def), and wild-type (C57BL/6J) mice. CD18-def mice are a hypomorphic strain that express CD11a/CD18 and CD11b/CD18 at a level that is ∼2% of normal, increasing to 16% of normal after activation of neutrophils with PMA (62). ICAM-1−/− and CD18-def mice had both been backcrossed for >10 generations on a C57BL/6J background.

All mice were obtained commercially and studied at 9 wk of age (Jackson Laboratories, Bar Harbor, ME), n = 8 per group. Mice received a single intraperitoneal injection of 6 mg/kg Escherichia coli LPS (Sigma, St. Louis, MO) at time 0. Blood was obtained for blood urea nitrogen (BUN) and cytokine measurements at time of LPS injection, and 2, 24, and 48 h later under continuous isoflurane/oxygen anesthesia. Mice were killed 48 h after LPS injection, with harvest of blood and kidney tissue. As controls, ICAM-1−/−, CD18-def, and wild-type mice were sham-injected with 150 mM saline and their blood and tissue were harvested as above (n = 5 per group). For time...
course experiments, wild-type mice were injected with LPS, and kidney was harvested 0, 3, 6, and 12 h later for analysis of mRNA and immunohistochemistry \((n = 6\) per group). BUN concentrations were determined with a Beckman CX5CE autoanalyzer. All experiments were done in accord with a written protocol approved by an independent review process conducted by the University of Chicago Institutional Animal Care and Use Committee.

**Depletion of neutrophils and blockade of ICAM-1 and VCAM-1.** For neutrophil depletion experiments, rat mAb Gr-1 was purchased (BD Pharmingen, San Diego, CA). Either Gr-1 or isotype control rat IgG2b was injected at a dose of 0.2 mg/mouse ip into wild-type mice, \(n = 10\) per group. Blood was obtained at time of Gr-1 injection and 24 h later for circulating neutrophil counts, as determined by Coulter counter, to confirm depletion. LPS was injected 24 h after Gr-1, and blood was obtained at that time and 2, 6, and 24 h later, with death of mice and harvest of tissue at 24 h after LPS. In an attempt to correct for the exaggeration of TNF release seen with neutrophil depletion, a third group of Gr-1-injected mice received low-dose LPS, 0.6 mg/kg, \(n = 8\), with a fourth comparison group receiving IgG followed by 0.6 mg/kg LPS.

In separate experiments to test the role of ICAM-1 and VCAM-1, rat mAbs YN1/1.7.4 \((18, 19)\) or M/K-2.7 \((19, 38)\) were injected into wild-type and CD18-def mice. Polyclonal rat IgG (Invitrogen, Carlsbad, CA) was injected into wild-type mice as a control group. Abs were injected 2 h before LPS, at a dose of 10 mg/kg ip, \(n = 6\) per group. Blood was obtained at time of LPS injection and 2, 6, and 24 h later, with death of mice and harvest of tissue at 24 h after LPS. YN1/1.7.4 or M/K-2.7 hybridomas were obtained from American Type Culture Collection (Manassas, VA) and grown in DMEM/10% FBS with 0.05 mM \(\beta\)-mercaptoethanol. Supernatant reactivity against vascular structures in frozen sections from LPS-injected mouse kidney was confirmed by immunohistochemistry, and IgG was purified using protein G affinity chromatography and concentrated to \(\approx 8\) mg/ml by Centricprep (Millipore, Billerica, MA). All injected Abs were either purchased in 5 mg/ml no azide/low endotoxin form or dialyzed into sterile 150 mM NaCl in endotoxin-free glassware.

**Pathology.** For routine histological analysis, kidneys were sectioned coronally, fixed in phosphate-buffered formalin, embedded in paraffin, and stained with periodic acid-Schiff base (PAS). Histological sections for each animal were assigned a semiquantitative score for tubular injury by a blinded observer, as previously described \((7)\). A blinded observer assigned a score ranging from 0 (no injury) to 3 (widespread injury) for each of 3 variables: tubular dilatation/flattening, tubular casts, and tubular degeneration/vacuolization. For each animal, 10 cortical high-power fields (HPF) were examined at random. For each variable within each field, a score of 0 was assigned when less than 5% of the tubules were affected, a score of 1 when 5–33% were affected, a score of 2 when 34–66% were affected, and a score of 3 when over 66% were affected. For immunohistochemistry, tissue was immediately frozen in OCT compound at \(-20^\circ\)C. Four-micrometer kidney cryostat sections were fixed with ether/ethanol, incubated with 0.3% \(\text{H}_2\text{O}_2\) for 30 min, and blocked with dilute horse serum. Sections were stained for neutrophils by sequential incubation with rat anti-mouse neutrophil (mAb 7/4, Serotec, Raleigh, NC) at 1:60 for 30 min followed by horseradish peroxidase (HRP)-conjugated rabbit anti-rat IgG (Sigma) at 1:60 for 30 min and diaminobenzidine reagent (Vector Laboratories, Burlingame, CA) for 10 min. A blinded observer counted the number of neutrophils per HPF and recorded the average of 10 fields for each sample. In an analogous fashion, frozen sections were stained with rat-anti-mouse VCAM-1 at 1:20, or hamster-anti-mouse ICAM-1 at 1:60, followed by HRP-anti-rat IgG or biotinylated rabbit-anti-hamster IgM at 1:100 and streptavidin-HRP (Abs purchased from BD Pharmingen). Methyl green counterstain was used as a final step with the ICAM-1 staining. Sections were scored as to the extent and intensity of VCAM-1 staining on a semiquantitative score ranging from 0 (minimal intensity) to 4 (maximum).

**Cytokine measurement.** TNF levels were determined from sera obtained at 0 and 2 h after LPS administration using commercially available ELISA kits for mouse TNF (R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions, on serum diluted 1:10.

**Real-time PCR.** A portion of frozen kidney was placed in TRIzol reagent (GIBCO BRL, Grand Island, NY), from which total RNA was purified according to the manufacturer’s instructions. To remove all traces of genomic DNA, samples were then treated with RNase-free RQ1 DNase (Promega, Madison, WI; 1 U per 4 \(\mu\)g RNA) in 10-\(\mu\)l reaction buffer (final concentration 40 mM Tris⋅HCl, 10 mM \(\text{MgSO}_4\), 1 mM \(\text{CaCl}_2\), pH 8.0) at 37°C for 30 min. This was followed by addition of 1 \(\mu\)l of 20 mM EGTA, pH 8.0, to stop the reaction, and incubation at 65°C for 10 min to inactivate the DNase. cDNA was generated from RNA using random hexamers as primers with the SuperScript first-strand synthesis kit (GIBCO BRL), according to the manufacturer's instructions, and diluted fivefold before analysis.

Real-time PCR was performed using the Prism 7700 reagent and the SybrGreen intercalating dye method with HotStar DNA polymerase (Applied Biosystems, Foster City, CA). Each reaction was conducted in a total volume of 50 \(\mu\)l with primers at 200 nM, 1 mM dNTPs, 3 mM \(\text{MgCl}_2\), and 10 \(\mu\)l of sample or standard cDNA. PCR was carried out with a hot start at 95°C (5 min) followed by 45 cycles at 95°C (15 s)/60°C (30 s). For each sample, the number of cycles required to generate a given threshold signal \((C_t)\) was recorded. Using a standard curve generated from serial dilutions of kidney cDNA, the ratio of ICAM-1 expression relative to GAPDH expression was calculated for each experimental animal and normalized relative to an average of ratios from the control (no LPS) group. Measurements of KC, MIP-2, and VCAM-1 mRNA expression were performed in an analogous fashion. Products of each reaction yielded a single band when run on agarose gel, confirming specific amplification. Primers were synthesized by Integrated DNA Technologies (Coralville, IA), with sequences as follows: GAPDH forward primer 5'-GGC AAA TTC AAC GGC ACA GT-3' and reverse primer 5'-CCC GAG AGT GGC TAT GA-3', MIP-2 forward primer 5'-CAC CAA CCA CCA GGC TAC A-3' and reverse primer 5'-GCC CTT GAG AGT GGC TAT GA-3', KC forward primer 5'-ACC GCC TGG CTT CTC TGT-3' and reverse primer 5'-TGG CTA TGA CTT CGG TTT GG-3', VCAM-1 forward primer 5'-CCC GAA CTC CTT GCA CTC TA-3' and reverse primer 5'-TGG GCC TCC ACC AGA CTG TA-3'.

**Statistics.** Data were analyzed with Minitab software (State College, PA). Unless noted otherwise, data are given as means ± SE. Groups were compared by two-tailed \(t\)-test, or ANOVA when more than two groups were compared. Tukey’s correction for multiple comparisons was used when comparing more than two groups. Because expression data obtained by real-time PCR were not normally distributed for any group, ANOVA was performed on log-transformed data. A \(P\) value ≤0.05 was considered significant.

**RESULTS**

**Induction of ICAM-1 within kidney.** To gain insight into changes in renal ICAM-1 expression soon after LPS is injected, when ARF is first established, wild-type mice were killed at various time points after LPS injection, and their kidneys were analyzed for ICAM-1 expression and neutrophil infiltration. At baseline, ICAM-1 expression is low, limited to weak staining in small arteries (Fig. 1, A–B). Likewise, few neutrophils are seen in kidney at baseline. However, within 3 h after LPS injection (6 mg/kg), both ICAM-1 mRNA expression, as quantitated by real-time PCR, and renal neutrophil infiltration were significantly increased. This parallel increase in renal ICAM-1 expression and neutrophil infiltration continued at 6 h and persisted at 48 h. Immunohistochemistry
confirmed that at 48 h after LPS administration renal ICAM-1 was strongly increased in peritubular capillaries, arterioles, and to a lesser extent within renal tubules (Fig. 1C).

**Effects of neutrophil depletion in LPS-induced ARF.** To study the role of renal neutrophilic infiltration in LPS-induced ARF, mice were injected with mAb Gr-1 to deplete circulating neutrophils. Twenty-four hours after Gr-1 injection, circulating neutrophil counts fell markedly, confirming depletion (84 ± 25 vs. 570 ± 170 PMN/μl in Gr-1 vs. control IgG group, *P* < 0.05). At this time point, LPS was injected at a dose of 6 mg/kg. Despite near absence of circulating neutrophils, Gr-1-treated mice experienced markedly increased mortality within 24 h (70 vs. 0%) and no protection against the ensuing ARF, with significantly higher BUN at 6 h after LPS (48.0 ± 7.9 vs. 39.5 ± 1.3 mg/dl, *P* < 0.05; Fig. 2A). As expected, Gr-1-injected mice had significantly fewer neutrophils present in kidney (1.4 ± 0.8 vs. 11.4 ± 1.3 PMN/HPF, *P* < 0.01; Fig. 2B). However, neutrophil-depleted mice had a profoundly exaggerated increase in serum TNF 2 h after LPS (16.2 ± 2.2 vs. 2.7 ± 0.6 ng/ml, *P* < 0.01; Fig. 2C) compared with control mice injected with IgG. Given the central role of TNF in LPS-induced ARF that has been previously demonstrated (7), and the fact that LPS exerts most of its renal effects indirectly, through TNF (9), we attempted to reduce this confounding effect of neutrophil depletion on TNF release by injecting neutrophil-depleted mice with a 10-fold lower dose of LPS, 0.6 mg/kg. Although Gr-1 still exaggerated LPS-induced TNF release compared with IgG controls (Fig. 2C), these neutrophil-depleted, low-dose LPS mice had 2-h TNF levels similar to non-neutrophil-depleted, high-dose LPS mice (2.2 ± 0.2 ng/ml; Fig. 2C). Despite this similar, relatively high level of TNF, these mice underwent a markedly lesser elevation in BUN at 24 h (32.6 ± 0.5 mg/dl; Fig. 2A), with minimal renal neutrophil infiltration and lesser histological injury. This minimal level of ARF and renal neutrophil infiltration in the Gr-1 low-dose LPS group was similar to what was seen in IgG low-dose LPS (data not shown), despite a significantly greater elevation in TNF (2.2 ± 0.2 vs. 0.48 ± 0.9 ng/ml, *P* < 0.01; Fig. 2C).

**Role of ICAM-1 in renal injury.** To study the pathogenic role of ICAM-1 in LPS-induced ARF, ICAM-1−/− and wild-type mice were studied. Because the principal ligands for ICAM-1 are CD11a/CD18 and CD11b/CD18, mice deficient in CD18 (CD18-def) were also studied in parallel. These mice are not absolutely deficient in CD18, but their granulocytes express CD18 at levels that are only 2% of normal, increasing to 16% of normal after stimulation of neutrophils with PMA (62).
significantly higher blood urea nitrogen (BUN) 6 h after LPS injection; were not protected against LPS-induced acute renal failure (ARF), with this rise was severe in wild-type mice, the level of BUN in the CD18-def group were also significantly protected against this pathologic injury (Fig. 4), as quantitated by cortical tubular injury scores (2.8 ± 0.6, 1.4 ± 0.4, and 3.2 ± 0.4 in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P < 0.05 vs. CD18-def mice and P = 0.13 vs. wild-type mice). Thus ICAM-1 deficiency conferred protection against both the functional and structural injury caused by LPS.

Neutrophilic infiltration. We hypothesized that mice deficient in ICAM-1 would have impaired leukocyte trafficking within the kidney following LPS. At baseline, neutrophils were sparse in the kidney and equivalent between wild-type, ICAM-1−/−, and CD18-def mice. As previously described, LPS administration induced a robust infiltration of the kidney with neutrophils (Fig. 5, A-B). In contrast to the concentration of neutrophils observed at the corticomedullary border seen in renal ischemia-reperfusion (22), neutrophils were distributed in a patchy manner throughout the renal cortex, and to a lesser extent within the renal medulla. In contrast to both wild-type and CD18-def mice, ICAM-1−/− mice had significantly fewer neutrophils within the kidney 48 h after LPS infiltration (10.2 ± 1.0, 4.6 ± 0.8, and 9.9 ± 1.0 PMN/HPF in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P < 0.05 for ICAM-1−/− vs. other groups; Fig. 5, C-D). Thus deficiency of ICAM-1, but not CD18, protected against LPS-induced neutrophilic infiltration.

Systemic and renal inflammation. We previously demonstrated the central role of TNF acting through its receptor TNFR1 in LPS-induced ARF (7). Therefore, we wanted to confirm that any protective role associated with ICAM-1 deficiency was not mediated through a difference in TNF activity. As expected, LPS administration caused a profound increase in circulating TNF levels, rising from undetectable at baseline in all three strains to a peak at 2 h. However, this was not significantly different among the different groups (2.70 ± 0.49, and 1.62 ± 0.05 in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P = NS; Fig. 6) and thus elevation in ICAM-1−/− mice was significantly less (143 ± 31.5 vs. 70.8 ± 24.4 mg/dl at 48 h, in wild-type vs. ICAM-1−/− mice, P < 0.05), demonstrating relative protection against LPS-induced ARF (Fig. 3). The levels of BUN in the CD18-def group were actually greater than that seen in wild-type mice, although not to a statistically significant degree (185 ± 16.6 mg/dl at 48 h). In addition to this decline in renal function, LPS induced modest renal tubular injury, most prominent in the renal cortex, as has been previously described (8).

Reduced expression of CD18 leads to a parallel decrease in CD11a and CD11b expression due to degradation, and these mice are phenotypically associated with deficiencies in neutrophil chemotaxis in response to intraperitoneal injection of thioglycollate (62). After injection with LPS (6 mg/kg), all mice experienced an acute rise in BUN indicative of ARF. While this rise was severe in wild-type mice, the level of BUN
cannot explain to the resistance to LPS-induced ARF seen in the ICAM-1−/− group.

Given that CD11a/CD18 and CD11b/CD18 are the only known ligands through which leukocytes bind to ICAM-1, it was notable that CD18-def mice were not protected against the neutrophil infiltration or ARF induced by LPS. Other investigators working with adhesion molecules have also noted inconsistencies with results obtained with knockout strategies and have implicated compensatory increases in other pathways to account for these observations (13, 48, 61). To determine whether a similar compensatory increase was present in the CD18-def group, we assayed kidney tissue for expression of neutrophil-attractant chemokines and VCAM-1 by real-time PCR. As shown in Table 1, LPS caused a large increase in mRNA expression of neutrophil-attractant chemokines MIP-2 (CXCL2) and KC (CXCL1) 48 h after LPS administration in wild-type kidney. Although LPS caused a similar increase in chemokine expression in ICAM-1−/− mice, this increase in MIP-2 and KC was significantly exaggerated in the CD18-def group (113 ± 51-, 106 ± 68-, and 330 ± 40-fold increase of KC in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P < 0.05; Table 1). Similarly, LPS strongly increased mRNA levels of VCAM-1 in wild-type and ICAM-1−/− kidney, but this increase was significantly greater in CD18-def mice (10.3 ± 6.0-, 8.82 ± 4.65-, and 38.8 ± 12.0-fold increase in wild-type, ICAM-1−/−, and CD18-def mice, respectively, P = 0.09). In support of this, LPS administration increased VCAM-1 protein expression within kidney in all groups, but to a significantly greater extent in CD18-def mice compared with the others, as assessed by semiquantitative immunohistochemistry scoring (data not shown).

Blockade of adhesion molecules. Given the intact neutrophil trafficking observed in CD18-deficient mice, the question arose as to whether residual leukocyte CD18 expression was sufficient to allow adhesion to ICAM-1. Similarly, given its up-regulation in CD18-def mouse kidney, the possibility of VCAM-1-mediated neutrophil infiltration was addressed (14, 51). In wild-type mice, anti-ICAM-1 neutralizing Ab YN1/1.7.4 successfully blocked LPS-induced ARF in parallel with a reduction in infiltrating neutrophils (Fig. 7, A-B). In contrast, anti-ICAM-1 blockade offered no protection against LPS-induced ARF or LPS-induced neutrophilic infiltration in CD18-def mouse. Pretreatment with anti-VCAM-1 mAb M/K 2.7 did not protect against LPS-induced ARF or LPS-induced neutrophilic infiltration in either wild-type or CD18-def mice, if anything showing a trend toward higher BUN and greater neutrophil infiltration, although this did not reach statistical significance (Fig. 7, C-D). Thus neither residual CD18 expression nor VCAM-1 upregulation can explain preserved neutrophil accumulation in CD18-def mice.

DISCUSSION

The ability to direct leukocytes, key effectors of the innate and adaptive immune response, to the site of inflammation is governed by a complex network of various chemotactic factors and adhesion molecules (12, 24). Initially, leukocyte rolling on endothelium is initiated by interaction between members of the
selectin family and their carbohydrate ligands. Subsequently, a transition from rolling to firm adhesion is governed by the interaction between $\beta_2$-integrins LFA-1 (CD11a/CD18) and/or Mac-1 (CD11b/CD18) on the leukocyte surface and ICAM-1 expressed on the surface of activated endothelial cells. ICAM-1 has also been shown to be expressed on other cell types during inflammation, such as renal tubular cells, which we confirmed in this study (28). Interference with this receptor-ligand interaction through a variety of approaches has been shown both to impair host response to various infections (48), yet to protect against many injurious effects of the immune system. Specifically, ICAM-1 has been found to play an important role in disease models of transplant rejection (36), radiation-induced pulmonary fibrosis (16), type I hypersensitivity (54), and atherosclerosis (3), among many disease models. It has been previously reported that ICAM-1$^-/-$ mice are resistant to LPS-induced mortality (64); although the mechanism of this protection is not known in great detail, the literature has noted a decrease in LPS-induced neutrophilic infiltration and organ damage within lung and liver in ICAM-1$^-/-$ mice (1, 20, 30), in lung and eye with use of anti-ICAM-1 neutralizing Abs (2, 30), and in lung and liver with use of an anti-CD11b neutralizing Ab (43). Similarly, protection against LPS-induced lung injury was noted in neutrophil-depleted mice (1). However, until the present study, the role of ICAM-1-mediated neutrophil adhesion in LPS-induced ARF has not been previously examined.

In comparison, much more is known about the role of leukocyte adhesion in IRI. Interruption of ICAM-1-mediated neutrophil infiltration has been shown to be protective in

![Fig. 5. PMN infiltration. A: at baseline, few neutrophils were present within the kidney in all groups, as seen on frozen kidney sections. B: LPS administration caused significant neutrophilic infiltration in WT mice at 48 h. C: while similar in CD18-def mice, neutrophilic infiltration was significantly lower in ICAM-1$^-/-$ mice. D: average neutrophils per high-power field (HPF). *P < 0.05 vs. WT and CD18-def groups. Magnification: ×400.](http://ajprenal.physiology.org/)

![Fig. 6. Serum TNF levels following LPS administration. At baseline, circulating TNF values were undetectable by sensitive ELISA in all 3 groups of mice. LPS injection caused a dramatic release of TNF into the circulation in all mice, peaking at ~2 h. However, there were no statistically significant differences in the extent of TNF release between WT, ICAM-1$^-/-$, or CD18-def mice; n = 8 per group.](http://ajprenal.physiology.org/)

**Table 1. Fold increase in RNA expression of selected chemokines and adhesion molecules as compared with sham-injected wild-type mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>MIP-2</th>
<th>KC</th>
<th>VCAM-1</th>
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<tbody>
<tr>
<td>Sham</td>
<td>1.00±0.17*</td>
<td>1.00±0.54*</td>
<td>1.00±0.41*</td>
</tr>
<tr>
<td>Wild-type</td>
<td>358±210</td>
<td>113±51</td>
<td>10.3±6.0</td>
</tr>
<tr>
<td>ICAM-1$^-/-$</td>
<td>269±170</td>
<td>106±68</td>
<td>8.82±4.65</td>
</tr>
<tr>
<td>CD18-def</td>
<td>976±252†</td>
<td>330±40*</td>
<td>38.8±12.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MIP-2, macrophage inflammatory protein-2; KC, keratinocyte-derived chemokine; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1. *P < 0.05 vs. all other groups. †P < 0.09 vs. wild-type group and <0.05 vs. other groups.
ischemic injury to heart, skeletal muscle, and brain (45, 46, 57). More relevant to the kidney, classic studies by Kelly and colleagues (22, 23) showed that both ICAM-1 mice or wild-type mice given anti-ICAM-1 neutralizing Ab were protected against renal IRI injury, in association with reduced neutrophil influx. However, a variety of other studies using neutrophil depletion and other strategies have yielded mixed results and collectively imply that neutrophils have a relatively lesser role in this model (47, 49, 58). More recently, the work of Rabb and colleagues (5) showed in several studies that the CD4 T cell is more likely to play a central role in renal ischemic injury. However, significant differences exist between the models of renal IRI and LPS-induced ARF; for example, the degree of histological damage is much greater in renal IRI, whereas renal hemodynamic changes and cytokine release may be more extensive with LPS-induced ARF (37).

While the data described above clearly support a pathogenic role of ICAM-1 in LPS-induced ARF, these data also show that the role of the neutrophil is not straightforward. Direct depletion of neutrophils with mAb Gr-1 led to a massive exaggeration in systemic TNF release after LPS and 70% mortality. This has been reported previously and has been ascribed to the paracrine action of an uncharacterized low molecular weight product of neutrophils which inhibits LPS-induced TNF release by macrophages (10, 11). Similarly, a genetic model of neutropenia has shown exaggerated TNF release in response to LPS (21). This inhibitory effect toward TNF release does not seem to involve integrin-ICAM-1 interaction, since TNF release and mortality were not exaggerated in ICAM-1−/− or CD18-def mice, nor in mice receiving anti-ICAM-1 Ab. Neutrophil-depleted mice were not protected against LPS-induced ARF, and in fact had significantly higher BUN levels 6 h after LPS. These data are consistent with a primary role of TNF that has been previously reported (7, 27), which is in fact greater than the direct role of LPS itself (9), and clearly indicates that TNF can cause renal injury through neutrophil-independent pathways. Possible ways that this may occur include hypotension from massive TNF-mediated induction of inducible nitric oxide synthase or increased TNFR1-mediated apoptosis. Although this confounding effect of neutrophil depletion makes a perfectly controlled experiment impossible, low-dose LPS given to neutrophil-depleted mice led to similar levels of TNF release (∼2.5 ng/ml) as seen with high-dose LPS in non-neutrophil-depleted mice and caused no mortality. At this “appropriate” level of TNF release following LPS, these mice were protected not only against renal neutrophil infiltration but also experienced minimal ARF. Taken together, these data suggest that while they serve to restrain systemic TNF release in response to LPS, neutrophils themselves may also later play an injurious role in LPS-induced ARF, given an equivalent degree of TNF release. A similar dual role of neutrophils was documented in the cecal ligation and puncture model of sepsis, where neutrophils are protective and reduce bacteremia early in the time course but later contribute to organ dysfunction and mortality (17).

A pathogenic role for neutrophil infiltration is also consistent with previous work in which interruption of TNFR1 or TLR4 signaling or inhibition of caspases protected against LPS-induced ARF in parallel with a reduction in renal neutrophil infiltration (7, 9, 15). More directly, hck mice, a model of impaired neutrophil activation, are protected against LPS-induced renal dysfunction (32). It should be noted that ICAM-1−/− mice were only partially protected against LPS-induced ARF, so it is likely that other mechanisms, such as
hemodynamic changes or damage from reactive oxygen species, remain relevant in this model. Additionally, ICAM-1-/- mice and mice receiving anti-ICAM-1 mAb still had some infiltration of the kidney with neutrophils after LPS injection, although to a significantly lesser extent than wild-type mice; thus other adhesion molecules, for example members of the selectin family, still allow for some leukocyte trafficking within the kidney in the setting of widespread inflammation. This is consistent with multiple other studies that have implicated leukocyte adhesion pathways independent of the CD18/ICAM-1 interaction and which have demonstrated that mediators of chemotaxis differ somewhat between different tissues (4, 40). Deficiency of ICAM-1 may have conferred protection against LPS-induced ARF not only by decreasing neutrophil adhesion, but also by abrogating signaling events that occur in endothelium upon ligation of ICAM-1 (6, 60), or possibly through other as yet unknown neutrophil-independent mechanisms. Last, ICAM-1 deficiency did not confer protection by reducing other mediators of inflammation, such as LPS-induced TNF release or renal chemokine expression. This is consistent with the understanding that ICAM-1 is one of several downstream effectors induced by TNF (44). Consistent with this, the modest degree of protection we described in ICAM-1-/- mice is much less than the degree of protection seen in TNFR1-/- mice (7).

Infiltration of the kidney with neutrophils or other leukocytes could lead to ARF in several different ways. Neutrophils attached to vessel walls could lead to reduced renal blood flow, as has been suggested by intravital microscopy studies (41, 42). This could happen from either direct physical obstruction of small peritubular capillaries, or possibly from damage to endothelial cells from the local release of reactive oxygen species, proteases, or cytokines, which could in turn trigger endothelial sloughing, vasoconstriction, or procoagulant effects. Depending on the extent to which neutrophils escape the vasculature and come in to contact with renal tubules, release of these injurious mediators could also result in direct tubular damage and consequent decrease in renal function (63). The experiments described above do not directly address which of these mechanisms are most relevant, so answering this question will require further study. We did observe that neutrophil infiltration was associated with increased tubular injury, which may have been due either to direct damage from infiltrating leukocytes and/or ischemia from upstream vascular obstruction.

Since the principal ligands for ICAM-1 are CD11a/CD18 and CD11b/CD18, we did not anticipate the lack of protection against LPS-induced ARF in CD18-/- mice. In contrast to our results showing preserved neutrophil infiltration within the kidney, this strain of CD18-/- mice does exhibit reduced neutrophil chemotaxis in chemical peritonitis (62), similar to ICAM-1-/- mice (55). The fact that CD18-/- mice still experienced robust neutrophil infiltration in response to LPS, in parallel with functional and structural kidney injury, remains consistent with a role for neutrophil-mediated kidney injury in LPS-induced ARF. It should be noted that this strain of mice used in our studies still expresses CD18 on circulating leukocytes at levels ~2% of wild-type, which may increase to 16% under inflammatory stimuli (62). However, interaction between residual CD11a/CD18 or CD11b/CD18 and ICAM-1 cannot explain preserved neutrophil recruitment after LPS, as blockade of ICAM-1 in CD18-/- mice did not block neutrophil influx. Because ICAM-1 has a short intracellular sequence lacking intrinsic kinase activity or Src homology domains, a signaling role for ICAM-1 independent of CD18 is less likely, although some authors have ascribed roles for ICAM-1 in processes such as angiogenesis or glutathione metabolism (25, 26). Another possibility is that ICAM-1 could mediate injury independent of leukocytes through binding of its ligand fibrinogen (31, 50). Such a role for ICAM-1 independent of neutrophil recruitment would still raise the question of what factors are responsible for neutrophil recruitment to the kidney, a process normally attributed to adhesion molecules and chemokines.

Alternatively, as with any genetically altered or mutant animal, compensatory mechanisms may have developed to compensate for the absence of normal CD18 expression. This might be expected to be more likely with CD18 deficiency, as opposed to ICAM-1 deficiency, since unlike ICAM-1, CD18 is expressed at fairly high levels at baseline, before an inflammatory stimulus (39). Multiple defects in adaptive immunity and in trafficking of various leukocyte types have been observed in CD18-deficient mice (33, 35). Other investigators described compensatory changes that preserve leukocyte migration or adhesion in genetically altered strains (13, 48, 61). As a possible example of this, we noted exaggerated expression of VCAM-1 and neutrophil-attracting chemokines MIP-2 and KC in response to LPS in CD18-def mice compared with wild-type mice. Since blockade of VCAM-1 with mAb M/K-2.7 did not protect CD18-def mice against LPS-induced neutrophil infiltration or ARF, VCAM-1 upregulation is not responsible for this preserved neutrophil infiltration. Therefore, increased synthesis of MIP-2 and KC may be a more likely explanation. It should also be noted that although we observed no exaggeration in LPS-induced TNF release in CD18-def mice, others have noted a greater intrinsic innate immune response in this strain (10).

In conclusion, these data support both an important protective role for neutrophils in inhibiting LPS-induced TNF release yet an adverse role for ICAM-1 in LPS-induced ARF. It should be noted that this LPS model is a rather simplified simulation of clinical sepsis in humans. In addition to LPS, a variety of bacterial products, such as fMLP and unmethylated bacterial DNA, may directly activate the innate immune system over a chronic or intermittent time course. To add to this complexity, clinical sepsis often occurs in a population with substantial comorbidities, yet in the setting of various supportive measures, such as hemodynamic drugs, mechanical ventilation, and antibiotics. Other investigators (37) used models such as cecal ligation and puncture in fluid-resuscitated aged mice to simulate septic ARF in humans, which could potentially demonstrate different results for the role of neutrophils and ICAM-1. It is hoped that greater insight into this process of leukocyte dynamics in sepsis may eventually lead to therapeutic strategies aimed at treatment and prevention of ARF. This may prove challenging, as neutrophils and other leukocytes clearly have an important role in combating the microorganisms responsible for sepsis; an anti-adhesion molecule strategy would be expected to have fewer adverse consequences in ARF associated with syndromes of sterile inflammation, such as ARF in the setting of pure ischemia or postsurgical states. Alternatively, greater understanding of the exact ways in which
adhesion molecules contribute to acute renal dysfunction could yield more specific and targeted therapies.

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GRANTS

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REFERENCES

34. Miyake K, Medina K, Ishihara K, Kimoto M, Auerbach R, Kincaide PW. A VCAM-like adhesion molecule on murine bone marrow stromal