Endotoxemic acute renal failure is attenuated in caspase-1-deficient mice

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Caspase-1-deficient (−/−) mice are protected against sepsis-induced acute renal failure (ARF). Mice were injected intraperitoneal with 2.5 mg of LPS that induces endotoxemic ARF. On immunoblot analysis of whole kidney, there was an increase in caspase-1 protein in LPS-treated mice compared with vehicle-treated controls. In LPS-treated mice, the glomerular filtration rate (GFR) was significantly higher in caspase-1−/− vs. wild-type mice at 16 and 36 h after LPS. To determine the mechanism of this protection, the caspase-1-activated cytokines IL-1β and IL-18 were investigated. IL-1β and IL-18 protein were significantly increased in the kidneys of LPS- vs. vehicle-treated mice. To determine the role of these cytokines, mice were treated with recombinant IL-1 receptor antagonist (IL-1Ra) or IL-18-neutralizing antiserum. In LPS-treated mice, GFR was not different in IL-1Ra-treated or IL-18-neutralizing antiserum-treated or combination therapy (IL-1Ra plus IL-18-neutralizing antiserum-treated) compared with control mice. In addition, tubular cell apoptosis, neutrophil infiltration, myeloperoxidase activity, caspase-3 activity, and calpain activity were not different between wild-type and caspase-1−/− mice with endotoxemic ARF. In LPS- vs. vehicle-treated wild-type mice, renal IL-1α was significantly increased. In both LPS- and vehicle-treated caspase-1−/− mice, renal IL-1α was very low. In summary, caspase-1−/− mice are functionally protected against endotoxemic ARF. Neutralization of IL-1β and IL-18 is not functionally protective. The role of the intracellular proinflammatory cytokine IL-1α in endotoxemic ARF merits further study.

the role of tumor necrosis factor (TNF) independent of inducible nitric oxide synthase in endotoxemic ARF in mice (19). However, the role of cytokines produced in the kidney in endotoxemic ARF has not been widely investigated.

The proinflammatory caspase-1 (formerly known as interleukin-1-converting enzyme or ICE) plays a major role in the cleavage of the precursor forms of the cytokines IL-1β and IL-18. Caspase-1 is remarkably specific for the precursors of IL-1β and IL-18 by making a single initial cut in each procytokine, which results in an active mature cytokine secreted into the extracellular space (7).

Caspase-1−/− mice are defective in production of the IL-1β and IL-18 (7, 11, 13, 20). While IL-1α is not a substrate for caspase-1, IL-1α production is also impaired in caspase-1−/− mice (20, 22). Caspase-1−/− mice are resistant to mortality from endotoxemic shock (22, 23). It has been proposed that the lack of proinflammatory cytokines may protect caspase-1−/− mice from endotoxemic shock (13). However, IL-1β−/− mice are normally sensitive to the lethal effects of LPS (9), whereas IL-18 neutralization during endotoxemia decreases mortality (28).

In the present study, caspase-1−/− mice were used to test the hypothesis that caspase-1 is a mediator of endotoxemic ARF. The fact that activation of the proinflammatory cytokines IL-1β, IL-18, and IL-1α is altered in caspase-1−/− mice makes these mice a very suitable model for further studying the role of cytokines in endotoxemic ARF. Thus the aims of the present study, in a nonhypotensive model of endotoxemic ARF, were to determine whether caspase-1−/− mice are protected against endotoxemic ARF and to explore the mechanisms of this protection, particularly the role of proinflammatory cytokines.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the Animal Ethics Review Committee at the University of Colorado Health Sciences Center. Caspase-1−/− mice backcrossed eight generations on a C57/BL6 strain were generously provided by Dr. H. Tsutsui (Dept. of Immunology and Zoology, Hyogo College of Medicine, Hyogo, Japan). These caspase-1−/− mice have been well characterized. They do not produce mature IL-1β and IL-18 (13, 17). The same background male mice (C57/BL6) served as wild-type controls (Jackson Laboratories, Bar Harbor, ME). Male mice aged 8–10 wk were used throughout the study. Mice were maintained on a standard rodent chow and had free access to water.

Endotoxemic ARF model in mice. Mice were intraperitoneally injected with 2.5 mg/kg LPS (LIST Biological Laboratories, Campbell, CA). Sixteen hours after LPS injection, renal function and blood parameters were assessed.

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pressure were measured. Mice were then killed. Blood was taken through cardiac puncture. One kidney was frozen in liquid nitrogen and the other kidney was fixed in 4% paraformaldehyde for histology.

**Measurement of glomerular filtration rate and mean arterial pressure.** The animals were anesthetized with pentobarbital sodium (60 mg/kg) and placed on a thermostatically controlled surgical table. A tracheotomy was performed in all mice. A steam of 95% oxygen was blown over the tracheal tube only if respiratory distress was noted. With the nonhypotensive dose of LPS used in the present study, none of the animals developed respiratory distress at 16 or 36 h. Catheters (custom pulled from PE-250) were placed in the jugular vein for maintenance infusion and carotid artery for blood pressure determinations. Mean arterial pressure (MAP) was measured via a carotid artery catheter connected to a TranspacIV transducer and monitored continuously using Windaq Waveform recording software (Dataq Instruments). An invasive maintenance infusion of 2.25% BSA in normal saline at a rate of 0.25 μl·g·body wt⁻¹·min⁻¹ was started 1 h before experimentation; 0.75% FITC-inulin was added to the infusion solution for the determination of the glomerular filtration rate (GFR) as described by Lotz et al. (25). A bladder catheter (PE-10) was used to collect urine. Two 30-min collections of urine were obtained under oil and weighed for volume determination. Blood for plasma inulin determination was drawn between urine collections. FITC in plasma and urine samples was measured using a Cytofluor 4000 series fluorescent plate reader (Perseptive Biosystems).

**Histological examination.** Paraformaldehyde (4%)-fixed and paraffin-embedded kidneys were sectioned at 4 μm and stained with periodic acid-Schiff (PAS) by standard methods. All histological examinations were performed by the renal pathologist without knowledge of the intervention. Histological changes due to acute tubular necrosis (ATN score) were evaluated in the outer stripe of the outer medulla and the cortex on PAS-stained tissue and were quantified by counting the percentage of tubules that displayed cell necrosis, loss of brush border, cast formation, and tubule dilatation as follows: 0 = none, 1 = <10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = >76%. At least 10 fields (×200) were reviewed for each slide.

Neutrophil infiltration was quantitatively assessed on PAS-stained tissue by the renal pathologist by counting the number of neutrophils per high-powered field (HPF; ×400). At least 10 fields were counted in the cortex and the outer stripe of the outer medulla for each slide.

Morphological criteria were used to count apoptotic cells on PAS-stained tissue by a pathologist experienced in the evaluation of renal apoptosis. Morphological characteristics included cellular rounding and shrinkage, nuclear chromatin compaction, and formation of apoptotic bodies (12). Apoptotic tubular cells were quantitatively assessed per HPF in the cortex and outer stripe of the outer medulla by the renal pathologist in a blinded fashion. At least 10 fields were counted for each slide.

**Cytokine assays.** The electrochemiluminescence (ECL) assays for IL-1β, IL-18, and IL-1α in whole kidney homogenates were performed as previously described in detail (8). Briefly, the kidney was homogenized in lysis buffer containing 10 mM N-2-hydroxyethylpiperazine-N’-ethane sulfonic acid, 150 mM NaCl, 1% (vol/vol) Igepal, 1 mM EDTA, 1 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 1 μg/ml soybean trypsin inhibitor; centrifuged at 2,500 rpm for 10 min; and the supernatant was then removed, placed in aliquots, and kept at −80°C until analyzed. Affinity-purified antiserum polyclonal antibodies (R&D Systems, Minneapolis, MN) labeled with biotin (IGEN, Gaithersburg, MD) and monoclonal antiserum antibodies (R&D Systems) labeled with ruthenium (II) tris(bipyridyl chelate) (IGEN) were used in each assay tube. 25 μl of the biotinylated antibody were incubated at room temperature with 25 μl of a 1 mg/ml solution of streptavidin-coated paramagnetic beads (Dynal, Lake Success, NY). To each assay tube, 25 μl of sample or standard were added, followed by 25 μl of the ruthenylated antibody (final concentration 1 mg/ml), and incubated overnight at room temperature. The reaction was quenched with 200 μl of PBS per tube, and the amount of chemiluminescence was determined using an Origen 1.5 analyzer (IGEN). A standard curve was constructed for each antibody.

IFN-γ measurements were performed with a commercially available ELISA kit from Endogen (Woburn, MA).

**Western blot analysis.** Kidney cortices were homogenized in radioimmunoprecipitation assay (RIPA) buffer, and Western blotting was performed using standard protocols as previously described (34). A goat anti-IL-18 polyclonal antibody (1:100) and a rabbit anti-caspase-1 polyclonal antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA) were used. Equal amounts of protein (100 μg) were loaded in each lane. Equal loading was confirmed by Coomassie blue staining of the membranes.

**IL-1 receptor antagonist.** A recombinant human IL-1 receptor antagonist (IL-1Ra) was a kind gift of Dr. D. Tracey (Upjohn, Kalamazoo, MI). It was dissolved in 0.02% azide in PBS. IL-1Ra (40 mg/kg) or vehicle was injected ip 30 min before and at 4, 8, and 12 h after LPS. IL-1Ra (10 mg/kg) blocks shocklike hemodynamic parameters and reduces circulating IL-1β and TNF-α levels in a model of gram-positive sepsis (1). A similar dose of IL-1Ra has been demonstrated to reduce neutrophil infiltration during ischemic ARF in mice (14).

**Rabbit anti-murine IL-18-neutralizing antiserum.** Rabbit anti-murine IL-18-neutralizing antiserum was obtained from a New Zealand rabbit immunized by intradermal injection of murine recombinant IL-18 in the presence of Hunter’s titermax adjuvant (7). The IL-18 antisem has been used in mice in vivo to block endogenous IL-18 (8, 28). Administration of IL-18 antisem protects against ischemic ARF in mice (26, 27). Rabbit anti-murine IL-18-neutralizing antiserum or vehicle (normal rabbit serum) was administered as follows: 200 μl ip 30 min before and 8 h after LPS injection.

**Myeloperoxidase assay.** Myeloperoxidase (MPO) activity (kinetic assay) was measured as described previously (16) with modifications (26).

**Caspase activity assay.** The activity of caspases was determined by use of fluorescent substrates as we previously described (4, 26). Renal cortex was mixed with a lysis buffer containing 25 mM NaHSO₄-HEPES, 2 mM dithiothreitol (DTT), 1 mM EDTA, 0.1% 3-(3-cholamidopropyl dimethylammonio)-1-pro-panesulfonate (CHAPS), 10% sucrose, 1 mM phenylmethylsulfonyl fluoride, and 1 μM pepstatin A, pH 7.2, and homogenized with 10 strokes in a glass-Teflon homogenizer. The lysate was then centrifuged at 4°C at 100,000 g in a Beckman Ti70 rotor for 1 h. The resultant supernatants were immediately frozen in liquid N₂ and stored at −70°C until use. Lysate protein was measured by the Bio-Rad DC protein assay kit with BSA as standards.

The caspase assay was then performed on this supernatant as follows: 200 μg protein (20–50 μl vol) were added to 10 μl of the substrate (final concentration, 50 μM). The volume was made up to 200 μl with the caspase assay buffer. The assay buffer for caspase-3 contained 250 mM KCl–HEPES, 50 mM KCl, 1 mM DTT, 1 mM EDTA, 0.1% CHAPS, pH 7.4. Ac-Asp-Glu-Val-Asp-7-amido-4-methyl coumarin (Ac-DEVDA-AMC) in 10% DMSO was used as a susceptible substrate for caspase-3 (35). The solution was preincubated for 10 min at 30°C before the substrate was added. The reaction was then initiated by addition of the substrate. Peptide cleavage was measured over 1 h at 30°C using a Cytofluor 4000 series fluorescent plate reader (Perseptive Biosystems) at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. An AMC standard curve was determined for each experiment. Caspase activity was expressed in nanomoles AMC released per minute of incubation time per milligram of lysate protein.

**Calpain activity.** Calpain activity was measured by use of fluorescent substrates as we previously described (5, 6) with modifications. N-succinyl-Leu-Tyr-7-Amino-4-methylcoumarin (Peptides International, Louisville, KY) was used as a susceptible substrate for calpain. A stock solution of 10 mM was prepared in 100% DMSO and stored at −20°C between use. Cytosolic extracts of whole kidneys were

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prepared as described in the caspase assay. The calpain assay was performed on the cytosolic extracts as follows: 100 µg protein (10–30 µl extract) were mixed with 10 µl of the substrate. The assay volume was made up to 200 µl with imidazole-HCl assay buffer. The imidazole-HCl buffer used contained 63.2 mM imidazole, 10 mM mercaptoethanol, pH 7.3, with or without 5 mM CaCl₂. In the assay performed without CaCl₂, the imidazole-HCl buffer containing 1 mM EDTA and 10 mM EGTA was used. The solution was preincubated for 10 min at 30°C before the substrate was added. The reaction was then initiated by addition of the substrate. Peptide cleavage was measured over 1 h at 30°C using a Cytofluor 4000 series fluorescent plate reader (Perseptive Biosystems) at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. An AMC standard curve was determined for each experiment. Calpain activity was determined as the difference between the calcium-dependent fluorescence and the non-calcium-dependent fluorescence. Calpain activity was expressed in nanomoles AMC released per minute of incubation time per milligram of lysate protein.

Statistical analysis. Nonnormally distributed data were analyzed by the nonparametric unpaired Mann-Whitney U-test. Multiple group comparisons were performed using a one-way ANOVA with post-test according to Newman-Keuls. Because unidirectional changes were expected with the intervention, i.e., improvement of the parameter examined, a one-way ANOVA was used. A P value of <0.05 was considered statistically significant. Values are expressed as means ± SE.

RESULTS

Caspase-1 protein expression increased during endotoxemic ARF. Western blot analysis was used to examine caspase-1 protein expression. Renal caspase-1 protein expression increased significantly in LPS-treated mice compared with the vehicle controls. As expected, its expression was absent in caspase-1 −/− mice with or without LPS (Fig. 1).

Caspase-1 −/− mice are protected against endotoxemic ARF. To determine whether the increase in caspase-1 protein expression plays a role in endotoxemic ARF, we used caspase-1 −/− mice. There was no significant difference in baseline glomerular filtration rate (GFR) between the vehicle (Veh)-treated wild-type (+/+ ) and Veh-treated caspase-1 −/− mice (n = 4–8). B: 16 h after LPS injection, caspase-1 −/− (LPS −/−) developed less severe ARF than wild-type controls (LPS +/+ ; *P < 0.01, n = 9–11). C: 36 h after LPS injection, caspase-1 −/− (LPS −/−) continue to have less severe ARF than wild-type controls (LPS +/+ ; *P < 0.05).

Fig. 1. Caspase-1 protein expression is increased in the kidney in endotoxemic acute renal failure (ARF). A: in wild-type mice, the pro form of caspase-1 (45 kDa) is absent in caspase-1 −/− mice. *P = 0.02 vs. vehicle-treated wild-type (+/+ ) mice.

Fig. 2. Caspase-1 −/− mice are protected against endotoxemic ARF. A: there was no significant difference in baseline glomerular filtration rate (GFR) between the vehicle (Veh)-treated wild-type (+/+ ) and Veh-treated caspase-1 −/− mice (n = 4–8). B: 16 h after LPS injection, caspase-1 −/− (LPS −/−) developed less severe ARF than wild-type controls (LPS +/+ ; *P < 0.01, n = 9–11). C: 36 h after LPS injection, caspase-1 −/− (LPS −/−) continue to have less severe ARF than wild-type controls (LPS +/+ ; *P < 0.05).
mice developed less severe ARF than wild-type mice. GFR (µl/min) was 171 ± 7.6 in caspase-1+/− mice, n = 11 vs. 96.4 ± 17 in wild-type mice, P < 0.05 (Fig. 2C).

Renal histology. An increased ATN score was a feature of the kidney histology (Fig. 3, A and B). Brush-border injury and tubule dilatation predominated over cell necrosis and cast formation in the ATN score. The ATN score was significantly increased in LPS-treated wild-type mice compared with vehicle-treated wild-type mice [2.2 ± 0.7 vs. 0.3 ± 0.1, P < 0.05, in cortex (Fig. 3C) and 1.8 ± 0.8 vs. 0, P < 0.05, in outer stripe of outer medulla (Fig. 3D), n = 7 per group]. While the ATN score in both cortex and outer stripe of outer medulla was decreased in LPS-treated caspase-1-deficient mice compared with LPS-treated wild-type mice, this did not reach statistical significance.

The number of tubular apoptotic cells was counted in the cortex and outer stripe of outer medulla. The apoptosis score (apoptotic tubular cells per HPF) was not different between the groups. The apoptosis score in the cortex was 0.04 ± 0.02 in vehicle-treated wild-type mice, 0.3 ± 0.2 in LPS-treated wild-type mice, and 0.2 ± 0.1 in LPS-treated caspase-1-deficient mice (n = 5 per group). The apoptosis score in the outer stripe of the outer medulla was 0.1 ± 0.02 in vehicle-treated wild-type mice, 0.4 ± 0.4 in LPS-treated wild-type mice, and 0.5 ± 0.5 in LPS-treated caspase-1-deficient mice (n = 5 per group).

Neutrophil infiltration was counted in the cortex and outer stripe of outer medulla. The number of neutrophils per HPF in the outer stripe of the outer medulla was increased in LPS-treated wild-type mice compared with vehicle-treated wild-type mice (0.6 ± 0.2 vs. 0, P < 0.05, n = 5). The number of neutrophils per HPF in the cortex was not different between LPS-treated wild-type mice and LPS-treated caspase-1-deficient mice (0.5 ± 0.3 vs. 0.9 ± 0.4, P = NS, n = 5). The number of neutrophils per HPF in both the outer stripe of outer medulla and cortex was not different in LPS-treated caspase-1-deficient mice compared with LPS-treated wild-type mice.

IL-1β and IL-18 protein in endotoxemic ARF. ECL was used to measure renal IL-1β and IL-18 protein during endotoxemic ARF. IL-1β protein (pg/mg) was significantly increased in LPS- vs. vehicle-treated mice (27.2 ± 2.3 vs. 14 ± 2.6, P < 0.05, n = 5; Fig. 4A). IL-18 protein (pg/mg) was significantly increased in LPS- vs. vehicle-treated mice (3.6 ± 2.2 vs. 1.5 ± 0.3, P < 0.05, n = 5; Fig. 4B).

IL-18 protein expression in endotoxemic ARF. An antibody that recognizes both the pro-IL-18 and the mature form was used in the Western blot analysis. The active form of IL-18 in the kidney was significantly increased in LPS-treated mice compared with vehicle-treated mice (Fig. 5).

Effect of IL-1Ra on endotoxemic ARF. IL-1Ra (40 mg/kg) or the vehicle was injected (ip) 30 min before and at 4, 8, and 12 h after LPS. GFR was measured at 16 h after LPS injection. There was no difference in GFR between vehicle-treated and IL-1Ra-treated mice after LPS injection (34 ± 7 vs. 66 ± 18 µl/min, n = 6, P = NS; Fig. 6A).

Effect of anti-murine IL-18-neutralizing antiserum on endotoxemic ARF. Rabbit anti-murine IL-18-neutralizing antiserum or normal rabbit serum (vehicle) was administrated as follows: 200 µl ip 30 min before LPS and 8 h after LPS injection. GFR was measured at 16 h after LPS injection. There was no difference in GFR between vehicle-treated and IL-18-neutralizing antiserum-treated mice after LPS injection (60.6 ± 18.2 vs. 41.1 ± 11.4, n = 5, P = NS; Fig. 6B).

Serum IFN-γ level was examined using the ECL method to ensure the dosage and in vivo effect of IL-18 antiserum. IL-18 antiserum significantly reduced IFN-γ levels in the serum. Serum IFN-γ level (pg/ml) was 22.2 ± 24.8 in controls, 856.7 ± 170.9 in endotoxemic mice (P < 0.01 vs. controls, n = 6), and 91.9 ± 10.0 in endotoxemic mice treated with IL-18 antiserum (P < 0.01 vs. endotoxemia alone, n = 6).

Effect of combination therapy with IL-1Ra- and IL-18-neutralizing antiserum on endotoxemic ARF. Combination therapy with both IL-1Ra (40 mg/kg ip 30 min before and at 4, 8, and 12 h after LPS) and IL-18 antiserum significantly reduced IFN-γ levels in the serum. Serum IFN-γ level (pg/ml) was 60.6 ± 18.2 in controls, 24.8 ± 18.0 in IL-18 antiserum-treated mice, and 6.5 ± 3.2 in combination therapy mice (P < 0.01 vs. controls, n = 6).
and 12 h after LPS) and IL-18AS (200 μl ip 30 min before LPS and 8 h after LPS injection) or vehicle (normal rabbit serum) was administered. Combination therapy did not protect against endotoxemic ARF. There was no difference in GFR between vehicle-treated and combination IL-1Ra and IL-18-neutralizing antiserum-treated mice compared with Veh-treated control mice. *P < 0.05 vs. Veh, n = 5. B: renal IL-18 increased significantly in LPS-treated mice compared with Veh-treated control mice. *P < 0.05 vs. Veh, n = 5.

MPO activity. There was no increase in MPO activity in the kidney in endotoxemic ARF. MPO activity (OD min⁻¹·mg⁻¹) in wild-type mice was 0.010 ± 0.003 in control mice vs. 0.012 ± 0.003 in endotoxemic ARF (n = 4, P = NS).

Caspase-3 activity. Caspase-3 activity (nmol min⁻¹·mg⁻¹) was 7.8 ± 1 in wild-type control mice, 15.8 ± 1.5 in wild-type mice with endotoxemic ARF (P < 0.001 vs. controls, n = 8), and 13.3 ± 1.3 in caspase-1 −/− mice with endotoxemic ARF (P = NS vs. wild-type endotoxemic mice, n = 8; Fig. 7).

IL-1α protein expression during endotoxemia. ECL was used to measure renal IL-1α protein expression during endotoxemic ARF. IL-1α (pg/mg) increased significantly in LPS-treated mice compared with vehicle-treated mice (19.4 ± 4.2 vs. 8.6 ± 1.3, n = 5, P < 0.01; Fig. 8). IL-1α (pg/mg) was very low in caspase-1 −/− mice that received vehicle for LPS vs. LPS (1.2 ± 0.4 vs. 0.9 ± 0.03, n = 5–9, P = NS).

Calpain activity. Pro-IL-1α is cleaved into a mature form by the cysteine protease calpain and not by caspase-1. The lack of IL-1α in the caspase-1-deficient mice may be due to reduced...
calpain. In this regard, we previously demonstrated that caspases downregulate the calpain inhibitor protein calpastatin in the kidney during ischemia-reperfusion (34). Thus in the present study, we measured calpain activity in the mice with endotoxic ARF. Calpain activity was not different between wild-type and caspase-1-deficient mice with endotoxic ARF, suggesting that a decrease in calpain is not the reason for the decrease in IL-1α in caspase-1-deficient mice.

The caspase-1 −/− mice demonstrated significant renal protection as assessed by GFR. The MAPs were no different between the wild-type and caspase −/− mice at either baseline or 16 h after the endotoxin administration. The GFR (μl/min) at 16 h after endotoxin administration was threefold higher in the caspase-1 −/− compared with wild-type mice (102 ± 15.3 vs. 33.0 ± 5.4, P < 0.01). While this degree of functional protection would avoid the clinical syndrome of ARF, it was clear that factors other than caspase-1 must contribute to the functional deterioration during endotoxemia in the caspase-1 −/− mice. Specifically, the GFR was still significantly below preendotoxemic levels. In this regard, earlier studies in this normotensive ARF model of endotoxemia have implicated renal nerve-mediated vasoconstriction (37), TNF-α (19), and oxygen radicals (38). These factors thus could contribute along with caspase-1 activation to the significant decrease in GFR in this normotensive model of endotoxemic ARF.

Further studies were thus undertaken to examine the potential mechanisms whereby caspase-1 activation could mediate the significant fall in GFR during endotoxemia. Earlier studies in a renal clamp model of ischemia had demonstrated that IL-18 antiserum afforded functional and histological protection (26, 27). The same IL-18 antiserum was therefore used in our normotensive endotoxic ARF model of endotoxemia have implicated renal nerve-mediated vasoconstriction (37), TNF-α (19), and oxygen radicals (38). These factors thus could contribute along with caspase-1 activation to the significant decrease in GFR in this normotensive model of endotoxemic ARF.

Discussions

Sepsis and endotoxemia are associated with an increased incidence of ARF. Moreover, ARF associated with sepsis has a substantially higher mortality than ARF in the absence of sepsis (29, 30). Endotoxemia is known to be associated with increased plasma cytokine levels. An upregulated protease during endotoxemia is caspase-1, which is specific for activating the precursors of proinflammatory cytokines IL-1β and IL-18 (7). In the present study, a normotensive model of endotoxemic ARF was demonstrated to have increased renal expression of caspase-1. Studies were therefore undertaken to examine the role of caspase-1 in this experimental murine model of endotoxemic ARF.

These caspase-1 −/− mice have a normal phenotype and have been shown to have decreased mortality during high-dose endotoxemia compared with wild-type mice (22). This severe endotoxemia, however, had a 100% mortality within 30 h, therefore suggesting multiorgan dysfunction (22). The purpose of the present study was to examine the role of caspase-1 in a nonlethal endotoxic model of ARF.

Gene targeting has produced caspase-1 −/− mice, which are defective in the production of active form of cytokines IL-1β and IL-18 and IL-1α (20, 22, 23). The proforms of IL-1β and IL-18 are known substrates for caspase-1. Caspase-1 converts the pro to the mature form of IL-1β and IL-18. Thus the caspase-1-deficient mice are deficient in the active (mature) form of IL-1β and IL-18. The original papers that described the caspase-1-deficient mice (20, 22) noted that these mice were also deficient in IL-1α. IL-1α lacks the aspartate cleavage sequence for caspase-1 and is thus not a substrate for caspase-1. It is still not known why caspase-1-deficient mice have less IL-1α, as pro-IL-1α is cleaved to its active form by the calcium-dependent cysteine protease calpain and not by caspase-1. It is possible that caspase-1-deficient mice have less calpain. Thus we measured calpain activity. Calpain activity (nmol·min⁻¹·mg⁻¹) was 362 ± 78 in wild-type control mice, 239 ± 169 in caspase-1 −/− control mice, 316 ± 95 in wild-type mice with endotoxic ARF (P = NS vs. controls, n = 7), and 159 ± 69 in caspase-1 −/− mice with endotoxic ARF (P = NS vs. wild-type endotoxic mice, n = 7).

Discussion

Sepsis and endotoxemia are associated with an increased incidence of ARF. Moreover, ARF associated with sepsis has a substantially higher mortality than ARF in the absence of sepsis (29, 30). Endotoxemia is known to be associated with increased plasma cytokine levels. An upregulated protease during endotoxemia is caspase-1, which is specific for activating the precursors of proinflammatory cytokines IL-1β and IL-18 (7). In the present study, a normotensive model of endotoxemic ARF was demonstrated to have increased renal expression of caspase-1. Studies were therefore undertaken to examine the role of caspase-1 in this experimental murine model of endotoxemic ARF.

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Gene targeting has produced caspase-1 −/− mice, which are defective in the production of active form of cytokines IL-1β and IL-18 and IL-1α (20, 22, 23). The proforms of IL-1β and IL-18 are known substrates for caspase-1. Caspase-1 converts the pro to the mature form of IL-1β and IL-18. Thus the caspase-1-deficient mice are deficient in the active (mature) form of IL-1β and IL-18. The original papers that described the caspase-1-deficient mice (20, 22) noted that these mice were also deficient in IL-1α. IL-1α lacks the aspartate cleavage sequence for caspase-1 and is thus not a substrate for caspase-1. It is still not known why caspase-1-deficient mice have less IL-1α, as pro-IL-1α is cleaved to its active form by the calcium-dependent cysteine protease calpain and not by caspase-1. It is possible that caspase-1-deficient mice have less calpain. In this regard, we previously demonstrated that caspases downregulate the calpain inhibitor protein calpastatin in the kidney during ischemia-reperfusion (34). Thus in the present study, we measured calpain activity in the mice with endotoxic ARF. Calpain activity was not different between wild-type and caspase-1-deficient mice with endotoxic ARF, suggesting that a decrease in calpain is not the reason for the decrease in IL-1α in caspase-1-deficient mice.

The caspase-1 −/− mice demonstrated significant renal protection as assessed by GFR. The MAPs were no different between the wild-type and caspase −/− mice at either baseline or 16 h after the endotoxin administration. The GFR (μl/min) at 16 h after endotoxin administration was threefold higher in the caspase-1 −/− compared with wild-type mice (102 ± 15.3 vs. 33.0 ± 5.4, P < 0.01). While this degree of functional protection would avoid the clinical syndrome of ARF, it was clear that factors other than caspase-1 must contribute to the functional deterioration during endotoxemia in the caspase-1 −/− mice. Specifically, the GFR was still significantly below preendotoxemic levels. In this regard, earlier studies in this normotensive ARF model of endotoxemia have implicated renal nerve-mediated vasoconstriction (37), TNF-α (19), and oxygen radicals (38). These factors thus could contribute along with caspase-1 activation to the significant decrease in GFR in this normotensive model of endotoxemic ARF.

Further studies were thus undertaken to examine the potential mechanisms whereby caspase-1 activation could mediate the significant fall in GFR during endotoxemia. Earlier studies in a renal clamp model of ischemia had demonstrated that IL-18 antiserum afforded functional and histological protection (26, 27). The same IL-18 antiserum was therefore used in our normotensive endotoxic ARF model of endotoxemia have implicated renal nerve-mediated vasoconstriction (37), TNF-α (19), and oxygen radicals (38). These factors thus could contribute along with caspase-1 activation to the significant decrease in GFR in this normotensive model of endotoxemic ARF.

Fig. 7. Caspase-3 activity is increased in endotoxemic ARF. Caspase-3 activity in the kidney was increased in mice with endotoxic ARF (LPS +/+ mice) compared with Veh-treated wild-type mice (⁎P < 0.001). There was no difference in caspase-3 activity between wild-type mice with endotoxic ARF (LPS +/+ mice) and caspase-1 −/− mice with endotoxic ARF (LPS −/− mice). Thus a decrease in caspase-3 does not explain the protection against endotoxemic ARF in caspase-1 −/− mice.

Fig. 8. IL-1α protein expression is increased in endotoxemic ARF and very low in caspase-1 −/− mice. ECL was used to measure renal IL-1α protein expression during endotoxemic ARF. In wild-type mice, IL-1α increased significantly in LPS-treated (LPS +/+ mice) compared with Veh-treated (Veh +/+ mice) mice (⁎P < 0.01 vs. Veh +/+ mice, n = 5). In caspase-1 −/− mice, IL-1α was virtually undetectable in LPS-treated (LPS −/− mice) and Veh-treated (Veh −/− mice) mice (⁎⁎P < 0.01 vs. Veh +/+ mice, ***P < 0.001 vs. LPS +/+ mice, n = 5–9).
endotoxemic ARF, the development of multitargeted inter-
ventions has been proposed (3). In the present study, however, combination therapy with both IL-18 antiserum and IL-1Ra was not protective.

Other mechanisms whereby increased caspase-1 activity may contribute to the significant decline in GFR during endo-
toxemia were therefore explored. Neutrophil infiltration on
histology was increased in the outer stripe of the outer medulla but not in the cortex in mice with endotoxemic ARF. However, MPO activity in the whole kidney was not increased in mice with endotoxemic ARF. Caspase-1-deficient mice that were protected against endotoxemic ARF did not have decreased neutrophil infiltration into the kidney as assessed by histology.

Next, we investigated the role of caspase-3, a major medi-
ator of apoptosis. LPS is known to induce an increase in caspase-3 in both cultured glomerular endothelial cells (1) and renal tubular cells (3). Caspase-1-deficient mice have less caspase-3 in response to cisplatin-induced ARF (10). Thus we evaluated the hypothesis that caspase-1 may activate caspase-3 with resultant tubular cell apoptosis in endotoxemic ARF. In the present study, an increase in the kidney of the executioner caspase-3, an important mediator of apoptosis (11), was ob-
erved in both the wild-type and caspase-1 −/− mice treated with LPS. However, the increase in renal caspase-3 in whole kidney was no different between the wild-type and caspase −/− mice, therefore not providing an explanation for the higher GFR at 16 h after LPS in the caspase-1 −/− mice. In addition, the amount of tubular cell apoptosis was not different between the groups.

Caspase-1 −/− mice are also known to be defective in the production of the IL-1α cytokine (20, 22, 23). IL-1α is rarely found in the extracellular compartment and therefore exerts its proinflammatory actions intracellularly or at the membrane (39). In vivo, IL-1α is not readily released from cells even on activation and unlike IL-1β, IL-1α is not detected in the serum of inflammatory and infectious diseases, with the exception of severe pathological conditions in which cell death results in release of the cytokine (39). IL-1α was overexpressed in various cells and activity was determined in the presence of saturating concentrations of IL-1Ra (39). In the presence of IL-1Ra, IL-1α activated NF-κB and AP-1 and released the cytokines IL-8 and IL-6 (39). These studies demonstrate that IL-1Ra does not block the function of IL-1α. In the present study, IL-1α was found to be significantly increased in the kidney in response to endotoxin in the wild-type but not caspase-1 −/− mice.

There is experimental evidence that renal vasoconstriction with intact tubular function may be the predominant pathoge-
netic factor in early endotoxemic ARF (33). In the mouse model of endotoxemic ARF described in the present study, we previously reported increased plasma concentrations of cate-
cholamines and activation of the renin-angiotensin-aldoste-
rone system (37). In the same study, renal denervation afforded considerable protection against the decrease in GFR (37). Thus it is possible that the attenuation of endotoxemic ARF de-
scribed in the present study may be due, in part, to less severe hemodynamic changes in the caspase-1-deficient mice. In addition, it is possible that modest changes in the ATN score, calpain activity, caspase-3 activity, and apoptosis combine to significantly improve renal function in the caspase-1-deficient mice.

In summary, caspase-1 −/− have higher GFRs in response to endotoxemia than wild-type mice. Although renal IL-18 and IL-1β were increased in the wild-type mice treated with endo-
toxin, no renal protection was demonstrated with either IL-18 antiserum or IL-1 receptor antagonist or the combination. In addition, the renal protection was not associated with decreased tubular necrosis, tubular apoptosis, neutrophil infiltra-
tion in the kidney, or decreased caspase-3 or calpain activity. The intracellular proinflammatory cytokine IL-1α, however, emerged as a potential mediator of the effect of caspase-1 in this normotensive, endotoxemic model of ARF. The further testing of this possibility awaits the future development of inhibitors of this intracellular cytokine (18).

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REFERENCES

1. Aiura K, Gelfand JA, Burke JF, Thompson RC, and Dinarello CA. Interleukin-1 (IL-1) receptor antagonist prevents Staphylococcus epider-
imidis-induced hypotension and reduces circulating levels of tumor necro-


4. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, and Mel-


7. Fantuzzi G, Puren AJ, Harding MW, Livingston DJ, and Dinarello CA. Interleukin-18 regulation of interferon-γ production and cell prolif-
eration as shown in interleukin-1β-converting enzyme (caspase-1)-defi-

8. Fantuzzi G, Reed DA, and Dinarello CA. IL-12-induced IFN-γ is de-


10. Faubel SG, Jlubanovic D, Reznikov LL, Somerset H, Dinarello CA, and Edelstein CL. Caspase-1-deficient mice are protected against cispla-


vation of interferon-γ inducing factor mediated by interleukin-1β con-


