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RESEARCH ARTICLE | *Mechanism and Treatment of Renal Fibrosis*

LPS removal reduces CD80-mediated albuminuria in critically ill patients with Gram-negative sepsis

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Netti GS, Sangregorio F, Spadaccino F, Staffieri F, Crovace A, Infante B, Maiorano A, Godeas G, Castellano G, Di Palma AM, Prattichizzo C, Cotoia A, Mirabella L, Gesualdo L, Cinnella G, Stallone G, Ranieri E, Grandaliano G. LPS removal reduces CD80-mediated albuminuria in critically ill patients with Gram-negative sepsis. *Am J Physiol Renal Physiol* 316: F723–F731, 2019. First published January 23, 2019; doi:10.1152/ajprenal.00491.2018.—LPS-induced sepsis is a leading cause of acute kidney injury (AKI) in critically ill patients. LPS may induce CD80 expression in podocytes with subsequent onset of proteinuria, a risk factor for progressive chronic kidney disease (CKD) frequently observed after AKI. This study aimed to investigate the therapeutic efficacy of LPS removal in decreasing albuminuria through the reduction of podocyte CD80 expression. Between January 2015 and December 2017, 70 consecutive patients with Gram-negative sepsis-induced AKI were randomized to either have coupled plasma filtration and adsorption (CPFA) added to the standard care ($n = 35$) or not ($n = 35$). To elucidate the possible relationship between LPS-induced renal damage, proteinuria, and CD80 expression in Gram sepsis, a swine model of LPS-induced AKI was set up. Three hours after LPS infusion, animals were treated or not with CPFA for 6 h. Treatment with CPFA significantly reduced serum cytokines, C-reactive protein, procalcitonin, and endotoxin levels in patients with Gram-negative sepsis-induced AKI. CPFA significantly lowered also proteinuria and CD80 urinary excretion. In the swine model of LPS-induced AKI, CD80 glomerular expression, which was undetectable in control pigs, was markedly increased at the podocyte level in LPS-exposed animals. CPFA significantly reduced LPS-induced proteinuria and podocyte CD80 expression in septic pigs. Our data indicate that LPS induces albuminuria via podocyte expression of CD80 and suggest a possible role of timely LPS removal in preventing the maladaptive repair of the podocytes and the consequent increased risk of CKD in sepsis-induced AKI.

acute kidney injury; albuminuria; CD80; chronic kidney disease; sepsis

INTRODUCTION

Acute kidney injury (AKI) in hospitalized patients has become increasingly common, in particular within the intensive care unit (ICU) population (22–67%) and is associated with poor long-term outcome (15, 49). Patients with severe AKI requiring initiation of renal replacement therapy have the highest in-hospital mortality rates, ranging from 45% to 70% (25, 27, 32, 50). Among survivors of this high-risk population, as many as 13–32% require dialysis at the time of hospital discharge (2, 3, 42). Limited data are available regarding recovery of sufficient kidney function to allow discontinuation of dialysis therapy following hospital discharge in patients with severe AKI (39), yet patients initiating in-hospital renal replacement therapy constitute a significant proportion of the incident dialysis population each year (26, 31, 40). Several reports in the past decade suggest a causal link between AKI and the consequent development of progressive chronic kidney disease (CKD) (16). Sepsis, particularly the severe form sustained by Gram-negative infections, is a major cause of AKI in ICU patients (52). Sepsis is a complex pathologic condition arising from the host response to an overwhelming infection. Gram-negative bacteria and the components of their walls, in particular the lipid A-containing lipopolysaccharide (LPS), play a major role in this setting (37). Indeed, LPS may induce uncontrolled cytokines release and activation of coagulation on endothelial cells leading to shock, multiple organ damage, and even death (34).

While the pathophysiology of sepsis-induced AKI has been widely investigated, the mechanisms underlining the transition between LPS-mediated AKI and the onset of progressive CKD are still poorly known.

Exposure to low-dose LPS, through direct stimulation of the Toll-like receptor-4 (TLR-4)/CD14 receptor, rapidly upregulates CD80 in podocytes in vivo, leading to nephrotic-range proteinuria (36). CD80 is a transmembrane protein expressed on the surfaces of B cells and other antigen-presenting cells. It works as a costimulatory signal and modulates T-cell activation by binding to either CD28 or CTLA-4. At podocyte level, CD80 expression, induced by various stimuli, causes actin

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reorganization, foot process (FP) effacement, and disruption of the slit diaphragm (SD), thereby modifying glomerular permselectivity and leading to proteinuria. An increased CD80 expression at podocyte level has been reported in several proteinuric glomerulopathies and is associated with poor response to therapy and worse renal outcome (9, 10, 23, 35).

In the last decade, a significant improvement in treatment of Gram-negative sepsis and septic-AKI has been obtained. Novel blood purification approaches such as direct hemoperfusion with polymyxin B (DHP-PMX) or citrate-based coupled plasma filtration adsorption (CPFA) therapy have been widely employed in ICU to treat severe sepsis and sepsis-related AKI. Both these therapies are able to remove endotoxins by direct adsorption of LPS onto polymyxin B-coated cartridge (48) or by efficient removal of the LPS-adaptor protein LBP (CPFA; Ref. 4).

This study aimed to investigate the therapeutic efficacy of LPS removal in decreasing albuminuria through the reduction of podocyte CD80 expression.

MATERIALS AND METHODS

Study population. The study population of the present prospective, single center, cohort study consisted of 70 consecutive patients with AKI and Gram-negative severe sepsis undergoing coupled plasma filtration and adsorption (CPFA) added to the standard care ($n = 35$) or not ($n = 35$), from January 1, 2015 to December 31, 2017 at the Intensive Care Unit of University Hospital "Ospedali Riuniti" of Foggia.

The present study involving human participants was approved by the local ethical committee (Decision No. 158/CE/2014 of September 03, 2014; Ethical Committee at the University Hospital "Ospedali Riuniti" of Foggia).

All procedures performed the present study were in accordance with the ethical standards of the Declaration of Helsinki, and all the enrolled patients provided written informed consent to participate to the present study.

All of the enrolled patients were 18 yr old or older. The diagnosis of AKI was defined according to K-DIGO 2012 Guidelines (21). The diagnosis of septic shock or severe sepsis was defined according to the Third Consensus Conference on Sepsis (43) and the severity of disease was assessed by Acute Physiology and Chronic Health Evaluation III (APACHE III) score (22). The diagnosis of Gram-negative sepsis required the presence of endotoxin levels >0.7. All the patients with Gram-positive sepsis were excluded from the study.

In all patients enrolled, the site of infection was identified in the first 12 h after diagnosis of sepsis. In detail, inclusion criteria were at least two of the systemic inflammatory response syndrome (SIRS) criteria and at least one organ dysfunction as defined by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference (1).

Patients were excluded from the study for the following reasons: life expectancy <30 days (as assessed by the attending physician); HIV infection; uncontrolled hemorrhage within 24 h before study entry; organ transplantation or end-stage renal disease requiring hemodialysis or peritoneal dialysis before study entry; history of sensitivity to anticoagulant and/or extracorporeal circulation; severe thrombocytopenia (<30,000 cells/mm^3) and/or granulocytopenia (<500 cells/mm^3); and an APACHE III score >30, a Sequential Organ Failure Assessment (SOFA) score >12, or >4 organ failures by a Goris score (13 22, 51).

A historical cohort of 24 patients with Gram-negative sepsis-induced AKI, treated with direct hemoperfusion with polymyxin B (DHP-PMX) was employed as the control group.

Extracorporeal treatment. All the patients were randomized 1:1 and assigned to receive different strategies of extracorporeal blood purification: CPFA added to the standard care (*group A*, $n = 35$) or hemofiltration (*group B*, $n = 35$). Patients of both groups received full intensive care management, including fluid resuscitation, vasopressors, antimicrobial therapy, ventilatory support, and appropriate surgical management, when required.

The historical cohort of 24 patients with Gram-negative sepsis-induced AKI was treated with direct hemoperfusion with polymyxin B (DHP-PMX) (*group C*).

CPFA was performed with the use of a four-pump monitor (Flexia, Bellco, Mirandola, Italy) consisting of a plasma filter (0.45-m^{2}polyethersulfone) and a following absorption on an unselective hydrophobic resin cartridge (surface of ~700 m^2/g) and a final passage of the reconstituted blood through a high-permeability 1.4-m^2 polyethersulfone hemofilter, in which convective exchanges may be applied in a postdilution fashion.

The postdilution reinfusion rate was set up to 4 l/h. The blood flow was maintained at 150–200 ml/min, while the plasma flow was controlled by a filtration fraction ranging from 10 to 18% of blood flow (8). The reinfusion solution, which was sterile and pyrogen-free, presented the following composition (in mmol/l): 140 Na, 1.5 K, 2 Ca, 0.75 Mg, 108 Cl, 35 bicarbonate, 4 acetate, and 5.55 glucose mEq/l. The anticoagulation protocol was based on continuous citrate infusion (24). CPFA was repeated daily for the first 3 days, lasting at least 10 h/session to assure the treatment of 0.15 liter of plasma·kg^{-1}·day^{-1}.

All patients in the historical control group underwent PMX treatment twice, on *days 0* and *1*, after diagnosis of severe sepsis. Blood flow rate was maintained at 80–120 ml/min. Each hemoperfusion session lasted for 2 h. Heparin was used as an anticoagulant.

Serum and urinary measurements. The main clinical and laboratory data were recorded daily. Serum levels of C-reactive protein (CRP), procalcitonin, endotoxin activity assay (EAA), and cytokines and urinary levels of creatinine, albumin, and proteins were measured at Clinical Pathology Laboratory of the University Hospital "Ospedali Riuniti" of Foggia using common laboratory assays. In detail, high-sensitive CRP was quantified by immunoturbidimetry (Beckman Coulter, Brea, CA). Procalcitonin was titrated by chemiluminescence immunoassay (VIDAS BRAHMS PCT assay; BioMerieux, Marcy-l'Etoile, France). EAA was measured by a chemiluminescent bioassay EAA (Spectral Medical, ON, Canada). The cytokines panel detection (IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, IL-4, IL-1α, VEGF, IFN-γ, monocyte chemoattractant protein-1, and EGF) was performed using a Protein Biochip Array (Randox Laboratory, Crumlin, UK).

Urinary assessment of creatinine, albumin, and proteins was performed by routine laboratory methods. Urinary CD80 measurements were performed using a commercially available ELISA kit (Thermo Fisher, Waltham, MA) as previously described (11, 28). All the results of urine albumin, proteins, and CD80 are reported as ratio and normalized to urinary creatinine excretion.

Animal model. The animal model of endotoxemia was induced, as previously described (4), after approval by the Ethical Committee of the Italian Ministry of Health, in 216.8 ± 0.7-mo-old female domestic swine weighting 58.4 ± 14.7 kg. The choice of female pigs was driven by the knowledge of a stronger activation of the innate immunity compared with male animals, as previously demonstrated (4). All the experiment procedures were performed in adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Briefly, the animals were randomized in three groups: control ($n = 7$), LPS ($n = 7$), and LPS + CPFA ($n = 7$). Under general anesthesia, LPS and LPS + CPFA animals were infused with a saline solution containing 300 μg/kg of LPS while control animals received 10 ml of sterile saline solution. Three hours after the infusion, LPS + CPFA animals were treated by CPFA as previously described for 6 h. Animals were euthanized at the end of treatment. Both control and LPS animals were euthanized 9 h after LPS infusion. A renal tissue sample was obtained after euthanization, and a portion

Table 1. *Main clinical and laboratory characteristics of the study population at the beginning of treatment*

	Total (n = 70)	Group A (n = 35)	Group B (n = 35)	P
Age, yr	65.9 ± 1.9	64.0 ± 2.2	66.8 ± 3.3	0.521
Sex (M/F)	41/29	20/15	21/14	0.575
CKD-EPI eGFR, ml/min	12.1 ± 4.8	13.0 ± 5.6	11.0 ± 3.9	0.201
APACHE III	66.7 ± 3.7	67.4 ± 3.8	64.2 ± 10.8	0.213
GCS	6.2 ± 0.7	6.0 ± 0.9	7.1 ± 1.6	0.750
Site of infection, n (%)				
Peritonitis/gastrointestinal tract	36 (51.4)	17 (48.6)	19 (54.3)	
Urinary tract infection	15 (21.4)	8 (22.9)	7 (20.0)	
Primary bacteriemia	9 (12.9)	5 (14.3)	4 (11.4)	
Pneumonia	4 (5.7)	2 (5.7)	2 (5.7)	
Colecistitis/colangitis	3 (4.3)	2 (5.7)	1 (2.9)	
Other	3 (4.3)	1 (2.9)	2 (5.7)	
Isolated microorganisms, n (%)				
Escherichia coli	41 (58.6)	21 (60.0)	20 (57.1)	
Klebsiella spp	17 (24.3)	9 (25.7)	8 (22.9)	
Proteus spp	5 (7.1)	2 (5.7)	3 (8.6)	
Pseudomonas	4 (5.7)	2 (5.7)	2 (5.7)	
Other/unknown	3 (4.3)	1 (2.9)	2 (5.7)	

Values are means ± SD. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; APACHE III, Acute Physiology and Chronic Health Evaluation III; F, female; M, male; GCS, Glasgow Coma Scale.

of each tissue specimen was immediately snap frozen in optimal cutting temperature medium (Sakura Finetek, Torrance, CA) and stored in liquid nitrogen. Urinary output was measured and urine samples were collected from all animals and stored at −80°C until use.

Tissue analysis. Confocal microscopy was performed on 5-μm-thick cryostat tissue sections of swine renal biopsies using a confocal laser-scanning microscope (TCS SP5; Leica Microsystems, Wetzlar, Germany), as previously described (45, 46). All the reagents were prepared in 0.05% Triton X-100-containing PBS to permeabilize cell membranes. Staining with primary hamster monoclonal anti-CD80 IgG antibody (clone 16-10A1; Thermo Fisher Scientific, San Diego, CA) and rabbit polyclonal anti-WT1 IgG antibody (clone C19; Santa Cruz Biotechnology, Santa Cruz, CA) and secondary Alexa Fluor 488-labeled goat anti-hamster IgG and Alexa Fluor 546-labeled goat anti-rabbit IgG, respectively (Molecular Probes, Eugene, OR), was performed following the manufacturers' instructions. Nuclei were counterstained with Topro-3 (Molecular Probes). The slides were then mounted in Gel Mount (Biomed) and sealed. Specific fluorescence quantification was performed as previously described (11, 45, 46).

Statistical analysis. Results are expressed as means ± SD unless otherwise stated. Statistical analyses were performed using the SPSS software (SPSS 17.0, Evanston, IL). Continuous variables were compared by paired or unpaired Student t-test or Mann-Whitney U-test, as appropriate. Frequencies were compared among groups by χ^2-test. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Between January 2015 and December 2017, 70 consecutive patients met the inclusion criteria for the present study and were assigned to two different blood purification treatments. In detail, 35 patients with sepsis related to Gram-negative bacteremia were treated with CPFA added to the standard care (*group A*), while the remaining 35 patients were treated only with hemofiltration (*group B*).

As shown in Table 1, the analysis of main clinical and laboratory characteristics of the study population at the beginning of treatment did not show statistically significant differences in age, sex distribution, and estimated glomerular filtration rate [Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI eGFR)], as well as in the APACHE III and Glasgow Coma Scale (GCS) score between the two groups. Moreover no statistical difference was observed in sites of infection and type of isolated microorganisms (Table 1).

Analysis of cytokine levels at beginning of the treatment did not show statistical differences between the two group (Fig. 1, *A–L*). However, treatment with CPFA significantly reduced the levels of IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, VEGF, and IFN-γ already after the first treatment, as compared with group treated with standard care (Fig. 1, *A–L*).

Accordingly, baseline serum CRP and Procalcitonin levels were high in Gram-negative septic patients of two groups (Fig. 2, *A* and *B*). Treatment with CPFA significantly reduced serum CRP and procalcitonin levels at 1 and 3 days, as compared with control group (Fig. 2, *A* and *B*).

Finally, we assessed the effect of septic status and the efficacy of proposed treatments on glomerular permeability to proteins. Extracorporeal treatment with CPFA significantly reduced the levels of EEA, as compared with control group (Fig. 3*A*).

Baseline proteinuria and albuminuria were significantly high in Gram-negative septic patients, but the reduction in circulating LPS levels by CPFA induced a reduction in glomerular permeability to plasma proteins, as demonstrated by the reduction of proteinuria and albuminuria levels (Fig. 3, *B* and *C*). Finally, we investigated the urinary excretion of CD80. Baseline urine CD80/creatinine ratio was elevated in Gram-negative septic patients. Removal of LPS by CPFA induced a statistically significant reduction in urinary CD80 excretion as compared with control group (Fig. 3*D*).

The analysis of the historical control group treated with direct hemoperfusion with polymyxin B (DHP-PMX) confirmed the beneficial effect of LPS removal on serum endotoxin activity and urinary glomerular permeability to proteins, as well as on reduction of urinary CD80 excretion (Fig. 4, *A–D*).

The analysis of main clinical and laboratory parameters before and after CPFA treatment did not show any significant difference between male and female septic patients in our study group (data not shown).

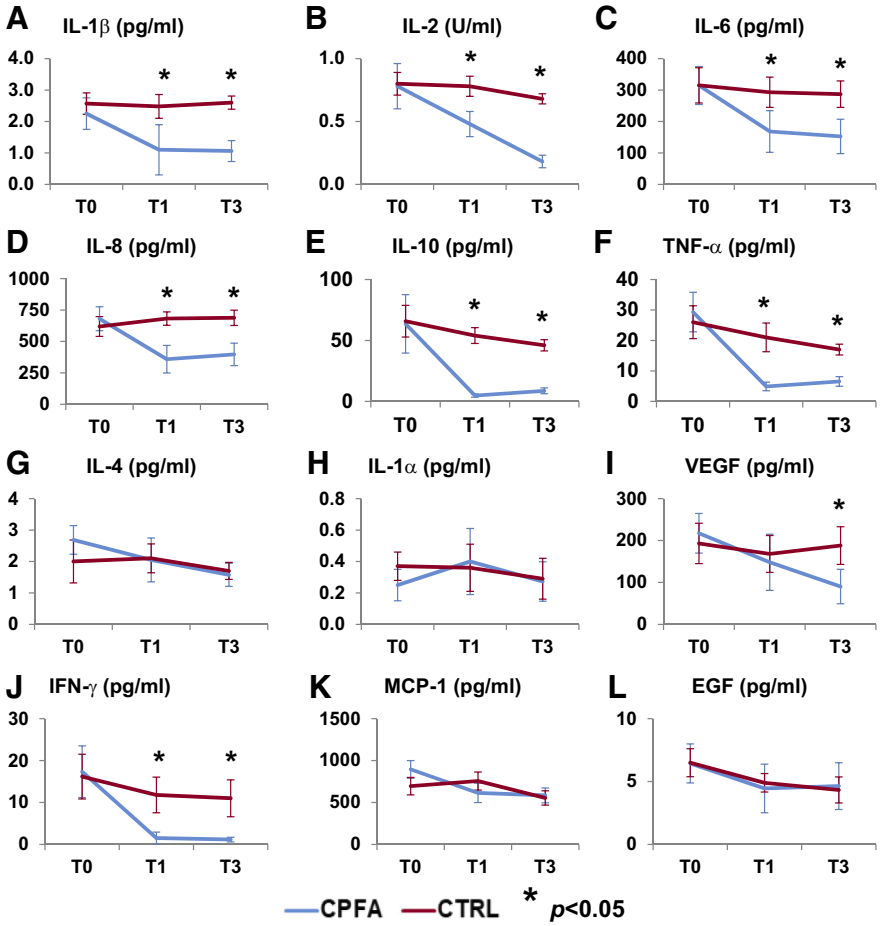

Fig. 1. Effects of extracorporeal treatments [coupled plasma filtration and adsorption (CPFA)] on cytokine removal. *A–F*, *I*, and *J*: Gram-negative septic patients treated with CPFA (blue line) showed a significant reduction of most of cytokine levels (IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, VEGF, and IFN-γ) as compared with control group (red line) (*$P < 0.05$ control group vs. CPFA group). *G*, *H*, *K*, and *L*: no statistically significant differences of cytokine levels were observed within the control group (red line) before and after standard therapy (IL-4, IL-1α, MCP-1, and EGF).

To elucidate the possible relationship between LPS-induced renal damage and subsequent increase in glomerular permeability to proteins in Gram-negative sepsis and CD80 expression, a swine model of LPS-induced AKI was set up. After 3 h from LPS infusion, endotoxemic animals were treated or not for 6 h with CPFA. Renal biopsies were performed at euthanize nine hours after LPS infusion in all the experimental groups. Confocal analysis of frozen renal tissues showed absence of CD80 glomerular expression in control pigs not exposed to LPS (Fig. 5, *A–D*). The experimental group exposed to LPS, but not treated with CPFA, showed marked increase of CD80 expression at the podocyte level, as demonstrated by the colocalization with the podocyte marker WT-1 (Fig. 5, *E–H*). CPFA treatment reduced podocyte expression of CD80 after LPS exposure, reaching a level comparable to the experimental group not exposed to LPS (Fig. 5, *I–L*), as shown by the image analysis (Fig. 5*M*).

Finally we evaluated the effect of CPFA treatment on LPS-induced increase in glomerular permeability to proteins. CPFA treatment, while reducing LPS levels and inhibiting CD80 induction at the podocyte level, was also able to reduce glomerular permeability to proteins (Fig. 5*N*, blue line).

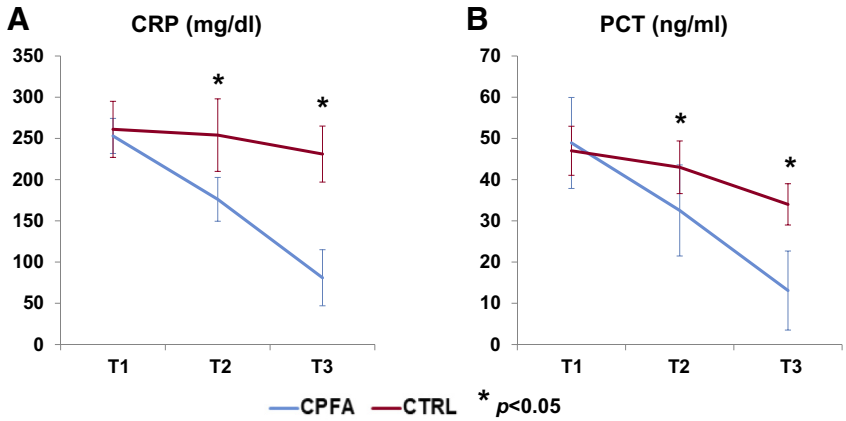

Fig. 2. Effects of extracorporeal treatments coupled plasma filtration and adsorption (CPFA) on markers of inflammation and sepsis. *A* and *B*: Gram-negative septic patients treated with CPFA (blue line) showed a significant reduction of most of serum C-reactive protein (CRP; *A*) and procalcitonin (PCT; *B*) levels as compared with control group (red line) (*$P < 0.05$ control group vs. CPFA group), while no statistically significant differences of cytokine levels were observed within the control group (redline) before and after standard therapy. T, time in days.

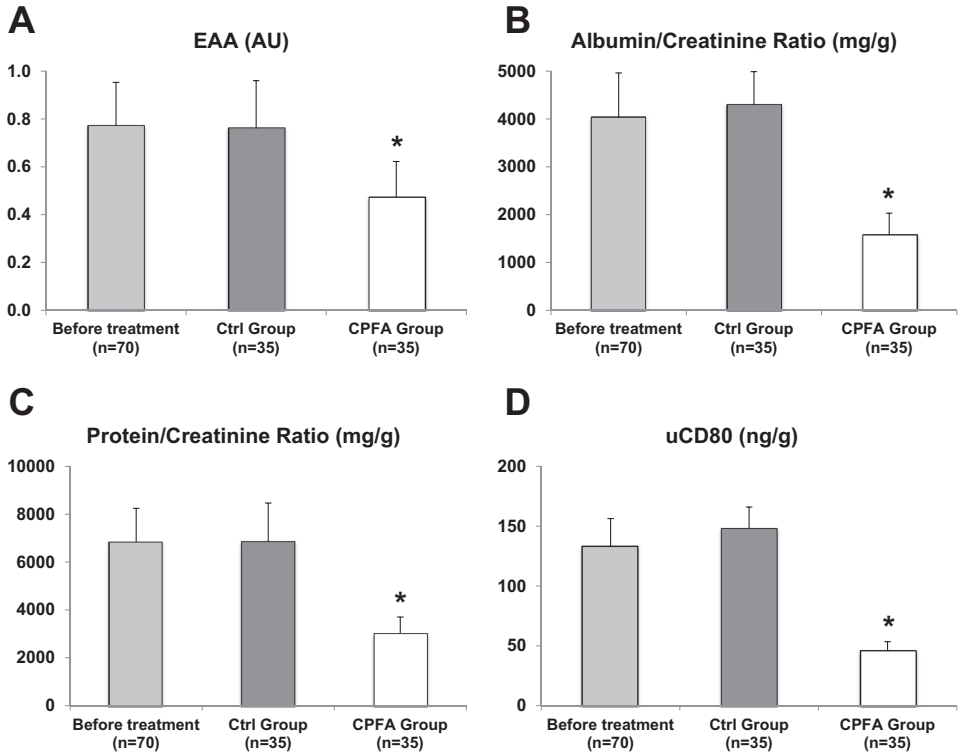

Fig. 3. Effects of coupled plasma filtration and adsorption (CPFA) on markers of Gram-negative infection and glomerular permeability. *A*: extracorporeal treatment with CPFA significantly reduced the levels of endotoxin activity assay [EAA; 0.77 ± 0.18 vs. 0.47 ± 0.15 arbitrary units (AU), $*P < 0.05$], while no differences were observed in the control group (0.77 ± 0.18 vs. 0.76 ± 0.20 arbitrary units, $P = 0.85$). *B* and *C*: reduction in circulating LPS levels by CPFA treatment induced a reduction in glomerular permeability to plasma proteins, as demonstrated by the reduction of proteinuria and albuminuria levels (6,834.6 ± 1,413.3 vs. 3,031.9 ± 670.8 mg/g of creatinine and 4,025.7 ± 935.5 vs. 1,581.7 ± 449.5 mg/g of creatinine for proteinuria and albuminuria before and after treatment, respectively, $*P < 0.05$); for instance, standard therapy did not affect glomerular permeability to proteins in the control group (6,834.6 ± 1,413.3 vs. 6,851.5 ± 1,621.8 mg/g of creatinine for proteinuria before and after treatment, $P = 0.61$; 4,025.7 ± 935.5 vs. 4,287.9 ± 829.3 mg/g of creatinine for albuminuria before and after treatment, $P = 0.84$). *D*: finally, the removal of LPS by CPFA induced a statistically significant reduction in urinary CD80 excretion in Gram-negative patients (133.2 ± 23.1 vs 46.5 ± 7.0 ng/g of creatinine for urinary CD80 before and after treatment, respectively, $*P < 0.05$, while no differences were observed after treatment in the control group (133.2 ± 23.1 vs 148.1 ± 17.8 ng/g of creatinine for urinary CD80 before and after treatment, $P = 0.96$).

DISCUSSION

In the present study, we demonstrated for the first time that LPS exposure in Gram-negative sepsis may induce albuminuria via podocyte expression of CD80. Selective removal of LPS reduced both albuminuria and CD80 expression in the experimental model as well as in the clinical setting, thus suggesting a possible role of this therapeutic approach in preventing the increased risk of progressive CKD in patients with septic AKI.

Among ICU patients, AKI is common, particularly the form associated with sepsis, and may lead to poor long-term outcomes (6, 38). Indeed, several observational studies report an increased incidence of progressive CKD among patients with a previous AKI. Current clinical practice guidelines recommend that patients should be followed-up for at least 3 mo to assess whether they may have developed CKD (21). Despite these recommendations, many patients with AKI do not receive a follow-up assessment nor do they receive appropriate care when kidney function has not recovered (14, 41), resulting in lost opportunities to intervene and potentially improve long-term outcomes (12). Many recent studies aimed to identify and screen patients at high risk of developing progressive CKD to improve outcomes for patients following AKI (7, 17). To this aim several prediction models have been built up, encompassing both clinical and laboratory variables (i.e., sex, age, and baseline serum creatinine). Among them, albuminuria has been proposed as an early marker of septic AKI (30) but also as a risk factor associated with lower rate of AKI recovery at 30 days after discharge (29). Recently, an investigation on a large Canadian cohort of patients confirmed albuminuria as one of the risk factors independently associated with CKD onset after AKI (18).

Our data confirm that during septic AKI due to Gram-negative infection, there is an increase glomerular permeability with subsequent albuminuria both in the experimental model and in the clinical clinical setting. In patients who survive after an AKI episode, albuminuria may represent a risk factor of progression toward CKD, as previously reported (33).

The pathophysiology of albuminuria onset during sepsis is still largely unclear. It has been suggested that the sepsis-related proinflammatory state alters tubular handling of filtered albumin and promotes albuminuria (20). Moreover, the release of several proinflammatory cytokines into the systemic circulation during sepsis might lead to a loss of endothelial cells barrier integrity and subsequent capillary leak. According to this hypothesis, albuminuria represents

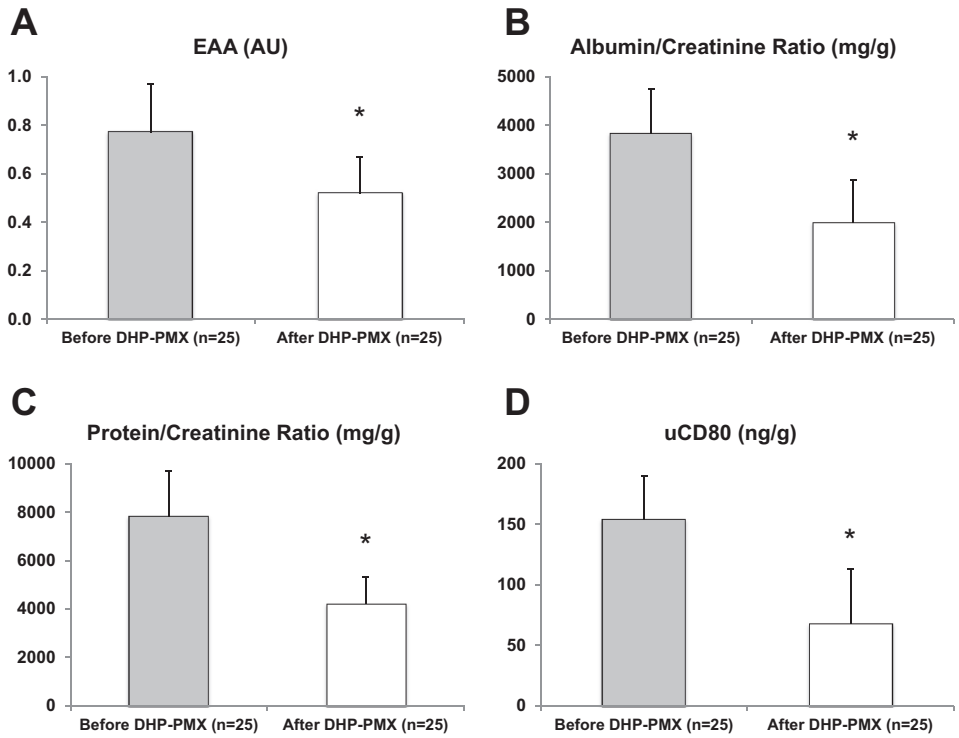

Fig. 4. Effects of direct hemoperfusion with polymyxin B (DHP-PMX) on markers of Gram-negative infection and glomerular permeability. *A*: extracorporeal treatment with DHP-PMX significantly reduced the levels of endotoxin activity assay [EAA; 0.77 ± 0.20 vs. 0.52 ± 0.15 arbitrary units (AU), $^*P < 0.05$]. *B* and *C*: the reduction in circulating LPS levels by DHP-PMX treatment induced a reduction in glomerular permeability to plasma proteins, as demonstrated by the reduction of proteinuria and albuminuria levels ($7,804.6 \pm 1,911.3$ vs. $4,209.1 \pm 1,080.9$ mg/g of creatinine and $3,825.7 \pm 935.1$ vs. $1,889.7 \pm 889.5$ mg/g of creatinine for proteinuria and albuminuria before and after treatment, respectively, $^*P < 0.05$. *D*: finally, the removal of LPS by DHP-PMX or CPFA induced a statistically significant reduction in urinary CD80 excretion in Gram-negative patients (154.2 ± 36.1 vs. 68.2 ± 45.1 ng/g of creatinine for urinary CD80 before and after treatment, respectively, $^*P < 0.05$).

the glomerular manifestation of this enhanced capillary permeability (19).

Another possible mechanism responsible of albuminuria is podocyte disruption and apoptosis. Podocytes have a peculiar structure and the slit diaphragms (SD) on the membranes of their FPs play an essential role in the glomerular filtration barrier. There is now growing evidence that several SD- and FP-associated molecules, including nephrin, podocin, and CD2-associated protein (CD2AP), are significantly downregulated during septic AKI (36).

More generally, LPS exposure may induce renal dysfunction and consequent maladaptive repair of septic AKI through suppression of mitochondrial biogenesis. In a mouse model of LPS-induced AKI, endotoxin exposure was able to disrupt mitochondrial homeostasis by downregulation of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and activation of the TLR-4/MEK/ERK pathway in the renal cortex (44).

The exposure to low-dose LPS in an animal model of sepsis, through direct stimulation of the TLR-4/CD14 receptor at podocyte level, may rapidly induce CD80 expression leading to nephrotic-range proteinuria (36). Indeed, CD80 expression on podocytes induces actin reorganization, FP effacement, and disruption of the SD complex due to sequestration of nephrin, CD2AP, and ZO-1, thereby modifying glomerular permselectivity and leading to proteinuria.

In our experimental model, exposure to LPS induced CD80 expression at podocyte level and an increase of proteinuria, while in septic patients a Gram-negative infection induced a significant urinary excretion of albumin and CD80. A selective removal of LPS with blood extracorporeal treatment not only reduced EAA but also decreased proteinuria along with CD80 expression and urinary excretion both in the animal model and in the clinical setting.

Of note this result was obtained not only in experimental group treated with CPFA but also in the historical control group treated with DHP-PMX. This finding underlines the beneficial effects of LPS removal, regardless of extracorporeal treatment choice, on serum endotoxin activity and urinary glomerular permeability to proteins, as well as on reduction of urinary CD80 excretion.

Our observation strongly suggests a link in experimental and clinical settings of a causal link between endotoxemia, CD80 expression by podocytes, and new onset albuminuria. LPS may also induce podocyte disruption through an indirect mechanism. It has been reported that during LPS-mediated sepsis, there is an increase of IL-1β and TNF-α released from activated macrophages infiltrating the glomeruli (19). These cytokines may directly suppress with a paracrine effect nephrin expression in podocytes, through the loss of nucleus-localized WT1, a transcriptional factor for upregulating nephrin gene (47). In our cohort of patients, the blood extracorporeal purification significantly reduces circulating levels of both IL-1β and TNF-α, among other proinflammatory cytokines. Thus we cannot exclude that the effect of LPS on CD80 expression and albuminuria may not be mediated by these two cytokines.

Whether LPS induces albuminuria through a direct or indirect action, our data clearly support the hypothesis that LPS effects on podocytes may represent a potential mechanism involved in maladaptive repair underlying the progression of AKI toward CKD (5). In this perspective, our observation may suggest that a timely removal of LPS through DHP-PMX and/or CPFA may reduce both urinary albumin and CD80 excretion, thus preventing the increased risk of progressive CKD in patients with septic AKI.

The sample size of our clinical study and the lack of follow-up data due to high mortality observed in our study group may significantly limits our observations, although their

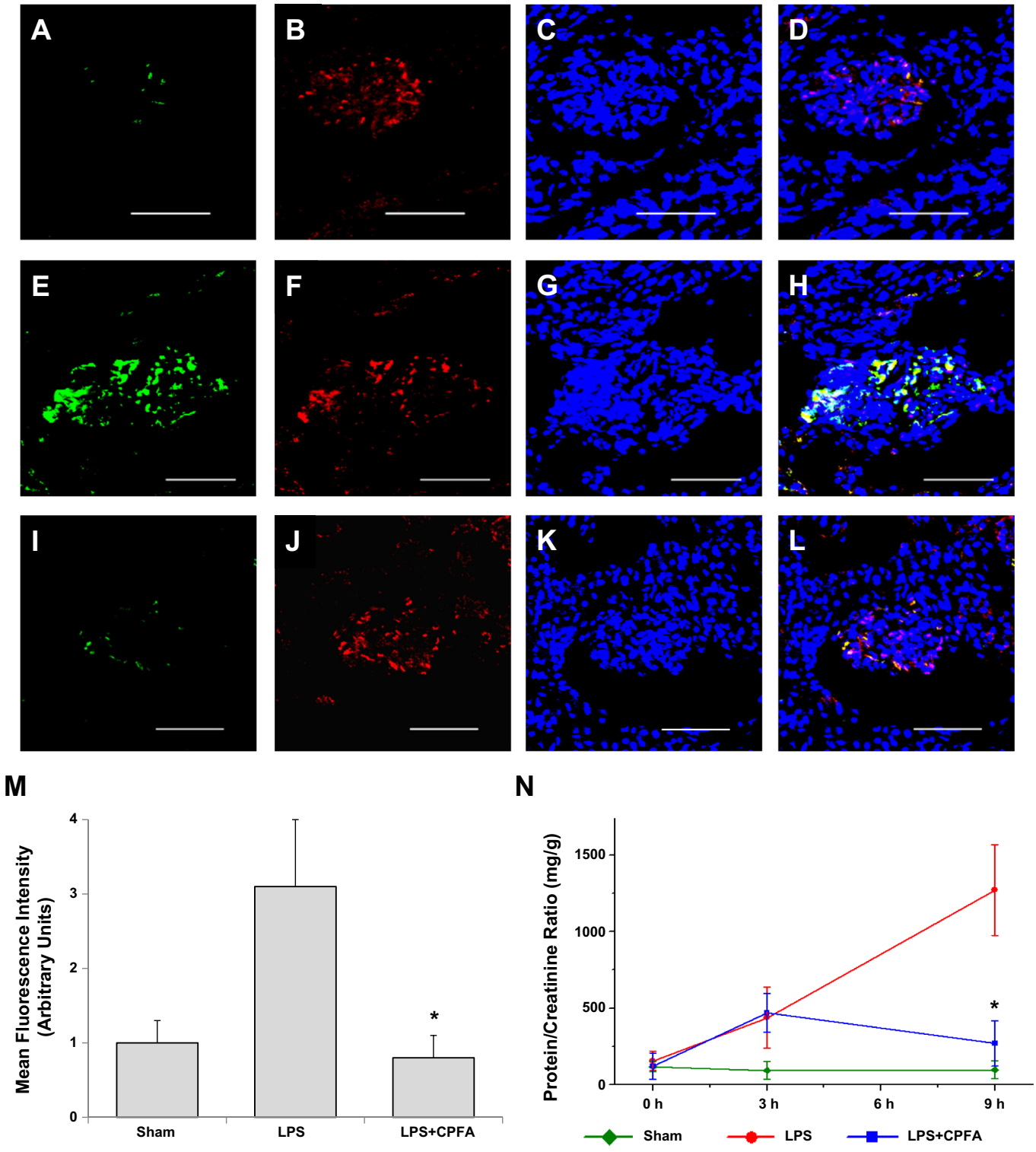

Fig. 5. Coupled plasma filtration and adsorption (CPFA) treatment reduces podocyte expression of CD80 and urinary protein excretion after LPS exposure in a pig model of acute renal damage. *A–D*: control pigs, which were not exposed to LPS (sham), did not express CD80 at podocyte levels. After 3 h from LPS infusion, endotoxemic animals were treated or not for 6 h with CPFA. *E–H*: the experimental group exposed to LPS, but not treated with CPFA, showed marked increase of CD80 expression (green) at the podocyte level, as demonstrated by the colocalization with the podocyte marker WT-1 (red). *I–L*: PFA treatment was able to reduce podocyte expression of CD80 (green) after LPS exposure, reaching a level comparable to the experimental group not exposed to LPS. Nuclei were stained with To-pro-3 (bar = 50 μm). *M*: analysis of mean fluorescence intensity (MFI) confirmed a reduction of CD80 expression after CPFA treatment in LPS-exposed pigs as compared with untreated septic group (0.8 ± 0.3 vs. 3.1 ± 0.9 arbitrary units for CPFA-treated septic pigs and LPS-exposed pigs, respectively; $*P < 0.02$). *N*: treatment with CPFAwas also able to reduce glomerular permeability to proteins in LPS-exposed pigs [268.8 ± 217.8 vs. 1,270.9 ± 895.7 mg/g of creatinine for proteinuria between CPFA-treated septic pigs (blue line) and LPS-exposed untreated pigs (red line), $*P < 0.05$].

confirmation in the animal model may support our conclusions. The strength of our swine model is represented by its several advantages over any rodent model used so far. Its main limit, on the other hand, is represented by the challenge to induce a multimicrobial sepsis, since pigs are particularly sensible to the severe hemodynamic instability featuring this model of severe sepsis.

Nevertheless, we believe that further clinical investigations are warranted to confirm our data.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.S.N. conceived and designed research; G.S.N., F. Sangregorio, A. Crovace, B.I., A.M., G. Godeas, G. Castellano, A.M.D.P., A. Cotoia, and L.M. performed experiments; G.S.N., F. Spadaccino, and C.P. analyzed data; F. Sangregorio, F. Spadaccino, B.I., A.M., G. Godeas, C.P., A. Cotoia, and L.M. interpreted results of experiments; F. Spadaccino and C.P. prepared figures; G.S.N., L.G., and G. Cinnella drafted manuscript; G.S., E.R., and G. Grandaliano edited and revised manuscript; G.S., E.R., and G. Grandaliano approved final version of manuscript.

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