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Lung inflammation is induced by renal ischemia and reperfusion injury as part of the systemic inflammatory syndrome

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Abstract
Introduction Ischemia and reperfusion injury (IRI) are mainly caused by leukocyte activation, endothelial dysfunction and production of reactive oxygen species. Moreover, IRI can lead to a systemic response affecting distant organs, such as the lungs.
Aim The objective was to study the pulmonary inflammatory systemic response after renal IRI.
Methods Male C57Bl/6 mice were subjected to 45 min of bilateral renal ischemia, followed by 4, 6, 12, 24 and 48 h of reperfusion. Blood was collected to measure serum creatinine and cytokine concentrations. Bronchoalveolar lavage fluid (BALF) was collected to determine the number of cells and PGE_2 concentration. Expressions of iNOS and COX-2 in lung were determined by Western blot. Gene analyses were quantified by real time PCR.
Results Serum creatinine increased in the IRI group compared to sham mainly at 24 h after IRI (2.57 ± 0.16 vs. 0.43 ± 0.07, $p < 0.01$). The total number of cells in BAL fluid was higher in the IRI group in comparison with sham, 12 h ($100 \times 10^4 \pm 15.63$ vs. $18.1 \times 10^4 \pm 10.5$, $p < 0.05$) 24 h ($124 \times 10^4 \pm 8.94$ vs. $23.2 \times 10^4 \pm 3.5$, $p < 0.05$) and 48 h ($79 \times 10^4 \pm 15.72$ vs. $22.2 \times 10^4 \pm 4.2$, $p < 0.05$), mainly by mononuclear cells and neutrophils. Pulmonary COX-2 and iNOS were up-regulated in the IRI group. TNF-α, IL-1β, MCP-1, KC and IL-6 mRNA expression were up-regulated in kidney and lungs 24 h after renal IRI. ICAM-1 mRNA was up-regulated in lungs 24 h after renal IRI. Serum TNF-α, IL-1β and MCP-1 and BALF PGE_2 concentrations were increased 24 h after renal IRI.
Conclusion Renal IRI induces an increase of cellular infiltration, up-regulation of COX-2, iNOS and ICAM-1, enhanced chemokine expression and a Th1 cytokine profile in lung demonstrating that the inflammatory response is indeed systemic, possibly leading to an amplification of renal injury.

Keywords Ischemia and reperfusion injury · INOS · COX-2 · IL-1β · Inflammation · Lung and kidney

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Introduction

Ischemia and reperfusion injury (IRI) is the main cause of acute renal failure (ARF) [1]. Although the mechanisms are

not well understood, inflammation plays a major role in its pathophysiology. The inflammatory response results in endothelial activation, enhancement in endothelial cell-leukocyte adhesion and a compromise of microvascular blood flow [2]. IRI up-regulates adhesion molecules in endothelial cells, promoting leukocyte adhesion and entrapment. Leukocytes can be activated by cytokines and reactive oxygen species (ROS). Different subgroups of leukocytes can contribute in different ways to IRI. Neutrophils have been the most widely studied inflammatory cell in IRI. Some studies have demonstrated that blocking the activation and migration of neutrophils ameliorates IRI [3–5]. Some works have demonstrated the role of CD4+ and CD8+ in renal IRI. Double knockout CD4/CD8 had renal protection from IRI [6]. Other studies suggest that the Th1 phenotype is deleterious and the Th2 phenotype is protective [7–9]. In addition to leukocyte and endothelial contribution to IRI, many inflammatory mediators are produced by the renal tubular epithelium thereby increasing inflammation. These include tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), interleukin-6 (IL-6), and chemokines, such as IL-8 and monocyte chemotactic protein-1 (MCP-1) [10–13].

Besides the local damage, these mediators could be involved in a systemic damage resulting from IRI. Studies have demonstrated that intestinal IRI is associated with acute lung injury, which is characterized by an increase in microvascular permeability and accumulation of neutrophils [14–16]. This increase in vascular permeability and neutrophil infiltration characterizes the adult respiratory distress syndrome (ARDS) in humans [17]. Acute renal failure is frequently combined with ARDS and this combination is associated with high mortality [18]. The mechanisms that result in this pulmonary dysfunction are not well established. In the present work, we intended to detail the pulmonary inflammatory systemic response in a mouse model of acute kidney injury.

Materials and methods

Animals

Isogenic male C57Bl/6, age 6–8 weeks (25–28 g) from our own facilities were housed in individual and standard cages and had free access to water and food. All procedures were previously reviewed and approved by the internal ethical committee.

Experimental model of IRI

Surgery was performed as previously described [19]. Mice were anesthetized with ketamine-xylazine (Agribrands do Brazil, São Paulo, Brazil). A midline incision was made and both renal pedicles were cross-clamped. In the ischemia period, animals were kept well hydrated with saline and at a constant temperature ($\sim$37°C) through a heating pad device. Forty-five minutes later, microsurgery clamps were removed, abdomen was closed in two layers and animals were placed in single cages, warmed by indirect light until complete recovery from anesthesia. Animals were kept under adjustable conditions until sacrifice at 4, 6, 12, 24 and 48 h of reperfusion. A total of 40 animals were used in this study. Eight animals were killed at each time point, where five were subjected to ischemia surgery, and three were subjected to the surgical procedure without clamping of renal pedicles (sham animals).

Analysis of renal function

Serum creatinine was used for evaluation of renal function after IRI. Blood samples were collected 6, 12, 24 and 48 h after ischemic surgery from the abdominal inferior vena cava immediately before induced death. Serum creatinine levels were determined by spectrophotometer (Spectramax190. Molecular Devices, Sunnyvale, CA, USA) readings at an absorbance of 520 nm using the modified Jaffé technique.

Bronchoalveolar lavage fluid

Bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. The animals were killed by an intraperitoneal injection of ketamine/xylazine (50 µl of a 100 mg/ml solution) at 4, 6, 12, 24 and 48 h of reperfusion. A tracheal cannula was inserted via a mid-cervical incision and the airways were washed twice with 1 ml of phosphate buffered saline (PBS, pH 7.4 at 4°C).

Total and differential cell count

BALF was centrifuged at 170*g* for 10 min at 4°C. The supernatant was removed and the cell pellet was resuspended in 0.5 ml of PBS. One volume of a solution containing 0.5% crystal violet dissolved in 30% acetic acid was added to nine volumes of the cell suspension. The total number of cells was determined by counting in a hematocytometer (Sigma-Aldrich, St. Louis, MO, USA). Differential cell counts were performed after cytocentrifugation (FANEM, Sao Paulo, Brazil) and staining with hematoxylin-eosin (H&E).

Western blot

Lung was collected after BAL and homogenized in 1 ml of cold PBS and lysed by sonication in ice-cold lyses buffer

containing 150 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaF, and 1 μg/ml leupeptin, followed by ultracentrifugation at 1,000*g* for 20 min at 4°C. Protein content in the supernatant of the lysed lung was determined using the BCA protein assay reagent kit (Thermo scientific pierce protein, Rockford, IL, USA), according to the manufacturer's protocol. Samples containing 20 μg protein were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane using the Bio-rad Mini-Gel system and trans-blot® SD-semidry Transfer cells (Bio-rad, Hercules, CA, USA). For immunoblotting, nitrocellulose membranes were incubated in Tris buffered saline with tween (TBS-T) (150 mM NaCl, 20 mM Tris, 1% Tween 20, pH 7.4) containing 5% non-fat milk dried milk, for 1 h. The blot was treated with a 1:1,000 dilution of rabbit polyclonal antibodies to COX-2 (Cayman Chemical, Ann Arbor, MI, USA) or rabbit antiserum to iNOS (Cayman Chemical, Ann Arbor, MI, USA) for 2 h at room temperature, then washed three times with TBS-T, and incubated with a 1:2,000 dilution of peroxidase-conjugated monoclonal anti-rabbit IgG (Cell Signaling technology, INC. Beverly, MA, USA) for 1 h at room temperature. Protein bands at 72 kDa (COX-2) or at 130 kDa (iNOS) were identified by comparison with Rainbow™ protein molecular weight markers (Amersham, Piscataway, NJ, USA). The immunocomplexed peroxidase-labeled antibodies were visualized by an ECL chemiluminescence kit following manufacturer's instruction (Amersham, Piscataway, NJ, USA) and exposed to photographic film (Kodak, Brazil). Finally, blots were stripped with 200 mM glycine, pH 3.0, for 10 min, washed with TBS-T three times for 30 min each, and reprobed with β-actin (Sigma, Chemical Co, St Louis, Mo, USA) 1:10,000, followed by anti-mouse secondary antibody (Cell Signaling technology, INC. Beverly, MA, USA) 1:2,000. The band densities were determined by densitometric analysis using the AlphaEaseFC™ software (Alpha innotech, San Leandro, CA, USA). Density values of bands were normalized to the total β-actin present in each lane and expressed as arbitrary units.

Quantification of prostaglandin (PG) E2

PGE_2 concentrations in BALF were determined by using EIA kits (Cayman Chemical Co., MI, USA), according to the method of Pradelles et al. [20]. Briefly, dilutions of the supernatants (1:10 and 1:100) were incubated with the conjugated eicosanoid-acetylcholinesterase and with the specific antiserum in 96-well plates precoated with anti-rabbit immunoglobulin G antibodies. After overnight incubation at 4°C, the plates were washed and the enzyme substrate (Ellman's reagent) was added for 60–120 min at 25°C. The optical density of the samples was determined at 412 nm in a microplate reader, and the concentration of eicosanoids was calculated from standard curve.

Gene profile

Lung and kidney samples were quickly frozen in liquid nitrogen. Total RNA was isolated from lung tissue using TRizol Reagent (Invitrogen, USA) methodology and RNA concentration was determined by spectrophotometer readings at an absorbance of 260 nm. First-strand cDNA were synthesized using the MML-V reverse transcriptase (Promega, Madison, WI, USA). All experimental protocols of real-time PCR were based on the manufacturer's recommendation using the TaqMan gold RT-PCR Core Reagents Kit (Applied Biosystems, Foster City, CA, USA). Primers and probes to hypoxanthine-guanine phosphoribosyltransferase (HPRT), IL-1β, tumor necrosis factor-alpha (TNF-α), IL-6, monocyte chemotactic protein-1 (MCP-1), IL-4, keratinocyte-derived chemokine (KC), chemokine C–C motif ligand 3 (CCL3) and intercellular adhesion molecule-1 (ICAM-1) were purchased from Applied Biosystems. Cycling conditions were as follows: 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. The amount of the target gene was normalized first to an endogenous reference (HPRT) and then relative to a calibrator (sample with the lowest expression, namely, sham-operated animals), using the $2^{-\Delta\Delta Ct}$ method. Hence, steady-state mRNA levels were expressed as an n-fold difference relative to the calibrator. Analyses were performed with the Sequence Detection Software 1.9 (SDS).

Cytokine assay

A Bioplex (Millipore, Billerica, MA, USA) mouse cytokine assay kit and the Bioplex 200 Suspension Array System/Luminex (Bio-Rad, Hercules, CA, USA) were used to measure TNF-α, IL-1β, MCP-1 and IL-6 in serum. The kit was used according to the manufacturer's instructions. The data were analyzed using Bio-Plex Manager software version 4.0. Standard curves ranged from 1.95 to 32,000 pg/ml. The lower limit of detection for each cytokine was as follows: 3.36 pg/ml for TNF-α, 5.53 pg/ml for IL-6, 5.56 pg/ml for IL-1β, and 20.6 pg/ml for MCP-1.

Statistical analyses

All data were described as mean ± SEM. Statistical evaluation of the data was carried out by a nonparametric test (Mann–Whitney test). A p value lower than 0.05 was considered to be significant. All statistical analyses were

performed with the aid of Sigma Stat Software 2.0 (Jandel Corporation, TX, USA).

Results

Renal dysfunction

C57Bl/6 mice were subjected to 45 min of a renal ischemic surgery. After 6, 12, 24 and 48 h of reperfusion, blood was collected to evaluate renal function by measurement of serum creatinine. The ischemic group presented a higher increase in serum creatinine in comparison to their controls (sham-operated animals), 6 h (1.3 ± 0.2 vs. 0.6 ± 0.1 mg/dl; $p < 0.05$), 12 h (1.7 ± 0.2 vs. 0.5 ± 0.2 mg/dl; $p < 0.05$), 24 h (2.57 ± 0.16 vs. 0.43 ± 0.07 mg/dl, $p < 0.05$) and 48 h (1.7 ± 0.5 vs. 0.6 ± 0.1 mg/dl) after reperfusion. These results showed that IRI caused a severe renal dysfunction (Fig. 1).

Cells in BALF after IRI

To analyze the systemic effects of renal IRI in the lungs, BALF analyses were performed at 4, 6, 12, 24 and 48 h after a renal ischemic surgery. No difference in total numbers of cells could be observed within 4 or 6 h of reperfusion, whereas, with 12, 24 and 48 h, the number of cells was significantly higher in ischemic groups in comparison to sham-operated animals (12 h: $100 \times 10^4 \pm 15.6$ vs. $18.1 \times 10^4 \pm 10.5$, $p < 0.05$; 24 h: $124 \times 10^4 \pm 8.9$ vs. $23.2 \times 10^4 \pm 3.5$, $p < 0.05$, 48 h: $79 \times 10^4 \pm 15.7$ vs. $22.2 \times 10^4 \pm 4.2$, $p < 0.05$). The differential number of cells showed that mononuclear cells were increased in the ischemic group compared to sham-operated at 12 h ($99.2 \times 10^4 \pm 15.2$ vs. $18 \times 10^4 \pm 1.2$; $p < 0.05$), 24 h ($114.9 \times 10^4 \pm 8.2$ vs. $23 \times 10^4 \pm 2.5$; $p < 0.05$) and 48 h ($77 \times 10^4 \pm 21$ vs. $22 \times 10^4 \pm 2.1$; $p < 0.05$). Neutrophil cells were increased only within 24 h ($9.1 \times 10^4 \pm 4.5$ vs. $0.2 \times 10^4 \pm 0.1$; $p < 0.05$) (Fig. 2).

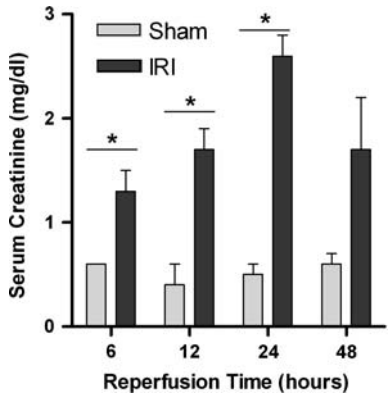

Fig. 1 Renal function**.** Renal ischemia and reperfusion injury (IRI) was carried out by 45 min bilateral clamping of renal pedicles, followed by 6, 12, 24 and 48 h of reflow. Serum creatinine was measured at reperfusion times. All serum creatinine levels were increased in IRI group when compared to sham-operated (control). Values were expressed by mean ± SEM ($n = 3$ sham; $n = 5$ IRI). Statistical analyses were performed by Mann–Whitney test. *$p < 0.05$ comparing to sham group

Expression of mRNA of adhesion molecule, cytokines and chemokines in kidney and lung after renal IRI

We assessed the expression of ICAM-1 by real-time PCR in the lungs and in the kidneys, an important adhesion molecule expressed by endothelial cells under activation. At 24 h after renal IRI, ICAM-1 was up-regulated in the lungs in comparison to sham (Fig. 3). Moreover, we investigated whether the expression of some chemokines important for migration of neutrophils and monocytes to injured sites would be involved in this acute injury. Indeed, MCP-1 and KC were highly up-regulated in lungs after renal ischemia. CCL3 did not increase significantly either in kidneys or in lungs after renal ischemia.

Expressions of IL-1β, TNF-α and IL-6 were up-regulated in both kidney and lung, simultaneously, in comparison to control, whereas IL-4 was down-regulated (Fig. 3).

Expression of COX-2 and iNOS in pulmonary tissue

Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), were quantified in lung after renal IRI by western blot. For this, lung was collected at 24 h after the renal ischemia surgery. The density of bands in western blot analysis demonstrated that both molecules were increased in ischemic groups at 24 h when compared to sham-operated animals (COX-2: $1{,}084 \pm 97.1$ vs. 129 ± 10 AU; iNOS: $1{,}172 \pm 126.2$ vs. 529 ± 59 AU) (Fig. 4).

In addition, we measured one of the COX-2 products, prostaglandin E2 (PGE_2), in BALF 24 h after renal ischemia surgery. We observed that PGE_2 was highly up-regulated after ischemia in comparison to control animals (Fig. 5).

Serum cytokines after renal IRI

After 24 h from renal IRI, pro-inflammatory mediators TNF-α, IL-1β, MCP-1 and IL-6, were quantified in serum. Serum levels of TNF-α, IL-1β, MCP-1 were significantly increased after IRI. Although serum IL-6 seems to increase after IRI, it was not statistically different from sham (Fig. 6).

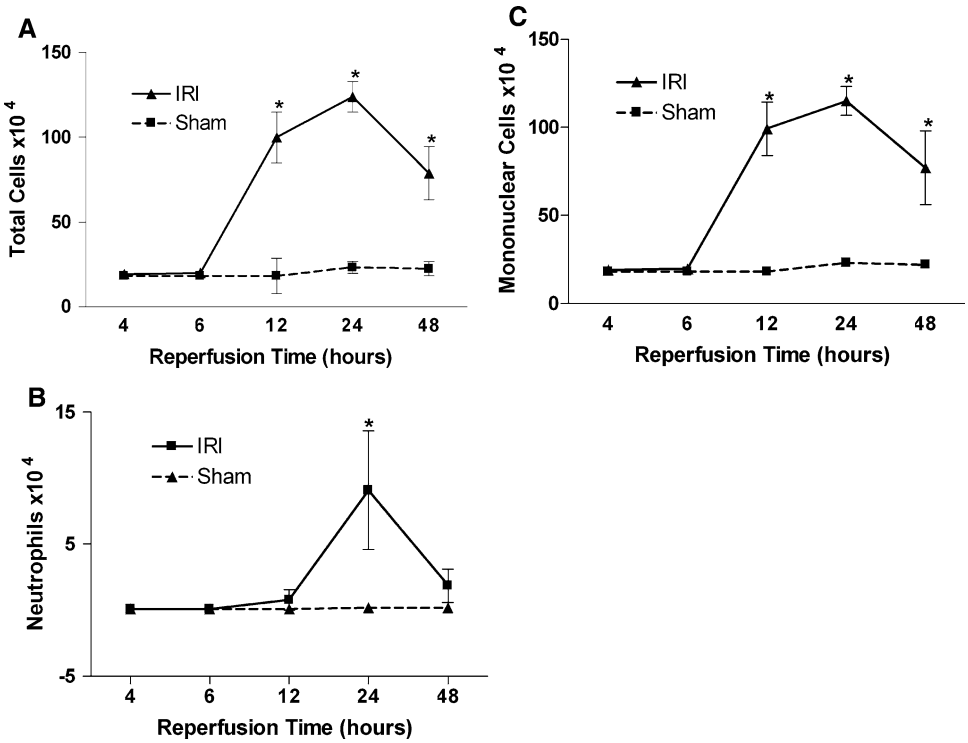

Fig. 2 Total cells in bronchoalveolar lavage fluid. After 4, 6, 12, 24 and 48 h from IRI surgery, bronchoalveolar lavage fluid (BALF) was processed and the total number of cells was determined by hematocytometer. Values were expressed by mean ± SEM ($n = 3$ sham; $n = 5$ IRI) and statistical analyses were performed by Mann–Whitney test. $^*p < 0.05$ comparing to sham-operated group

Discussion

ARF is associated with decreased allograft survival in transplanted kidney recipients and with high mortality in patients with native kidneys [21, 22]. The major cause of ARF is ischemia and reperfusion injury (IRI). The pathogenesis of renal IRI is complex and still not fully understood, but inflammation is currently accepted as an important pathogenic component [2]. IRI results in endothelial and leukocyte activation, production of ROS, tubular cell death and release of inflammatory mediators, such as cytokines and chemokines [2]. Besides the local damage caused by IRI, distant organs can also be affected [23–25]. Although many studies have been performed to demonstrate the systemic effect of IRI, the mechanism is not well established. In this work we used a mouse model of renal IRI to study these inflammatory systemic effects in the lungs. C57Bl/6 mice were subjected to 45 min of renal ischemia injury, and lungs were studied at different time points of reperfusion.

In order to analyze the cellular infiltration in the lungs after renal IRI, we performed BALF analyses at different times after reperfusion. We demonstrated that the cellular infiltrate in the lungs was increased simultaneously to the rise in serum creatinine levels. Moreover, it was mainly constituted of mononuclear and neutrophil cells. Another study demonstrated that there was a neutrophil accumulation in the lungs following intestinal IRI [15]. In our model, the amount of mononuclear cells was found to be increased at an early time point (12 h after reperfusion) instead of neutrophils. This could imply that resident mononuclear cells actually first migrate to the pulmonary alveolus, elevating the cell number count. A few hours later, neutrophils and more mononuclear cells migrate from the bloodstream to the lungs. Although another possibility is that neutrophils are migrating first from bloodstream to the lungs but reaching the bronchial space after mononuclear cells. This kinetic might be influenced by the secretion of different chemokines and by the state of chemokine receptor on those cells [26]. Here, we could see that KC, a neutrophil-induced chemokine, and MCP-1, macrophage chemokine, were highly up-regulated in kidneys and in lungs after renal ischemia. It is not well understood which mechanism leads to cellular infiltration in the lungs after a renal IRI. One possibility is the contribution of alveolar endothelial cell (EC) activation in the lungs after the systemic release of pro-inflammatory cytokines.

EC activation is a hallmark of renal IRI [2] and is always present in other ischemic organs. Once activated, the EC up-regulates some molecules and changes its anti-coagulant phenotype, promoting adhesion and favoring transmigration of polymorphonuclear cells. We assessed endothelial activation by analyzing the expression of a specific adhesion molecule, ICAM-1, in the lung after renal IRI. We observed that 24 h after renal IRI, ICAM-1 mRNA expression was up-regulated in lungs, suggesting an endothelial activation. We also studied two molecules that could be associated with endothelial dysfunction and cellular infiltration: COX-2 and

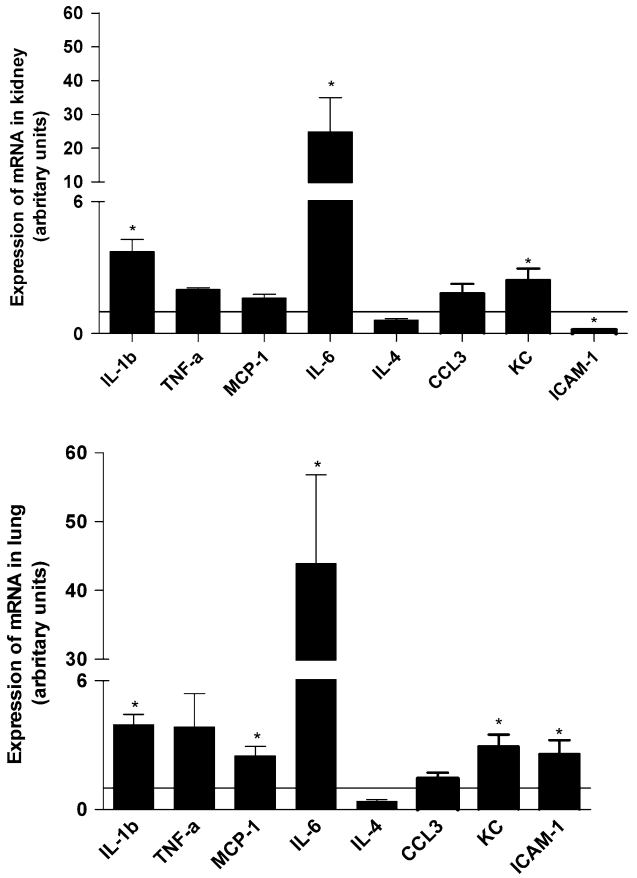

Fig. 3 Cytokine expression in kidney and lung after IRI. Interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-4 (IL-4), chemokine C–C motif ligand 3 (CCL3), keratinocyte-derived chemokine (KC) and intercellular adhesion molecule-1 (ICAM-1) messenger RNA (mRNA) expression were evaluated by real-time polymerase chain reaction (PCR). Kidneys and lungs were harvested 24 h after the ischemic insult. Samples were normalized by the endogenous gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) and then the sham group was used as a calibrator, using $2^{-\Delta\Delta Ct}$ method. *Bars* represent mean ± SEM ($n = 5$ IRI). *Solid line* represents $2^{-\Delta\Delta Ct}$ from sham group ($n = 3$). Statistical analyses were performed by Mann–Whitney test. *$p < 0.05$ comparing to sham-operated group

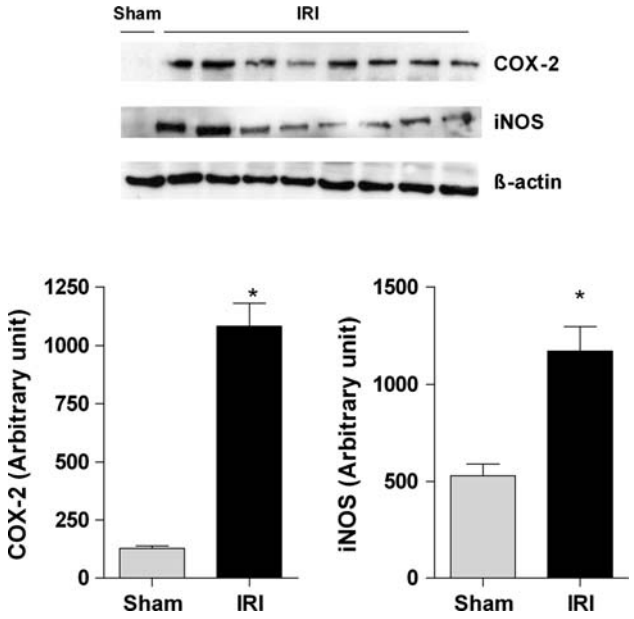

Fig. 4 Expressions of COX-2 (**a**) and iNOS (**b**) in pulmonary tissue. Lungs were collected 24 h after renal IRI to quantify the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) by western blot. Graphs represent the density values of bands that were determined by densitometric analysis and normalized by the total β-actin. Expression of COX-2 and iNOS was increased in the IRI group. All data were expressed by mean ± SEM ($n = 3$ sham; $n = 5$ IRI) and statistical analyses were performed by Mann–Whitney test. *$p < 0.05$ comparing to the sham-operated group

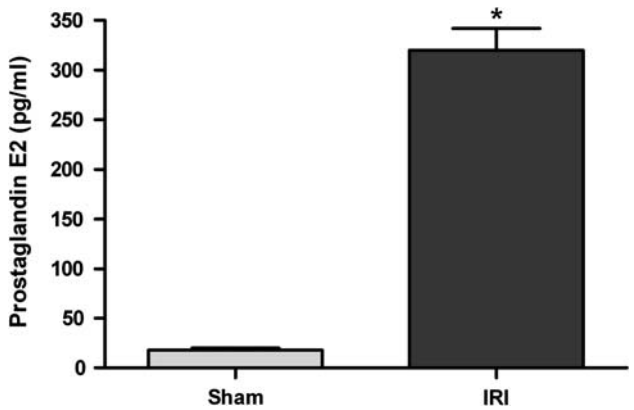

Fig. 5 Prostaglandin E2 in BALF. After 24 h from renal IRI, bronchoalveolar lavage fluid (BALF) was collected and prostaglandin E2 concentration was measured as described in "Materials and methods". All data were expressed by mean ± SEM ($n = 3$ sham; $n = 5$ IRI) and statistical analyses were performed by Mann–Whitney test. *$p < 0.05$ comparing to sham-operated group

iNOS. COX is a key enzyme to generate prostaglandins from arachidonic acids and iNOS to produce nitric oxide (NO). Levels of both enzymes are low in EC but readily up-regulated by inflammatory cytokines such as IL-1β and TNF-α [27, 28]. We have previously demonstrated that COX-2 is important to renal damage after IRI, since the blockage of COX-2 ameliorates IRI [29]. Although the role of NO in IRI is still controversial, previous studies have demonstrated that both NOS isoforms exert different modulator effects in lung after an intestinal IRI. The constitutive NOS is protective in intestinal IRI, whereas the inducible NOS is responsible for the lung dysfunction [30–32]. In addition, other authors have described that COX-2 and iNOS are up-regulated in ischemic kidneys and have a deleterious participation in renal IRI [33, 34]. Studies have demonstrated that in a sepsis-induced acute lung injury, the iNOS produced by neutrophils and macrophages, but not produced by the endothelial cells, play an important role in the microvascular dysfunction [35, 36]. Here, we observed an

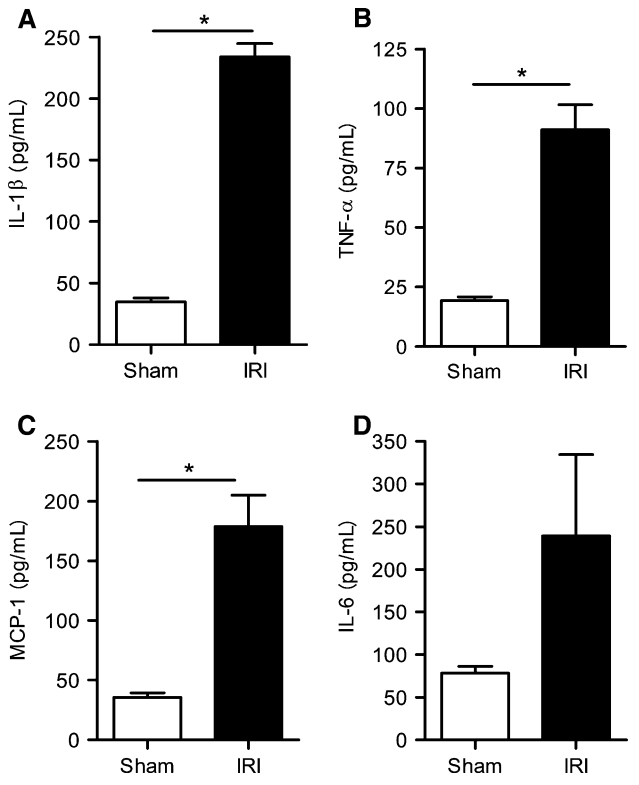

Fig. 6 Serum cytokines 24 h after renal IRI. Interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1) and interleukin-6 (IL-6) (**a**–**d**) concentrations in serum (pg/ml) were quantified by Bioplex, as described in "Materials and methods". Values were expressed by mean ± SEM ($n = 3$ sham; $n = 5$ IRI). Statistical analyses were performed by Mann–Whitney test. *$p < 0.05$ comparing to the sham-operated group

endothelial activation and up-regulation of ICAM-1, COX-2 and iNOS in the lungs after renal IRI, which might be contributing to cellular infiltration. Although further studies are necessary to determine in this model whether this iNOS is being produced by endothelial cells, neutrophils or macrophages.

In addition, PGE_2, one of the products formed by COX-2, was increased in BALF 24 h after renal IRI. Lipid mediators such as prostaglandins are involved in the pathogenesis of asthma where they contribute to early events such as inflammatory cellular infiltration, bronchial hyper-reactivity and mucus secretion [37]. The production of prostaglandins in the airways is well known and documented, but it is not clear whether these mediators play a deleterious or a beneficial role in airway diseases. PGE2 was shown to have a protective effect on allergen-induced [38] and aspirin-induced asthma [39], but other studies have pointed out an inverse correlation between eosinophilic airway inflammation and PGE2 concentration in induced sputum from asthmatic subjects, which supported a possible anti-inflammatory role of PGE2 [40]. Moreover, several studies have suggested that PGE2 may enhance eosinophil survival, providing a potential pro-inflammatory activity [41, 42]. Landgraf et al. [43] showed that the use of prostaglandin synthesis inhibitors reduced significantly the influx of neutrophils, eosinophils and lymphocytes in the BALF of sensitized C57Bl/6 mice, 24 h after the second antigen challenge. Until now, no study has reported the systemic increase in expression of COX-2 in lungs after a renal IRI.

Some studies have demonstrated that a Th1 pattern in IRI is deleterious, while a Th2 pattern is protective. IL-4, a major Th2 cytokine, is a protective factor in renal IRI, since IL-4 KO mice had an impaired renal function compared to wild-type [9]. We analyzed in the kidneys and the lungs the expression of some pro-inflammatory Th1 cytokines and chemokines, such as IL-1β, TNF-α, MCP-1 and IL-6, and a Th2 cytokine, IL-4. We observed that expressions of all pro-inflammatory cytokines analyzed were increased in the kidneys and lungs 24 h after renal IRI, whereas IL-4 expression was diminished. It suggests that, likely in the kidneys, the systemic response that affects lungs is a Th1 pattern. In addition to that, we measured IL-1β, TNF-α and MCP-1 in the serum and observed that they were increased in the serum 24 h after renal IRI. We did not observe a significant increase in serum IL-6 24 h after IRI. According to a previous study, IL-6 reaches its peak in serum by 4 and 8 h after renal IRI, decreasing to baseline levels by 24 h [44]. Some works have suggested that IL-1β and TNF-α could lead to damage in distant organs by the lymphatic thoracic duct. After an intestinal IRI in rats the ligation of the thoracic lymphatic duct decreased the serum levels of IL-1β and TNF-α when compared to rats with intact duct. Consequently, the damage in pulmonary tissue was reduced [45, 46].

Taking all data into consideration, we assume that these pro-inflammatory mediators, TNF-α, IL-1β and MCP-1, released by the ischemic kidney might reach the lungs, induce inflammation, up-regulate COX-2 and iNOS expressions, and ultimately contribute to the accumulation and to activation of neutrophils and mononuclear cells. In addition, we have demonstrated that the pattern of cytokines and chemokines expressed in the lungs is similar to that expressed in kidneys after ischemia injury.

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Conflict of interest statement The authors have no conflict of interest.

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