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Modulation of inflammatory response by selective inhibition of cyclooxygenase-1 and cyclooxygenase-2 in acute kidney injury

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Abstract
Objective and design This work explored the role of inhibition of cyclooxygenases (COXs) in modulating the inflammatory response triggered by acute kidney injury.
Material C57Bl/6 mice were used.
Treatment Animals were treated or not with indomethacin (IMT) prior to injury (days -1 and 0).
Methods Animals were subjected to 45 min of renal pedicle occlusion and sacrificed at 24 h after reperfusion. Serum creatinine and blood urea nitrogen, reactive oxygen species (ROS), kidney myeloperoxidase (MPO) activity, and prostaglandin E2 (PGE_2) levels were analyzed. Tumor necrosis factor (TNF)-α, t-bet, interleukin (IL)-10, IL-1β, heme oxygenase (HO)-1, and prostaglandin E synthase (PGES) messenger RNA (mRNA) were studied. Cytokines were quantified in serum.
Results IMT-treated animals presented better renal function with less acute tubular necrosis and reduced ROS and MPO production. Moreover, the treatment was associated with lower expression of TNF-α, PGE_2, PGES, and t-bet and upregulation of HO-1 and IL-10. This profile was mirrored in serum, where inhibition of COXs significantly decreased interferon (IFN)-γ, TNF-α, and IL-12 p70 and upregulated IL-10.
Conclusions COXs seem to play an important role in renal ischemia and reperfusion injury, involving the secretion of pro-inflammatory cytokines, activation of neutrophils, and ROS production. Inhibition of COX pathway is intrinsically involved with cytoprotection.

Keywords Cyclooxygenase · Renal ischemia and reperfusion injury · Cytokines · Heme oxygenase 1

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Introduction

Eicosanoids are compounds derived from eicosatetraenoic or arachidonic acid (AA), an essential fatty acid with 20 carbon atoms and four double bonds, one of the major components of the membrane phospholipids of mammals. Activation of phospholipases generates free AA which can be converted to prostanoids, leukotrienes, and epoxyeicosatrienoic acid by specific enzymes [1]. Prostanoids are generated via cyclooxygenases [2] or prostaglandin H (PGH) synthases, which possess two catalytic functions:

one related to COX activity, which incorporates molecular oxygen into AA, generating prostaglandin G2 (PGG_2), and the other related to peroxidase activity, which reduces PGG_2 to PGH_2. Two isoforms of COX are well described. COX-1 is the constitutive enzyme originally found in bovine prostate [3]. Ferreira and Vane in 1971 suggested that the mechanism of the anti-inflammatory effect of nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin (IMT) was through inhibition of prostaglandins synthesis [4]. With the discovery in 1990 of a COX induced by inflammatory stimuli (COX-2) a series of compounds were developed which showed greater selectivity for this enzyme. The polypeptide sequences of COX-1 and COX-2 are approximately 60% similar, although their regulation is quite different [3]. However, it was later found that COX-2 is expressed constitutively in certain tissues (vascular endothelium, respiratory epithelium, central nervous system) and is thus responsible for prostanoids synthesis for homeostatic functions [5].

Blockade of COX has been associated with functional and histological improvement after ischemia and reperfusion injury (IR) in various organs [6–9]. In brain IR, Candelario-Jalil et al. [9] demonstrated that inhibition of COX-2 isoforms was associated with less cell damage and leukocyte infiltration. We have previously confirmed that COXs are upregulated after renal IR and that therapy with IMT prior to injury induced protection. Moreover, COX inhibition was also able to lessen renal fibrosis triggered by acute injury [10, 11].

The mechanism involved in the protection induced by COX inhibition is not completely elucidated. Here, we hypothesized that immune modulation is one possible mechanism. To address this question, we performed acute renal injury in mice pretreated with IMT and investigated the cytokines profile in serum and pro-inflammatory-related molecules in renal tissue. We anticipated that COX inhibition would lead to downregulation of pro-inflammatory cytokines concomitantly with induction of cytoprotection and improvement in renal function and histology.

Materials and methods

Animals

Isogenic male C57BL/6 mice, aged 8–12 weeks (25–28 g), were purchased from the Animal Facilities Center of the Federal University of São Paulo (UNIFESP), Brazil. All animals were housed in individual standard cages and had free access to water and food. All procedures were previously reviewed and approved by the internal Ethics Committee of the institution.

Experimental model of renal IR

Surgery was performed as previously described [10]. Mice were briefly anesthetized with ketamine-xylazine (intraperitoneal; 100 mg/kg, 10 mg/kg; Agribrands do Brazil, São Paulo, Brazil). A midline incision was made and both renal pedicles were clamped for 45 min. During the procedure, animals were kept at constant temperature (37°C) by a heated pad device. Exposed viscera were kept well hydrated with 200 µl 0.9% saline solution. Subsequently, microsurgery clamps were removed, the abdomen was closed, and animals were placed in single cages, warmed by indirect light until completely recovered from anesthesia. Animals were kept under adjustable conditions (temperature and humidity) until sacrifice, according to experimental protocol at 24 h after renal reperfusion.

Drug

Initially, a dose–response curve was established to determine the best dose of IMT (Sigma, St. Louis, MO, USA). For this, three doses were chosen: 0.5, 2, and 5 mg/kg. Animals ($N = 6$) were divided into different groups and treated with the stated doses 1 day before and at the day of surgery. Animals treated with the highest dose presented a significant decrease in serum creatinine at 24 and 48 h of reperfusion (Fig. 1). So, for further experiments mice were treated ($N = 8$) or not with IMT ($N = 8$) at 5 mg/kg/day [12, 13] as previously described. Sham-operated animals ($N = 5$) were included as controls.

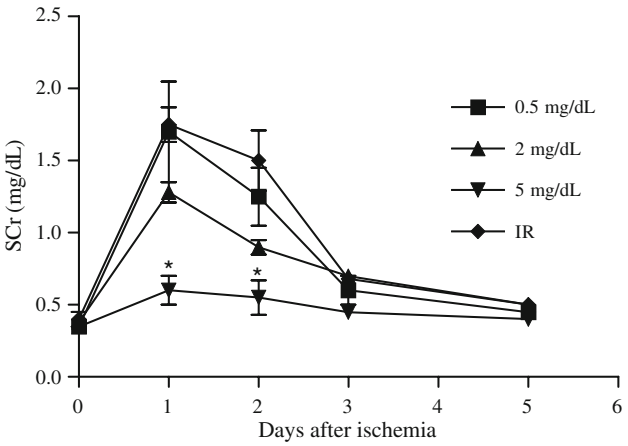

Fig. 1 Dose-response curve of indomethacin. Renal function (serum creatinine, SCr) was assessed 24, 48, 72, and 120 h after reperfusion in animals treated with three different doses of indomethacin (0.5, 2, and 5 mg/kg). Animals only subjected to ischemia were also studied and sacrificed at the same reperfusion time. In each experiment, a total of six animals were studied. Results are expressed as mean and standard deviation (SD). Statistical significance was tested by using a *t*-test. $*P < 0.05$ in IR group versus animals treated with 5 mg/kg, at 24 and 48 h after reperfusion

Functional analyses

Blood and kidney samples were collected at 24 h after IR. *Assessment of renal function:* Blood was collected for blood urea nitrogen (BUN) and serum creatinine measurements. Five hundred microliters were collected from cava vein by 13 × 4.5-gauge needle under anesthesia. After that, blood was centrifuged at 9,696×*g* for 10 min to separate serum, which was then frozen at −20°C until use. Kidneys were harvested for histological and mRNA analyses at the end point. *Renal morphometric analyses*: Morphometric evaluation was performed in a blinded manner by a single observer. The presence of acute tubular necrosis (ATN) was estimated in 4-μm-thick hematoxylin and eosin-stained sections. All microscopic fields of each slide were examined at final magnification of 250×. Tubular injury was evaluated using a semiquantitative scale, on which the percentage of cortical and outer medulla tubules showing epithelial necrosis was assigned a score as follows: 0 for ≤10%, 1 for 10–25%, 2 for 26–75%, and 3 for ≥75%. The mean of all area analyzed was plotted and compared between groups.

Reactive oxygen species production

To study reactive oxygen species (ROS) production, kidney tissue extracts were incubated with 2′7′-dichlorofluorescein diacetate (DCFH-DA 0.3 mM, Sigma, St. Louis, MO, USA) in phosphate-buffered saline (PBS; Sigma, Co., USA), with agitation for 30 min at 37°C in a shaking water bath. Then 2 ml ethylenediamine tetraacetic acid (EDTA, Sigma, St. Louis, MO, USA) was added to terminate the reaction. After centrifugation, erythrocytes were removed by hypotonic lysis, and the cell pellet was resuspended in 1.0 ml 3 mM EDTA in PBS. Intracellular DCFH fluorescence was studied by flow cytometry; its value relates to ROS production. Histograms of fluorescence intensity were constructed for each tube and the geometric mean of the fluorescence intensity (mean fluorescence intensity, MFI) of DCFH in the population of cells was determined. Samples were acquired in a fluorescence-activated cell sorting (FACS) Calibur flow cytometer (BD Biosciences, USA).

Gene profiles

Kidney samples were quickly frozen in liquid nitrogen. Total RNA was isolated from kidney tissues using TRIzol reagent (Invitrogen, USA) methodology and RNA concentration was determined by spectrophotometer absorbance readings at 260 nm. First-strand complementary DNAs (cDNAs) were synthesized using Moloney murine leukemia virus (MML-V) reverse transcriptase (Promega, USA). Reverse-transcription polymerase chain reaction (RT–PCR) was performed using the TaqMan Green real-time PCR assay (Applied Biosystem, USA) and primers specific to pro- (TNF-α, Mm99999068_m1; t-bet, Mm01299454_g1; IL-1β, Mm01336189_m1) and anti-inflammatory cytokines (IL-10, Mm99999062_m1; HO-1, Mm00516007_m1, a protective gene). Prostaglandin E synthase 2 (PGES, Mm01327277_g1) mRNA was also quantified. Hypoxanthine phosphoribosyl transferase (HPRT) was used for housekeeping (Mm01545399_m1). Analyses were performed by using Sequence Detection Software 1.9 (SDS). mRNA expression was normalized to HPRT abundance. Values are expressed relative to a reference sample (the calibrator), i.e., IMT-treated sham-operated samples. Triplicates of each sample were done. The relative expression of mRNA expression was calculated by $2^{-\Delta\Delta CT}$. All the experimental samples are expressed as *n*-fold difference relative to the calibrator.

Bio-Plex

For the cytokine assays, custom Bio-Rad (Bio-Rad laboratories, Hercules, CA) Bio-Plex cytokine analysis kits were used in conjunction with the Bio-Plex system array reader according to the manufacturer's directions. The specific cytokines interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin 12 p70 (IL-12 p70), granulocyte monocyte colony stimulating factor (GM-CSF), and interleukin 10 (IL-10) were chosen to be representative of injury in ischemia. Samples were run in triplicate in each assay. Standard curves for each of the analyzed substances were included in each run, and sample concentrations were calculated using Bio-Plex Manager software.

Quantification of prostaglandin (PG)E_2

Prostaglandin E2 (PGE_2) concentrations were determined by using EIA kits (Cayman Chemical Co., MI, USA), according to the method described by Pradelles et al. in 1985 [14]. Renal tissues were homogenized with a Polytron homogenizer (Kinematica AG, Littau-Lucerne, Switzerland) in 1 ml 0.1 M phosphate buffer pH 6.2 containing 0.5% hexadecyltrimethyl ammonium bromide and 5 mM EDTA (Sigma, St. Louis, MO, USA). The samples were then sonicated five times (each for 10 s) at 40 Hz and centrifuged at 3,000×*g* for 30 min at 4°C. 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

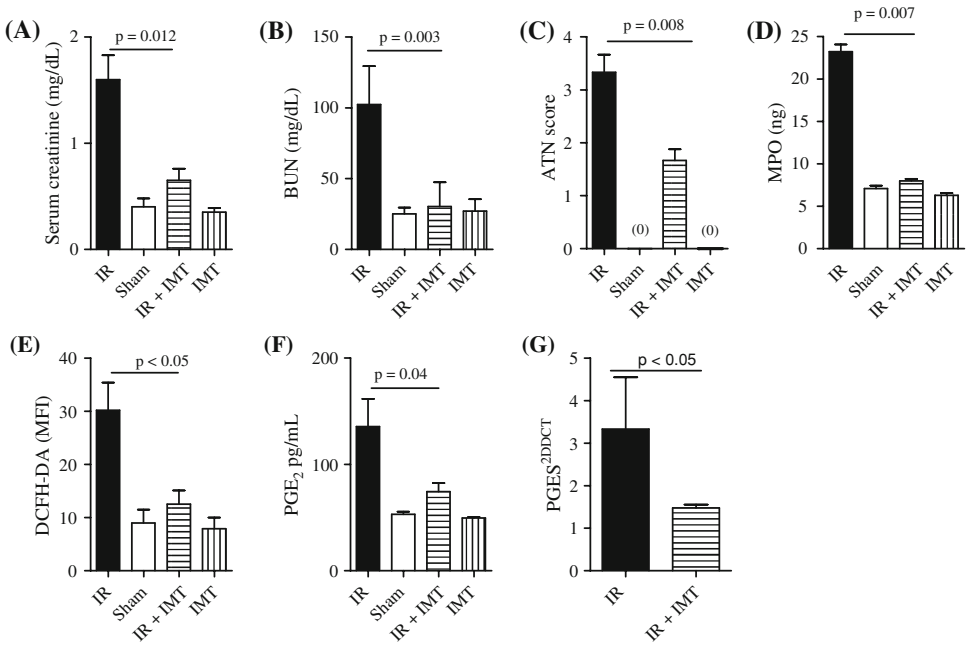

Fig. 2 Functional data. Renal function (serum creatinine in panel **a**; BUN in panel **b**) and percentage of tubular necrosis (ATN) (**c**) were assessed 24 h after reperfusion in animals treated or not with indomethacin. At this time, MPO (**d**), ROS estimated by DCFH fluorescence values (**e**), PGE_2 (**f**), and PGES (**g**) were measured in kidney samples. Data are presented as mean and standard deviation (DP). In each experiment, a total of eight animals were studied. In sham-operated and IMT-treated sham-operated groups, five animals were studied. Results are expressed as mean and standard deviation (SD). Statistical significance was tested by using a *t*-test. *BUN* blood urea nitrogen, *IMT* indomethacin, *PGE2* prostaglandin E2, *PGES* prostaglandin E synthase, *MPO* myeloperoxidase, *ROS* reactive oxygen species, *IR* ischemia and reperfusion, *ATN* acute tubular necrosis

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 the standard curve.

Myeloperoxidase activity

To quantify the neutrophil infiltration in the renal tissue we assayed myeloperoxidase (MPO) activity in renal homogenates. Renal tissues were homogenized with a Polytron homogenizer (Kinematica AG, Littau-Lucerne, Switzerland) in 1 ml 0.1 M phosphate buffer pH 6.2 containing 0.5% hexadecyltrimethyl ammonium bromide and 5 mM EDTA (Sigma, St. Louis, MO, USA). The samples were then sonicated five times (each for 10 s) at 40 Hz and centrifuged at $3{,}000 \times g$ for 30 min at 4°C. MPO activity was assayed in 10-ml aliquots of the supernatants by measuring changes in absorbance at 460 nm resulting from decomposition of H_2O in the presence of θ-dianisidine in a final volume of 1.5 ml as previously described by Henson et al. in 1978 [15].

Statistics

Data are expressed as mean and standard deviation (SD). Parameter tests were used to compare the data since the values presented normal distributions. PCR data are presented as ratios of analyzed genes to HPRT mRNA, in arbitrary units (AU). Significant difference was considered when $P < 0.05$.

Results

Cyclooxygenases inhibition ameliorates acute renal dysfunction

Initially, we assessed whether inhibition of COXs could improve renal function outcome after IR. Since we observed that the highest tested dose of IMT was able to significantly decrease the serum creatinine at 24 and 48 h of reperfusion, this dose was chosen for further experiments (Fig. 1). Animals were treated or not with IMT and subjected or not to IR and sacrificed after 24 h of reperfusion. As demonstrated in Fig. 2, IMT-treated animals indeed presented significantly lower serum creatinine (as previously demonstrated) and BUN than nontreated animals. Moreover, acute tubular necrosis index corroborated the functional data (Fig. 2, panels A, B, and C).

Next, we quantified neutrophil infiltration by measuring MPO and ROS by flow cytometry in the tissue 24 h after reperfusion. IMT-treated animals presented lower MPO and

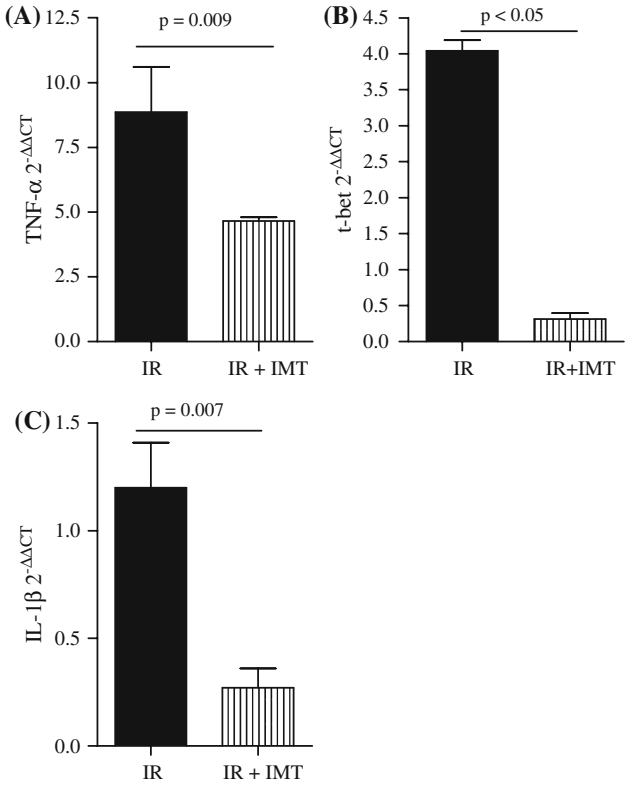

Fig. 3 Pro-inflammatory molecules. At 24 h after reperfusion, animals treated with IMT or not, and subjected to renal IR, were investigated for levels of mRNA of TNF-α (**a**), t-bet (**b**), and IL-1β (**c**). Data are represented as mean and standard deviation (DP). In each experiment, a total of eight animals were studied. In sham-operated groups, five animals were studied (data not shown). Results are expressed as mean and standard deviation (SD). Statistical significance was tested by using a *t*-test. *IMT* indomethacin, *IR* ischemia and reperfusion

ROS activities than nontreated animals (Fig. 2, panels D and E) at 24 h after reperfusion.

The inhibition of serum PGE_2 levels and mRNA of PGES indicated that IMT therapy worked accordingly (Fig. 2, panel F and G). Sham-operated animals presented similar values to normal control mice (data not shown).

Impaired production of Th1-related cytokines in IMT-treated animals subjected to renal IR

Renal IR is an inflammatory syndrome characterized by production of Th1-related cytokines and cellular infiltration [16, 17]. Initially, we observed the expression of t-bet in renal tissue after ischemia. t-bet is a Th1-related transcript factor and has already been documented in renal IR [16]. TNF-α has recently been associated with systemic damage of intestinal IR [18, 19]. The inhibition of COX is associated with lower expression of t-bet and TNF-α (Fig. 3), corroborating the idea that ROS generated during renal IR could indeed stimulate mononuclear cells to produce TNF-α [20]. Furthermore, IMT-treated animals also presented reduced expression of IL-1β compared with nontreated animals (Fig. 3, panel C).

Additionally, we investigated a broad profile of cytokines in serum after reperfusion. Strikingly, IMT therapy induced profound suppression of Th1-related cytokines, most markedly for TNF-α, IFN-γ, and IL-12 p70. This inhibition was followed by a subsequent increase in serum levels of IL-10. Although IL-10 mRNA was enhanced in IMT-treated animals, this did not reach statistical significance. GM-CSF was not suppressed by IMT treatment (Fig. 4).

Inhibition of cyclooxygenases is associated with a cytoprotective phenotype

Inflammation is a center point in renal IR pathogenesis. Therefore, we investigated key molecules already described in this model and ascribed to be cytoprotective [21]. Indomethacin-treated animals subjected to renal IR showed significantly high expression of IL-10 and HO-1 mRNA (Fig. 5).

Discussion

Acute renal injury is a multifaceted entity that evolves through different stages, culminating in organ failure. Its pathogenesis is complex, with participation of the natural and adaptive arms of immune response [22–27]. Very early after reperfusion, ROS are produced and released, leading to cell activation and death. Endothelial cells are one of the primary targets of ROS, whose action in these cells includes changes toward pro-inflammatory phenotype [2, 28–35]. ROS seem to be mainly produced by activated neutrophils, and the role of these cells is well established in renal IR [36–39]. Furthermore, ROS production has been linked to COXs activity after injury [40–45]. Besides induction of oxidative burst, enzymes such as hydrolases and elastases secreted by neutrophils are also involved in tissue destruction after reperfusion [36].

Besides the involvement of innate immune cells, T-lymphocytes are also implicated in the pathogenesis of IR [24, 46, 47]. Specifically, CD4+ Th1 T-lymphocytes seem to be the most detrimental [16, 17]. The mechanism of action of these cells is still under debate; although it seems to involve an antigen-independent activation [48], T cell receptor (TCR) $\alpha\beta$-knockout CD4+ T-cells are protected from renal IR [20].

Here, we investigated whether COX inhibition would affect the inflammatory response triggered by renal IR. Therefore, animals were pretreated or not with IMT, a nonselective inhibitor of COX, and subjected to renal IR. At the peak of renal dysfunction, animals were sacrificed

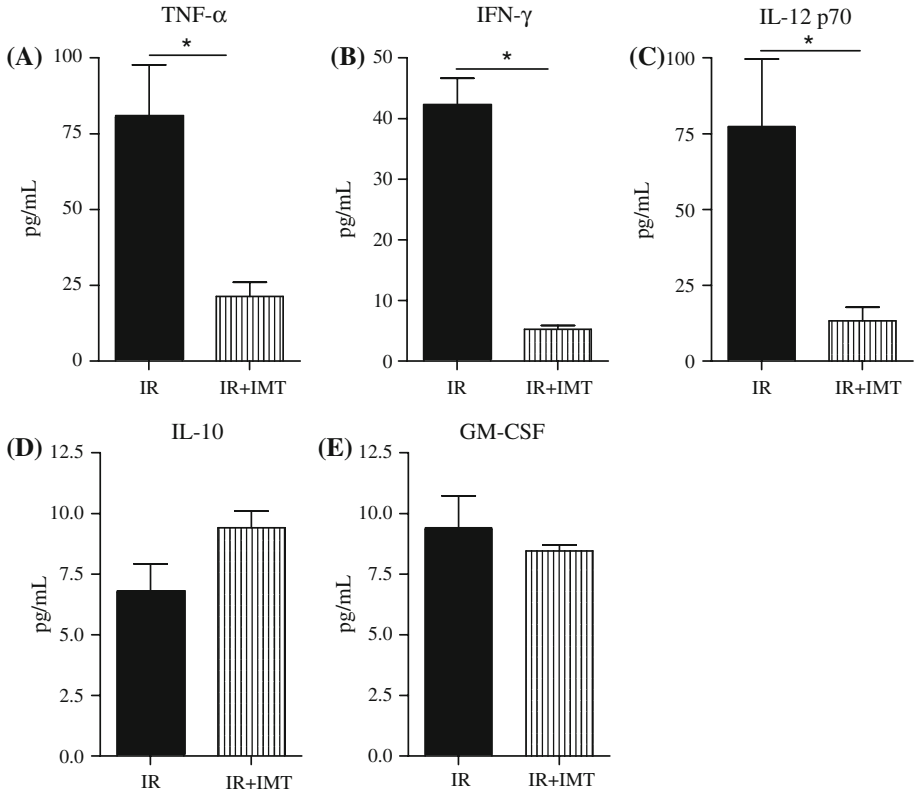

Fig. 4 Serum cytokine profile. At 24 h after reperfusion, animals treated with IMT or not, and subjected to renal IR, were investigated for levels of serum cytokines: TNF-α (**a**), IFN-γ (**b**), IL-12 p70 (**c**), IL-10 (**d**), and GM-CSF (**e**). Data are presented as mean and standard deviation (DP). In each experiment, a total of eight animals were studied. In sham-operated groups, five animals were studied (data not shown). Results were expressed as mean and standard deviation (SD). Statistical significance was tested by using a *t*-test. $*P < 0.05$ in IMT-treated group versus nontreated group. *IMT* indomethacin, *IR* ischemia and reperfusion

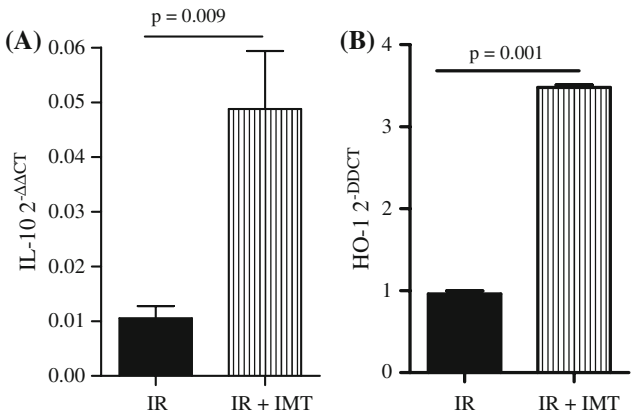

Fig. 5 Anti-inflammatory molecules. At 24 h after reperfusion, animals treated with IMT or not, and subjected to renal IR, were investigated for levels of mRNA of IL-10 (**a**) and HO-1 (**b**). Data are expressed as mean and standard deviation (SD). In each experiment, a total of eight animals were studied. In sham-operated groups, five animals were studied (data not shown). Statistical significance was tested by using a *t*-test. *IMT* indomethacin, *IR* ischemia and reperfusion

and studied for different parameters of cell activation. Animals subjected to renal IR presented severe dysfunction that was ameliorated by previous treatment with IMT, as already described in previous works [6, 10, 11, 49]. Inhibition of COXs was proved to be effective by the lower levels of PGE_2 in IMT-treated animals, demonstrating the effectiveness of the dose used. PGES coverts PGH_2 to PGE_2. PGE_2 is the primary PG produced in every tubular segment, having a special role in renin secretion and NaCl transport. Previous data have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit PGE_2 levels in humans and in animals [50, 51].

Furthermore, treatment with IMT promoted significant inhibition of MPO and ROS. Arachidonic acid (AA) can be metabolized by COX into prostanoids, by lipoxygenase (LO) into 5(S)-, 12(S)-, and 15(S)-hydroxyeicosatetraenoic acid (HETE), and by cytochrome P450 (CYP) into 18-, 19-, and 20-HETE and epoxyeicosatrienoic acids. In this process, PG endoperoxide (PGH_2) synthase participates in the generation of PGs. PGH_2 synthase has a cyclooxygenase component which catalyzes the addition of two oxygen molecules into AA to form PGG_2. After that, a reduction of the 15-hydroperoxy group of PGG_2 leads to the generation of PGH_2. This is a highly oxygen-consuming process, in which ROS may be generated. However, the released oxygen radicals can cause degradation of PG hydroperoxidase itself [52]. PGH_2 is then converted to other metabolites [53, 54]. Although recently Kunz and colleagues demonstrated that COX-2 was not a major source of ROS after cerebral ischemia, in our renal model IMT did decrease ROS production, arguing that COX expression by renal cells may be an important source of ROS. This discrepancy could be illustrated by the fact that the inhibition

of COX may accelerate the synthesis of 5-HETE, which also generates ROS. In this sense, COX inhibition could also be associated with ROS production. Indeed, NSAIDs have been associated with apoptosis of tumoral cells due to oxidative stress [55]. In our model, conversely, we observed inhibition of ROS, and this difference could be applied to the renal model, where the PGs are generated at higher levels.

Next, we investigated whether COX inhibition could suppress the expression of some pro-inflammatory molecules. Renal IR is associated with a broad profile of cytokines, both pro- and anti-inflammatory. TNF-α is upregulated after renal, intestinal, cerebral, and lung ischemia [18, 19]. In intestinal IR, TNF-α is implicated in the systemic inflammation that culminates in lung injury [18, 19, 56, 57]. Our data shows that COX inhibition is able to suppress TNF-α in renal tissue after ischemia [58]. More interestingly, COX inhibition was able to suppress a broad range of cytokine in serum, including IFN-γ and IL-12 p70. Inhibition of TNF-α by COX inhibitors or by drugs that simultaneously inhibited COX has been shown previously [59–66]. Moreover, TNF-α production induced by COX seems to be dependent on the nuclear factor kappa-B (NF-κB) pathway and is associated with upregulation of other cytokines such as IL-6 and IL-1β, all inhibited in our model [59, 62]. Interesting, we also observed a reduction in expression of IL-1β in IMT-treated animals. IL-1β has been consistently associated with upregulation of PGE_2 in several models including renal cells [67, 68]. Altogether, these results illustrate the potential of COX inhibition to decrease extrarenal damage induced by IR; since COX upregulation is an early event after IR, the blockade of the AA pathway is able to impair the inflammation that occurs subsequently.

In the past decade it has become increasingly clear that the cytoprotective response instigated by an injury is crucial for determining the final functional outcome of an injured organ. Many molecules present cytoprotective action depending on the microenvironment. HO-1 has been largely studied and characterized as an anti-inflammatory, anti-apoptotic, and antiproliferative molecule (reviewed by [21]). In renal IR, many authors have already demonstrated that HO-1 is upregulated [69–71], and its prior induction by chemical reagents or gene therapy is associated with better functional and histological damage after IR [71]. Here, we showed that the protection seen in IMT-treated animals is followed by upregulation of HO-1 and IL-10. Many drugs seem to induce HO-1, such as rapamycin and statins [71, 72], although the exact mechanism involved in this gene expression regulation is not solved.

COX inhibition was also associated with IL-10 production. IL-10 and Th2-related cytokines are related to protection in acute renal injury [16]. IL-10-knockout mice presented higher expression of COX-2 in areas surrounding tumor [73]. Berg and colleagues showed that IL-10-knockout mice stimulated with low dose of lipopolysaccharide (LPS) expressed high levels of COX-2 mRNA [74]. Our data shows that COX inhibition is associated with higher expression of IL-10, which could imply a cytokine-dependent mechanism of protection or simply that less tissue injury evolves less Th1-related cytokines, and therefore higher IL-10 expression. This issue needs to be addressed in a future study.

Previous data demonstrated that COXs are involved in the pathogenesis of IR. Here, we add some insight into the mechanism of this complex process and present evidence that it might involve modulation of the immune response.

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References

1. Camara NO, Martins JO, Landgraf RG, Jancar S. Emerging roles for eicosanoids in renal diseases. Curr Opin Nephrol Hypertens. 2009;18:21–7.
2. Kanellis J, Paizis K, Cox AJ, Stacker SA, Gilbert RE, Cooper ME, et al. Renal ischemia–reperfusion increases endothelial VEGFR-2 without increasing VEGF or VEGFR-1 expression. Kidney Int. 2002;61:1696–706.
3. Appleby SB, Ristimaki A, Neilson K, Narko K, Hla T. Structure of the human cyclo-oxygenase-2 gene. Biochem J. 1994;302(Pt 3):723–7.
4. Ferreira SH, Moncada S, Vane JR. Indomethacin and aspirin abolish prostaglandin release from the spleen. Nat New Biol. 1971;231:237–9.
5. Komhoff M, Grone HJ, Klein T, Seyberth HW, Nusing RM. Localization of cyclooxygenase-1 and -2 in adult and fetal human kidney: implication for renal function. Am J Physiol. 1997;272:F460–8.
6. Lozano H, Wu D, Bassuk J, Aria J, Kurlansky P, Lamas GA, et al. The effects of prostaglandin inhibition on whole-body ischemia–reperfusion in swine. Am J Emerg Med. 2008;26:45–53.
7. Hamada T, Tsuchihashi S, Avanesyan A, Duarte S, Moore C, Busuttil RW, et al. Cyclooxygenase-2 deficiency enhances Th2 immune responses and impairs neutrophil recruitment in hepatic ischemia/reperfusion injury. J Immunol. 2008;180:1843–53.
8. Knight S, Johns EJ. Renal functional responses to ischaemia–reperfusion injury in normotensive and hypertensive rats following non-selective and selective cyclo-oxygenase inhibition with nitric oxide donation. Clin Exp Pharmacol Physiol. 2008;35:11–6.
9. Candelario-Jalil E, Gonzalez-Falcon A, Garcia-Cabrera M, Leon OS, Fiebich BL. Post-ischaemic treatment with the cyclooxygenase-2 inhibitor nimesulide reduces blood–brain barrier disruption and leukocyte infiltration following transient focal cerebral ischaemia in rats. J Neurochem. 2007;100:1108–20.
10. Feitoza CQ, Camara NO, Pinheiro HS, Goncalves GM, Cenedeze MA, Pacheco-Silva A, et al. Cyclooxygenase 1 and/or 2 blockade ameliorates the renal tissue damage triggered by ischemia and reperfusion injury. Int Immunopharmacol. 2005;5:79–84.

11. Feitoza CQ, Sanders H, Cenedeze M, Camara NO, Pacheco-Silva A. Pretreatment with indomethacin protects from acute renal failure following ischemia–reperfusion injury. Transplant Proc. 2002;34:2979–80.
12. Kondo F, Kondo Y, Gomez-Vargas M, Ogawa N. Indomethacin inhibits delayed DNA fragmentation of hippocampal CA1 pyramidal neurons after transient forebrain ischemia in gerbils. Brain Res. 1998;791:352–6.
13. Ko JK, Tang F, Cho CH. Co-regulation of mucosal prostanoids and substance P by indomethacin in rat stomachs. Life Sci. 1997;60:PL 277–81.
14. Pradelles P, Grassi J, Maclouf J. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: an alternative to radioimmunoassay. Anal Chem. 1985;57:1170–3.
15. Henson PM, Zanolari B, Schwartzman NA, Hong SR. Intracellular control of human neutrophil secretion. I. C5a-induced stimulus-specific desensitization and the effects of cytochalasin B. J Immunol. 1978;121:851–5.
16. Marques VP, Goncalves GM, Feitoza CQ, Cenedeze MA, Fernandes Bertocchi AP, Damiao MJ, et al. Influence of TH1/TH2 switched immune response on renal ischemia–reperfusion injury. Nephron Exp Nephrol. 2006;104:e48–56.
17. de Paiva VN, Monteiro RM, Marques Vde P, Cenedeze MA, Teixeira Vde P, dos Reis MA, et al. Critical involvement of Th1-related cytokines in renal injuries induced by ischemia and reperfusion. Int Immunopharmacol 2009;9(6):668–72.
18. Cavriani G, Domingos HV, Soares AL, Trezena AG, Ligeiro-Oliveira AP, Oliveira-Filho RM, et al. Lymphatic system as a path underlying the spread of lung and gut injury after intestinal ischemia/reperfusion in rats. Shock. 2005;23:330–6.
19. Cavriani G, Oliveira-Filho RM, Trezena AG, da Silva ZL, Domingos HV, de Arruda MJ, et al. Lung microvascular permeability and neutrophil recruitment are differently regulated by nitric oxide in a rat model of intestinal ischemia–reperfusion. Eur J Pharmacol. 2004;494:241–9.
20. Savransky V, Molls RR, Burne-Taney M, Chien CC, Racusen L, Rabb H. Role of the T-cell receptor in kidney ischemia–reperfusion injury. Kidney Int. 2006;69:233–8.
21. Camara NO, Soares MP. Heme oxygenase-1 (HO-1), a protective gene that prevents chronic graft dysfunction. Free Radic Biol Med. 2005;38:426–35.
22. Thurman JM. Triggers of inflammation after renal ischemia/reperfusion. Clin Immunol. 2007;123:7–13.
23. Versteilen AM, Di Maggio F, Leemreis JR, Groeneveld AB, Musters RJ, Sipkema P. Molecular mechanisms of acute renal failure following ischemia/reperfusion. Int J Artif Org. 2004;27:1019–29.
24. Ysebaert DK, De Greef KE, De Beuf A, Van Rompay AR, Vercauteren S, Persy VP, et al. T cells as mediators in renal ischemia/reperfusion injury. Kidney Int. 2004;66:491–6.
25. Bohmova R, Viklicky O. Renal ischemia–reperfusion injury: an inescapable event affecting kidney transplantation outcome. Folia Microbiol (Praha). 2001;46:267–76.
26. Bonventre JV. Complement and renal ischemia–reperfusion injury. Am J Kidney Dis. 2001;38:430–6.
27. Lien YH, Lai LW, Silva AL. Pathogenesis of renal ischemia/reperfusion injury: lessons from knockout mice. Life Sci. 2003;74:543–52.
28. Fernandes Bertocchi AP, Campanhole G, Wang PH, Gonçalves GM, Damião MJ, Cenedeze MA, et al. A Role for galectin-3 in renal tissue damage triggered by ischemia and reperfusion injury. Transpl Int 2008;21(10)999–1007.
29. Leemreis JR, Versteilen AM, Sipkema P, Groeneveld AB, Musters RJ. Digital image analysis of cytoskeletal F-actin disintegration in renal microvascular endothelium following ischemia/reperfusion. Cytometry A. 2006;69:973–8.
30. Schlichting CL, Schareck WD, Weis M. Renal ischemia–reperfusion injury: new implications of dendritic cell-endothelial cell interactions. Transplant Proc. 2006;38:670–3.
31. Vinas JL, Sola A, Genesca M, Alfaro V, Pi F, Hotter G. NO and NOS isoforms in the development of apoptosis in renal ischemia/reperfusion. Free Radic Biol Med. 2006;40:992–1003.
32. Farrar CA, Wang Y, Sacks SH, Zhou W. Independent pathways of P-selectin and complement-mediated renal ischemia/reperfusion injury. Am J Pathol. 2004;164:133–41.
33. Ishimura T, Fujisawa M, Isotani S, Iijima K, Yoshikawa N, Kamidono S. Endothelial nitric oxide synthase expression in ischemia–reperfusion injury after living related-donor renal transplantation. Transpl Int. 2002;15:635–40.
34. Kanellis J, Mudge SJ, Fraser S, Katerelos M, Power DA. Redistribution of cytoplasmic VEGF to the basolateral aspect of renal tubular cells in ischemia–reperfusion injury. Kidney Int. 2000;57:2445–56.
35. Caramelo C, Espinosa G, Manzarbeitia F, Cernadas MR, Perez Tejerizo G, Tan D, et al. Role of endothelium-related mechanisms in the pathophysiology of renal ischemia/reperfusion in normal rabbits. Circ Res. 1996;79:1031–8.
36. Heinzelmann M, Mercer-Jones MA, Passmore JC. Neutrophils and renal failure. Am J Kidney Dis. 1999;34:384–99.
37. Maenpaa CJ, Shames BD, Van Why SK, Johnson CP, Nilakantan V. Oxidant-mediated apoptosis in proximal tubular epithelial cells following ATP depletion and recovery. Free Radic Biol Med. 2008;44:518–26.
38. Plotnikov EY, Kazachenko AV, Vyssokikh MY, Vasileva AK, Tcvirkun DV, Isaev NK, et al. The role of mitochondria in oxidative and nitrosative stress during ischemia/reperfusion in the rat kidney. Kidney Int. 2007;72:1493–502.
39. Yamanobe T, Okada F, Iuchi Y, Onuma K, Tomita Y, Fujii J. Deterioration of ischemia/reperfusion-induced acute renal failure in SOD1-deficient mice. Free Radic Res. 2007;41:200–7.
40. Nakamura T, Sakamoto K. Reactive oxygen species up-regulates cyclooxygenase-2, p53, and Bax mRNA expression in bovine luteal cells. Biochem Biophys Res Commun. 2001;284:203–10.
41. Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Matsuo T, et al. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. Diabetes. 2003;52:2570–7.
42. Barbieri SS, Eligini S, Brambilla M, Tremoli E, Colli S. Reactive oxygen species mediate cyclooxygenase-2 induction during monocyte to macrophage differentiation: critical role of NADPH oxidase. Cardiovasc Res. 2003;60:187–97.
43. Kunz A, Anrather J, Zhou P, Orio M, Iadecola C. Cyclooxygenase-2 does not contribute to postischemic production of reactive oxygen species. J Cereb Blood Flow Metab. 2007;27:545–51.
44. Chen JJ, Huang WC, Chen CC. Transcriptional regulation of cyclooxygenase-2 in response to proteasome inhibitors involves reactive oxygen species-mediated signaling pathway and recruitment of CCAAT/enhancer-binding protein delta and CREB-binding protein. Mol Biol Cell. 2005;16:5579–91.
45. Sugiyama T, Yoshimoto T, Sato R, Fukai N, Ozawa N, Shichiri M, et al. Endothelin-1 induces cyclooxygenase-2 expression and generation of reactive oxygen species in endothelial cells. J Cardiovasc Pharmacol. 2004;44(Suppl 1):S332–5.
46. Bonventre JV, Zuk A. Ischemic acute renal failure: an inflammatory disease? Kidney Int. 2004;66:480–5.
47. Pinheiro HS, Camara NO, Noronha IL, Maugeri IL, Franco MF, Medina JO, et al. Contribution of CD4 + T cells to the early mechanisms of ischemia–reperfusion injury in a mouse model of acute renal failure. Braz J Med Biol Res. 2007;40:557–68.

48. Grigoryev DN, Liu M, Hassoun HT, Cheadle C, Barnes KC, Rabb H. The local and systemic inflammatory transcriptome after acute kidney injury. J Am Soc Nephrol. 2008;19:547–58.
49. Ito Y, Katagiri H, Ishii K, Kakita A, Hayashi I, Majima M. Effects of selective cyclooxygenase inhibitors on ischemia/reperfusion-induced hepatic microcirculatory dysfunction in mice. Eur Surg Res. 2003;35:408–16.
50. Brenner DE, Harvey HA, Lipton A, Demers L. A study of prostaglandin E2, parathormone, and response to indomethacin in patients with hypercalcemia of malignancy. Cancer. 1982;49:556–61.
51. Scherzer P, Wald H, Rubinger D, Popovtzer MM. Indomethacin and sodium retention in the rat: role of inhibition of prostaglandin E2 synthesis. Clin Sci (Lond). 1992;83:307–11.
52. Miyamoto T, Ogino N, Yamamoto S, Hayaishi O. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. J Biol Chem. 1976;251:2629–36.
53. Bonventre JV, Nemenoff R. Renal tubular arachidonic acid metabolism. Kidney Int. 1991;39:438–49.
54. Samuelsson B, Goldyne M, Granstrom E, Hamberg M, Hammarstrom S, Malmsten C. Prostaglandins and thromboxanes. Annu Rev Biochem. 1978;47:997–1029.
55. Adachi M, Sakamoto H, Kawamura R, Wang W, Imai K, Shinomura Y. Nonsteroidal anti-inflammatory drugs and oxidative stress in cancer cells. Histol Histopathol. 2007;22:437–42.
56. Chen LW, Chang WJ, Chen PH, Liu WC, Hsu CM. TLR ligand decreases mesenteric ischemia and reperfusion injury-induced gut damage through TNF-alpha signaling. Shock 2008.
57. Adibhatla RM, Hatcher JF. Secretory phospholipase A2 IIA is up-regulated by TNF-alpha and IL-1alpha/beta after transient focal cerebral ischemia in rat. Brain Res. 2007;1134:199–205.
58. Bertolini A, Ottani A, Sandrini M. Dual acting anti-inflammatory drugs: a reappraisal. Pharmacol Res. 2001;44:437–50.
59. Yamamoto K, Arakawa T, Taketani Y, Takahashi Y, Hayashi Y, Ueda N, et al. TNF alpha-dependent induction of cyclooxygenase-2 mediated by NF kappa B and NF-IL6. Adv Exp Med Biol. 1997;407:185–9.
60. Huang ZF, Massey JB, Via DP. Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in human in vitro differentiated macrophages. Biochem Pharmacol. 2000;59:187–94.
61. Chen CC, Sun YT, Chen JJ, Chiu KT. TNF-alpha-induced cyclooxygenase-2 expression in human lung epithelial cells: involvement of the phospholipase C-gamma 2, protein kinase C-alpha, tyrosine kinase, NF-kappa B-inducing kinase, and I-kappa B kinase 1/2 pathway. J Immunol. 2000;165:2719–28.
62. Kniss DA, Rovin B, Fertel RH, Zimmerman PD. Blockade NF-kappaB activation prohibits TNF-alpha-induced cyclooxygenase-2 gene expression in ED27 trophoblast-like cells. Placenta. 2001;22:80–9.
63. Weaver SA, Russo MP, Wright KL, Kolios G, Jobin C, Robertson DA, et al. Regulatory role of phosphatidylinositol 3-kinase on TNF-alpha-induced cyclooxygenase 2 expression in colonic epithelial cells. Gastroenterology. 2001;120:1117–27.
64. Yoshida H, Kubota T, Krueger JM. A cyclooxygenase-2 inhibitor attenuates spontaneous and TNF-alpha-induced non-rapid eye movement sleep in rabbits. Am J Physiol Regul Integr Comp Physiol. 2003;285:R99–109.
65. Harizi H, Norbert G. Inhibition of IL-6, TNF-alpha, and cyclooxygenase-2 protein expression by prostaglandin E2-induced IL-10 in bone marrow-derived dendritic cells. Cell Immunol. 2004;228:99–109.
66. Payvandi F, Wu L, Haley M, Schafer PH, Zhang LH, Chen RS, et al. Immunomodulatory drugs inhibit expression of cyclooxygenase-2 from TNF-alpha, IL-1beta, and LPS-stimulated human PBMC in a partially IL-10-dependent manner. Cell Immunol. 2004;230:81–8.
67. Kojima F, Naraba H, Sasaki Y, Beppu M, Aoki H, Kawai S. Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. Arthritis Rheum. 2003;48:2819–28.
68. Kohan DE. Interleukin-1 regulation of collecting duct prostaglandin E2 and cyclic nucleotide accumulation. J Lab Clin Med. 1994;123:668–75.
69. Feitoza CQ, Goncalves GM, Bertocchi AP, Wang PW, Damiao MJ, Cenedeze MA, et al. A role for HO-1 in renal function impairment in animals subjected to ischemic and reperfusion injury and treated with immunosuppressive drugs. Transplant Proc. 2007;39:424–6.
70. Tsuchihashi S, Zhai Y, Fondevila C, Busuttil RW, Kupiec-Weglinski JW. HO-1 upregulation suppresses type 1 IFN pathway in hepatic ischemia/reperfusion injury. Transplant Proc. 2005;37:1677–8.
71. Goncalves GM, Cenedeze MA, Feitoza CQ, Wang PM, Bertocchi AP, Damiao MJ, et al. The role of heme oxygenase 1 in rapamycin-induced renal dysfunction after ischemia and reperfusion injury. Kidney Int. 2006;70:1742–9.
72. Gueler F, Park JK, Rong S, Kirsch T, Lindschau C, Zheng W, et al. Statins attenuate ischemia–reperfusion injury by inducing heme oxygenase-1 in infiltrating macrophages. Am J Pathol. 2007;170:1192–9.
73. Shattuck-Brandt RL, Varilek GW, Radhika A, Yang F, Washington MK, DuBois RN. Cyclooxygenase 2 expression is increased in the stroma of colon carcinomas from IL-10(-/-) mice. Gastroenterology. 2000;118:337–45.
74. Berg DJ, Zhang J, Lauricella DM, Moore SA. Il-10 is a central regulator of cyclooxygenase-2 expression and prostaglandin production. J Immunol. 2001;166:2674–80.