Administration of Neural Precursor Cells Ameliorates Renal Ischemia-Reperfusion Injury

Pamella Huey Mei Wang^a Telma T. Schwindt^b Gabriela Filoso Barnabé^b
Fabiana L.T. Motta^b Patricia Semedo^a Felipe Caetano Beraldo^c
Marilda Mazzali^c Marlene Antonia dos Reis^d Vicente de Paula Antunes Teixeira^d
Alvaro Pacheco-Silva^{a,e} Luis Eugênio A. de Mello^b Niels O.S. Câmara^{a,f}

^aLaboratório de Imunologia Clínica e Experimental, Division of Nephrology, and ^bLaboratory of Neurophysiology, Division of Physiology, Universidade Federal de São Paulo, São Paulo, ^cDivision of Nephrology, Universidade Estadual de Campinas, Campinas, ^dDivision of Pathology, Universidade Federal do Triângulo Mineiro, Minas Gerais, ^eInstituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, and ^fLaboratory of Transplantation Immunobiology, Department of Immunology, Universidade de São Paulo, São Paulo, Brazil

Key Words

Neurospheres • Neural precursor cells • Ischemia and reperfusion injury, renal • Immunomodulation

Abstract

In this study we evaluated whether administration of stem cells of neural origin (neural precursor cells, NPCs) could be protective against renal ischemia-reperfusion injury (IRI). We hypothesized that stem cell outcomes are not tissue-specific and that NPCs can improve tissue damage through paracrine mechanisms, especially due to immunomodulation. To this end, Wistar rats (200–250 g) were submitted to 1-hour ischemia and treated with NPCs (4×10^6 cells/animal) at 4 h of reperfusion. To serve as controls, ischemic animals were treated with cerebellum homogenate harvested from adult rat brain. All groups were sacrificed at 24 h of reperfusion. NPCs were isolated from rat fetus telencephalon and cultured until neurosphere formation (7 days). Before administration, NPCs were labeled with carboxyfluorescein diace-

tate succinimidylester (CFSE). Kidneys were harvested for analysis of cytokine profile and macrophage infiltration. At 24 h, NPC treatment resulted in a significant reduction in serum creatinine (IRI + NPC 1.21 ± 0.18 vs. IRI 3.33 ± 0.14 and IRI + cerebellum 2.95 ± 0.78 mg/dl, $p < 0.05$) and acute tubular necrosis (IRI + NPC $46.0 \pm 2.4\%$ vs. IRI $79.7 \pm 14.2\%$, $p < 0.05$). NPC-CFSE and glial fibrillary acidic protein (GFAP)-positive cells (astrocyte marker) were found exclusively in renal parenchyma, which also presented GFAP and SOX-2 (an embryonic neural stem cell marker) mRNA expression. NPC treatment resulted in lower renal proinflammatory IL-1 β and TNF- α expression and higher anti-inflammatory IL-4 and IL-10 transcription. NPC-treated animals also had less macrophage infiltration and decreased serum proinflammatory cytokines (IL-1 β , TNF- α and INF- γ). Our data suggested that NPC therapy improved renal function by influencing immunological responses.

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Introduction

Acute renal failure (ARF) mainly develops following ischemic or toxic insults, whereby damage is not only caused by loss of blood regulation, but also by production and regulation of multiple cytokines [1]. Development of improved therapies able to limit this immunological response can have a relevant impact on ARF. This quest extends to a new field of research, the stem cell therapy studies. However, full comprehension of the mechanisms underlying stem cell therapy has yet to be achieved, although it has been hypothesized that these may act through dedifferentiation, paracrine modulation or cell fusion [2–4]. In renal insults, consensus data rely on more beneficial effects after mesenchymal stem cell administration and action through inflammatory modulation [5].

Furthermore, stem-cell plasticity is remarkable. Mesenchymal stem cell, once believed necessary for hematopoietic stem cell support, are now known to differentiate into several tissues, including neural cells [6]. Their broad plasticity may determine their therapeutic potential. We believe that stem cell benefits are not tissue-specific and have hypothesized that stem cells of neural origin could be protective against a renal insult, chiefly through paracrine mechanisms.

A direct link between neural molecules and renoprotection is being discovered. Growth and transcription factors similar to those essential to the nervous system may be operating in the kidney and be induced after stimuli, such as an ischemic episode [7]. Moreover, exogenous administration of glial cell line-derived neurotrophic growth factor was shown to be protective against renal IRI [8].

In this study, we analyzed whether neural precursor cells (NPCs) could be protective against renal IRI. NPCs are grown in suspension, giving rise to cell aggregates also known as neurospheres. NPCs represent both neural stem cells and neural progenitor cells that coexist within neurospheres [9]. Within the neurospheres, the neural stem cells are able to self-renew and after asymmetrical divisions can give rise to neural progenitors [10]. The neural progenitor cells are more restricted to glial or neuronal lineage and have reduced self-renewal capability. Thus, neurospheres are heterogeneous cell aggregates of precursor cells in a wide range of differentiation and commitment stages. We had previously observed that within rat neurospheres, approximately 25% of the cells were β -tubulin III-positive (neurons or neuronal progenitors) and 20% glial fibrillary acidic protein (GFAP)-pos-

itive (astrocytes or glial progenitors) [11]. We also showed that only 30% of the cells were nestin-positive (neural stem cells). Nestin expression cannot be found in 100% of cells because its expression is downregulated upon differentiation. To observe the differentiation capability of neurospheres, they have to be plated on a substrate that allows adhesion and migration in the absence of mitogens. In this condition, neurospheres spontaneously give rise to functional neurons, astrocytes and oligodendrocytes [12–14].

Because neurospheres produce growth factors [15] and cytokines [16–19], they can also modify tissue immune responses. Our hypothesis is that NPC administration can improve renal IRI by immunomodulation. We believe that this process is not tissue-specific and depends on paracrine mechanisms. Herein, we found that NPC treatment improved renal function after IRI, mainly due to polarization of the immune response into an anti-inflammatory pattern.

Methods

Animals

Male Wistar rats (220–250 g) were purchased from the Center of Development of Experimental Models for Biology (CEDEME – UNIFESP), São Paulo, Brazil. All procedures were previously reviewed and approved by the internal Ethics Committee on Animal Research of the Universidade Federal de São Paulo (UNIFESP).

Harvesting and Culturing Rat NPCs

NPCs were obtained from Wistar rat fetuses 14.5 days post-conception (embryonic day 14.5), as previously described [20]. Briefly, telencephalons were dissected and incubated with trypsin-EDTA solution (Gibco, Paisley, UK) for 10 min at 37°C. Cells were dissociated and filtered in a 40- μ m cell strainer (BD Bioscience, San Jose, Calif., USA). The culture media contained 70% DMEM, 30% F12, 1% PSA, 2% B27 (Gibco), 20 ng/ml EGF (Sigma, St. Louis, Mo., USA), 20 ng/ml FGF-2 (R&D, Abingdon, UK), and 5 μ g/ml heparin (Sigma). Cells were seeded in T25 flasks (100,000 cells/ml) and maintained at 37°C in a 5% CO₂ atmosphere. Neurospheres were cultured for 10–14 days before administration.

In vitro Differentiation Studies

For NPC plating, coverslips were coated with poly-L-lysine (0.1 mg/ml in water) and laminin solution (50 μ g/ml in PBS; Sigma) at 37°C for 30 min. Spheres were then plated in a differentiation medium containing 70% DMEM, 30% F12 and 2% B27. During a 7-day period the cells that migrated from the neurospheres spontaneously differentiated into neurons, astrocytes and oligodendrocytes. Cells were fixed in 4% PFA and permeabilized in PBS 0.1 M/5% goat serum/0.1% Triton X-100. They were then incubated overnight at 4°C with primary antibodies against β -tubulin III (mouse IgG2b, 1:200; Sigma) to visualize neurons, GFAP (rabbit IgG, 1:300; DakoCytomation, Glostrup, Denmark) for as-

trocytes and Galactocerebroside-C (Gal-C; mouse IgG, 1:200; Chemicon, Oxfordshire, UK), an oligodendrocyte marker. Secondary antibodies Alexa 546 or Alexa 488 (Molecular Probes, Eugene, Oreg., USA) were used. Cells were viewed under a fluorescence microscope (Nikon, Tokyo, Japan).

Experimental Model of Renal IRI

Surgery was performed as previously described [21]. Rats were anesthetized with ketamine-xylazine (Agribands do Brazil, São Paulo, Brazil). Renal pedicles were cross-clamped for 1 h. During this time animals were kept well hydrated and at a constant temperature (~37°C). After surgery, animals were placed in single cages and warmed by indirect light until complete recovery from anesthesia. Animals were kept under adjustable conditions until sacrifice at 24 h of reperfusion. Control animals were subjected to the surgical procedure without renal vessel occlusion (sham).

NPC Administration

Prior to administration, neurospheres growing in suspension were disrupted by trypsin-EDTA in order to form single cell suspensions. Cell viability was tested by trypan blue and propidium iodide. The percentage of viable cells was always superior to 90%. Only viable cells were considered to determine the final cell density used for treatment. Approximately 4×10^6 of NPCs in single suspension were given to each animal 4 h after the insult. Initially, several administration routes were tested (intravenous, i.v., intraperitoneal, i.p., and intracortical, i.c.). After this, all treatments used the i.v. route. To act as controls some animals were treated with adult rat cerebellum homogenate, known to be a stem cell-free zone [22–24]. NPCs and cerebellum cells were labeled with 3 μ M carboxyfluorescein diacetate succinimidylester (CFSE) in constant agitation for 30 min at 37°C, before administration.

Animals Groups

Animals were assigned as follows: group 1 (IRI, n = 10) = animals subjected to IRI; group 2 (IRI + NPC, n = 10) = animals subjected to IRI and treated with NPCs; group 3 (IRI + cerebellum, n = 10) = animals subjected to IRI and treated with cerebellum cells isolated from adult rat brain; group 4 (n = 10) = sham-operated animals, and group 5 (n = 10) = sham-operated animals treated with NPCs. Surgeries were performed as follows: groups 1–3 underwent surgical procedures on the same day, and groups 4 and 5 the next day (2 animals/group).

Renal Function

Serum creatinine was measured to evaluate renal function. Blood samples were collected at 0, 4 and 24 h from the vascular plexus. At 4 h, blood was collected immediately before NPC treatment. Serum creatinine was measured on a Cobas Mira Plus (Roche, Mannheim, Germany), using the modified Jaffé technique, as previously described [21].

Histological Morphometric Analyses

Kidney formaldehyde-fixed paraffin sections were stained with hematoxylin and eosin. The extent of acute tubular necrosis (ATN) morphology in the outer medulla was examined by blinded morphometric assessment using a computer-assisted imaging system with an Olympus BX40F-3 microscope (Olympus Optical Company, Tokyo, Japan) and the software program KS300 (Zeiss, Thornwood, N.Y., USA). The results are expressed as a ratio of the

injured area to total area ($145,846.7 \mu\text{m}^2$). Twenty-five random fields were counted at an original magnification $\times 835$, almost covering all outer medulla, corresponding to an area of $3,646,167.5 \mu\text{m}^2$, for each animal, per slide. For morphometric analyses, ATN was analyzed 24 h after reperfusion. Tubular necrosis pattern included: loss of cytoplasmatic and nuclear membrane integrity, loss of brush border, vacuolization of tubular epithelial cells, and presence of intratubular debris.

Immunohistochemistry

Immunohistochemistry was performed using the ABC method.

GFAP marker: Kidney sections were incubated overnight with primary polyclonal antibody against GFAP (1:500; DakoCytomation) followed by incubation with secondary biotinylated anti-rabbit antibody (from donkey, 1:100; Amersham Biosciences, Buckinghamshire, UK) and streptavidin-HRP for 2 h each (BD Bioscience). Cultured NPCs differentiated into astrocyte (GFAP-positive cells), and adult rat brain sections (data not shown) were used as positive controls.

ED-1 marker: Slides were incubated with primary ED-1 antibody (Serotec, Oxford, UK), followed by incubation with the specific biotinylated secondary antibody. Macrophages were counted in 15 random cortical and 10 medullar tubule-interstitial HPFs ($\times 400$). The HPFs ($\times 400$) were grid-lined (10×10), and the number of stained grid fields was counted in each HPF using the KS 300 Imaging System (Carl Zeiss Microimaging, Jena, Germany). The results are expressed as the percentage of positive fields at the cortex and medulla.

RT-PCR

Kidney samples were quickly frozen in liquid nitrogen. Total RNA was isolated by the TRIzol Reagent (Invitrogen, Carlsbad, Calif., USA) methodology, and first-strand cDNA was synthesized by MML-V reverse transcriptase (Promega, Wisc., USA), according to the manufacturer's protocol. RT-PCR was performed for GFAP (sense) 5'-AAG AGT GGT ATC GGT CCA AGT TTG-3', (anti-sense) 5'-CAG TTG GCG GCG ATA GTC AT-3' and SOX-2 (sense) 5'-GTC AAG AGG CCC ATG AAT GC-3', (anti-sense) 5'-TCT CGG TCT CCG ACA AAA GTT T-3'. The house-keeper gene hypoxanthine guanine phosphoribosyltransferase (HPRT; sense) 5'-CTC ATG GAC TGA TTA TGG ACA GGA C-3' and (anti-sense) 5'-GCA GGT CAG CAA AGA ACT TAT AGC C-3' was used as endogenous control. Cycling conditions were: 10 min at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 58°C and 30 s at 72°C. Resulting fragments were analyzed in a 2.0% agarose gel electrophoresis. For this assay, cultured neural stem cells and adult rat brain were used as controls.

Flow Cytometry

Kidney, lung, spleen and liver were harvested at 24 h, homogenized and filtered by a 0.45- μ m filter. Blood was also collected at 24 h. We used cultured NPCs labeled and non-labeled with CFSE and organs harvested from the sham and non-treated ischemic group as controls. 3.5 ml of FACS buffer was added to each sample. Then, samples were centrifuged at 1,400 rpm for 10 min at 4°C, the supernatant was removed and pellets resuspended in 4 ml of FACS buffer. The samples were analyzed in the FACS-Canto (Beckton Dickinson, Mountain View, Calif., USA) cytometer with the aid of Cell Quest software.

Fig. 1. NPC differentiation. NPCs were grown in suspension and gave rise to cell aggregates called neurospheres (A). For differentiation, NPCs were plated on coverslips coated with poly-L-lysine (0.1 mg/ml in water) and laminin solution (50 μ g/ml in PBS; Sigma, USA) at 37°C for 30 min and cultivated in a differentiation medium containing 70% DMEM, 30% F12 and 2% B27, for 7 days. During this period the migrating cells spontaneously differentiated into neurons, astrocytes and oligodendrocytes. Detection of differentiated cells was made by immunofluorescence. Cells were fixed in 4% PFA and permeabilized in PBS 0.1 M/5% goat serum/0.1% Triton X-100. They were then incubated with primary antibodies against β -tubulin III (mouse IgG2b, 1:200) for neurons (B), GFAP (rabbit IgG, 1:300) for astrocytes (C), and Gal-C (mouse IgG, 1:200) for oligodendrocytes (D). Secondary antibodies Alexa 546 or Alexa 488 (Molecular Probes) were used.

Cytokine mRNA

Real-time PCR was performed using the following TaqMan PCR assays: IL-1 β (Mm00434228_m1), IL-4 (Mm00445259_m1), IL-10 (Mm00439616_m1), TNF- α (Mm00443258_m1), and the housekeeper gene HPRT (Mm00446968_m1; Applied Biosystems, Foster City, Calif., USA). We used the cycling conditions described by the manufacturer (Applied Biosystems). mRNA relative quantification was performed using the comparative threshold method (described in details in User Bulletin 2; PerkinElmer, Applied Biosystems, Foster City, Calif., USA, 1997). The target gene amount was normalized by HPRT and then compared to a calibrator (non-treated sham-operated animals) using the formula $2^{-\Delta\Delta C_t}$. All data were expressed as an n-fold difference related to the expression of matched controls (sham). Analyses were performed with Sequence Detection Software 1.9 (SDS).

ELISA

Cytokine were measured in serum collected at 24 h of reperfusion using custom BioRad (Bio-Rad Laboratories, Hercules, Calif., USA) BioPlex cytokine analysis kits in conjunction with the BioPlex system array reader, according to the manufacturer's instructions. The specific cytokines (IL-1 β , TNF- α and IFN- γ)

Fig. 2. Different routes of NPC administration. Intraperitoneal, intravenous and intracortical administrations were tested. All NPC administration routes reduced serum creatinine levels at 24 h of reperfusion.

were chosen based on our PCR data 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 BioPlex Manager software.

Statistical Analyses

All data are described as mean + SEM. Different results among groups were compared using the ANOVA. Significance was considered when $p < 0.05$. All statistical analyses were performed with the aid of SigmaStat Statistical Software 2.0 (Jandel Corporation, Tex., USA).

Results

NPC Treatment

Before treatment, NPC plasticity was observed through in vitro differentiation into neurons and astrocytes (fig. 1). For treatment, several administration routes were tested. All administration routes presented a significant reduction in serum creatinine levels (i.p. 2.28 ± 0.30 mg/dl; i.v. 1.21 ± 0.18 mg/dl, and i.c. 1.79 ± 0.20 mg/dl vs. IRI 3.33 ± 0.14 mg/dl, $p < 0.05$; fig. 2). The i.v. administration was chosen for all of the following assays. No difference between sham and sham+NPC creatinine levels was observed at 24 h of reperfusion (sham+NPC 0.53 ± 0.08 mg/dl, and sham 0.63 ± 0.09 , $p < 0.05$).

Fig. 3. Tissue response after NPC administration. Renal function at different reperfusion times was estimated by serum creatinine levels (**A**). At 4 h of reperfusion, and immediately before NPC or cerebellum cell administration, all ischemic groups had similar serum creatinine levels. However, at 24 h of reperfusion, only the NPC-treated group presented a significant reduction in this parameter. Renal function results were corroborated by histological analyses of renal parenchyma collected at 24 h of reperfusion (**B**). The extension of acute tubular necrosis (ATN) morphology in the outer medulla was examined by blinded morphometric assess-

ment using an Olympus BX40F-3 microscope (Olympus Optical Company) and KS300 software program (Zeiss, Thornwood, N.Y., USA). The results are expressed as a ratio of the injured area versus total area ($145,846.7 \mu\text{m}^2$). The tubular necrosis pattern included: loss of cytoplasmatic and nuclear membranes integrity, loss of brush border, vacuolization of tubular epithelial cells, and presence of intratubular debris. The IRI+NPC group had a lower percentage of ATN. No difference was observed between IRI and IRI+cerebellum groups concerning serum creatinine levels or percentage of ATN.

Renal Function

Immediately before treatment (4 h of reperfusion), all groups subjected to renal IRI had similar serum creatinine levels (IRI 1.46 ± 0.07 mg/dl, IRI+NPC 1.44 ± 0.07 mg/dl and IRI+cerebellum 1.30 ± 0.20 mg/dl; fig. 3). At 24 h, only the IRI+NPC-treated group presented a significant reduction in serum creatinine in comparison to the other ischemic groups (IRI+NPC 1.21 ± 0.18 vs. IRI 3.33 ± 0.14 and IRI+cerebellum 2.95 ± 0.78 mg/dl, $p < 0.05$; fig. 3A).

The better renal function of the IRI+NPC rats was corroborated by their lower percentage of ATN assayed by morphometric analyses after hematoxylin-eosin staining (IRI+NPC $46.0 \pm 2.4\%$ vs. IRI $79.7 \pm 14.2\%$ and IRI+cerebellum $78.9 \pm 12.5\%$, $p < 0.05$; fig. 3B).

NPC Intrarenal Localization after Treatment

Different techniques were used to demonstrate NPC migration and survival in ischemic kidneys. All assays were performed in kidneys collected at 24 h. Immunohistochemistry against GFAP had positive staining only in ischemic kidneys from the NPC-treated group (fig. 4A). Sham-operated animals treated with NPC, non-treated

IRI group and ischemic animals treated with cerebellum cells had no GFAP or other neural marker-positive staining (data not shown). GFAP is a marker of astrocytes or glial progenitors, thus it was able to identify NPCs in a latter differentiation stage. The presence of SOX-2 and GFAP mRNA was also observed exclusively in the IRI NPC-treated group (fig. 4B). SOX-2 is an embryonic neural stem cell marker and its positive expression in kidney homogenates indicated that NPCs at the beginning of their differentiation also migrated to the injured kidneys.

To analyze the approximately number of NPCs that were able to migrate to the ischemic kidneys we treated the rats with NPC-CFSE-positive cells and estimated their migration by flow cytometry. CFSE is a fluorescent staining dye that labeled all NPCs regardless of their differentiation. After NPC treatment, flow cytometry revealed 6.94% NPC-CFSE-positive cells in kidney extracts (fig. 4C). At 24 h, no NPC-CFSE-positive cells were observed in blood, spleen or liver. In lungs, 0.99% of the cells were CFSE-positive, while in the liver 0.04% were positive. Since these values were very low they were considered equal to zero (data not shown).

Fig. 4. NPC homing to ischemic kidneys. Kidneys were collected at 24 h of reperfusion. Immunohistochemistry found GFAP-positive cells (indicated by arrows) only in the NPC+IRI group (**A**). NPC genes were observed in renal parenchyma by RT-PCR. For this assay, cultured NPCs were used as control. SOX-2 (embryonic neural stem cell marker) and GFAP mRNA expression were only observed in the NPC-treated group (**B**). Lane 1 = Cultured

NPC; lane 2 = adult rat brain; lane 3 = IRI, and lane 4 = IRI+NPC. After NPC-CFSE administration, flow cytometry demonstrated 6.94% CFSE-positive cells in renal extracts collected at 24 h of reperfusion (**C**). No NPC-CFSE-positive cells were observed in blood, spleen or liver. In lungs, 0.99% of the cells were CFSE-positive, while in the liver 0.04% were positive (data not shown). Since these values were very low, they were considered equal to zero.

Cytokine Profile

We analyzed whether NPC treatment could induce an anti-inflammatory pattern. Cytokine mRNA was measured in kidney homogenate by RT-PCR. The IRI+NPC group had significantly lower renal expression of IL-1 β and TNF- α , and higher transcription of IL-4 and IL-10, in comparison to the other ischemic groups (fig. 5). Sham and sham+NPC groups had similar values which differed from all ischemic groups (data not shown). Similarly, no difference was observed between IRI and IRI+cerebellum groups.

Furthermore, we measured the expression of some proinflammatory cytokines in serum collected at 24 h of

reperfusion. NPC treatment significantly reduced the circulatory levels of IL-1 β , INF- γ and TNF- α (fig. 6). No difference was observed between IRI and IRI+cerebellum groups. Sham and sham+NPC animals presented values close to the detection limit of the test (data not shown).

Macrophage Infiltration

Renal IRI is associated with macrophage infiltration. Therefore, we evaluated whether NPCs could decrease this cell infiltration. Kidney samples were analyzed 24 h after reperfusion. We observed that NPCs significantly reduced macrophage infiltration in the renal cortex and medulla (fig. 7).

Fig. 5. Renal cytokine expression. Cytokine profile was measured by real-time PCR. The proinflammatory pattern was assayed by IL-1β and TNF-α expression, and the anti-inflammatory pattern by IL-4 and IL-10. NPC treatment significantly reduced IL-1β (A)

and TNF-α (B) expression and increased IL-10 (C) and IL-4 (D) transcription observed in the ischemic non-treated and ischemic treated with cerebellum cell groups. No difference was observed between IRI and IRI+cerebellum groups.

6

Fig. 6. Circulatory cytokine expression. Cytokines were measured in serum collected at 24 h of reperfusion using custom ELISA BioPlex cytokine analysis. The specific cytokines IL-1β, TNF-α and IFN-γ were chosen based on our PCR data 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 BioPlex Manager Software. NPC treatment significantly reduced the circulatory levels of IL-1β, IFN-γ and TNF-α. No difference was observed between IRI and IRI+cerebellum groups. Sham-operated animals presented values close to the detection limit of the test (data not shown). NSC = Neural stem cell.

7

Fig. 7. Macrophage infiltration. Kidney samples harvested at 24 h after reperfusion were analyzed for macrophage infiltration. Slides were incubated with primary antibody ED-1 (Serotec), followed by incubation with the specific biotinylated secondary antibody. Reaction was continued using an ABC detection kit (Dako, Glostrup, Denmark) and diaminobenzidine tetrahydrochloride color developer (Dako, Carpinteria, Calif., USA). Macrophages were counted in 15 random cortical and 10 medullar tubule-interstitial HPFs (×400). The HPFs (×400) were grid-lined (10 × 10), and the number of stained grid fields was counted in each HPF using the KS 300 Imaging System (Carl Zeiss Microimaging, Jena, Germany). Results are expressed as percentage of positive fields in the cortex and medulla. NPC treatment significantly reduced macrophage infiltration in the renal cortex and medulla.

Discussion

IRI is one of the main causes of ARF, and new therapies to ameliorate this injury are extremely important. In this vein, stem cell therapy is gaining increased attention.

In cerebral ischemia, NPC protection has been attributed to maximum trophic factor activation, which probably augmented NPC migration in positive feedback [25]. Thus, a reciprocal interaction between NPCs and the ischemic environment may be crucial for its protective action. However, little is known on how NPCs work. The present study demonstrates that in renal ischemia, NPC administration can be beneficial. It was observed that NPC treatment reduced serum creatinine and morphological damage. To our knowledge, this study was the first attempt to show that protection may not be tissue-specific.

Stem cell therapeutic potential also relies on homing ability to injured sites, which may depend on chemotactic properties, probably through chemokine, selectin or integrin interactions in a similar process or manner to that of leukocytes [26]. We demonstrated that NPCs migrated and survived exclusively in ischemic kidneys until sacrifice at 24 h. On the other hand, fully differentiated cells derived from adult cerebellum were not able to migrate to the injury site and did not promote any improvement in renal function. We believe that inflammation represents the key danger signal for orchestrating recruitment and long-term survival of NPCs.

Stem cell benefits are also associated with immune response suppression through modulation of T cells [27], B cells [28], NK cells [29] and antigen-presenting cell [30] functions. In this manner, paracrine modulation has become increasingly plausible. In renal IRI, an inflamma-

tory syndrome is present, and TCD4+ lymphocytes have been identified as major immunological effectors of this pathology [31]. Renoprotective responses are achieved after Th2 differentiation [32]. Renal protection was found after Th1 component deletion and deleterious effects in the absence of Th2 molecules [33]. The Th1 pattern is triggered by Tbet transcription factor activation and IL-12, IL-1 β and IFN- γ production. Meanwhile, the Th2 pattern is characterized by activation of the transcription factor GATA-3 followed by IL-4, IL-5, IL-6, IL-10 and IL-13 production. In this study, NPC treatment increased anti-inflammatory molecule expression (IL-4 and IL-10) and significantly diminished the proinflammatory IL-1 β and TNF- α renal transcription and IL-1 β , INF- γ and TNF- α circulatory levels. Thus, NPC treatment was able to modify the immune response found in renal IRI, unbalancing it to an Th2 pattern which is protective against renal IRI [32, 34]. NPC immune modulation properties have also been shown in experimental models of nervous system disorders [35] and in vitro [16–19].

Although we were unable to describe all processes involved in NPC protection, their ability to remain in the ischemic kidney and protect against the insult was remarkable, indicating that there is a lot more to learn about stem cell plasticity.

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