Transplant Immunology 27 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Donor preconditioning with rabbit anti-rat thymocyte immunoglobulin ameliorates ischemia reperfusion injury in rat kidney transplantation☆,☆☆

Federico Cicora [a,b,c], Javier Roberti [d,*], Natalia Lausada [a], Pedro González [e], Diego Guerrieri [c,f], Pablo Stringa [a], Paola Cicora [c], Daniela Vásquez [c], Ivana González [c], Gustavo Palti [b], Dante Intile [c], Clemente Raimondi [a]

[a] Faculty of Medicine, Universidad Nacional de La Plata, La Plata, Argentina
[b] Transplant Unit, German Hospital of Buenos Aires, Buenos Aires, Argentina
[c] Fundación para la Investigación y Asistencia de la Enfermedad Renal (FINAER), La Plata, Argentina
[d] Faculty of Humanities, Universidad de Belgrano, Buenos Aires, Argentina
[e] Comisión de Investigaciones Científicas, Buenos Aires, Argentina
[f] Faculty of Medicine, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 13 February 2012
Received in revised form 20 March 2012
Accepted 21 March 2012

Keywords:
Kidney transplantation
Donor preconditioning
Apoptosis
Ischemia reperfusion injury
Thymoglobulin

ABSTRACT

A major concern in transplantation is the preservation of organ function. Ischemia time and microcirculatory disturbance of the organ cannot be avoided and may result in ischemia reperfusion injury (IRI), increasing the risk of delayed graft function (DGF) and acute and chronic rejection. Anti-thymocyte immunoglobulin (rATG) is a polyclonal antibody preparation with multiple effects when administered to recipients. Our objective has been to evaluate whether the administration of rATG to kidney donors instead of recipients, in an experimental model of syngeneic rat transplantation, ameliorates IRI and facilitates immediate graft function recovery. Urea and creatinine levels and necrosis severity scores were significantly lower in kidneys from donors that had received rATG (urea: control: 211 ± 8 mg/dl *vs.* treatment: 110 ± 15 mg/dl, $p<0.001$; creatinine: control: 4.6 ± 0.24 mg/dl *vs.* treatment: 2.6 ± 0.22 mg/dl, $p<0.001$; necrosis severity scores: control: 2.3 *vs.* treatment: 1.6, $p<0.05$). TUNEL staining showed 80 ± 13 positive cells in control group and 9 ± 3 ($p<0.001$) in treatment group. *In situ* expression of proinflammatory cytokines TNF-α, IL-6, IL-21 and TGF-β1 was reduced in rATG group ($p<0.01$); the same was observed for KIM-1 and caspase 8 ($p<0.001$). Cytoprotective genes Bcl2 and HO-1 were upregulated *in situ* in treatment group ($p<0.001$). *In situ* expression of IL-17, caspase 9, IL-23a, CxCl3 and ICAM1 showed no difference between groups ($p>0.05$). Findings suggest ATG administered to donors may ameliorate the IRI process in kidney transplantation, expressed by lower necrosis and apoptosis scores and the improvement of renal function, which may be explained through the diminished *in situ* expression of inflammatory mediators.

1. Introduction

A major concern in renal transplantation is the preservation of organ function. Ischemia time and microcirculatory disturbance of the organ cannot be avoided and may result in ischemia reperfusion injury (IRI). IRI is associated with graft dysfunction, which increases the risk of delayed graft function (DGF) and acute rejection [1]. On the one hand, the transplant outcome inevitably depends on the amount of tissue damage caused by IRI and immune attacks; however, it also depends on regenerative and restorative processes [2]. IRI has been reported to be associated with a longer hospitalization time and decreased long-term graft survival rates [1].

Various lines of research have attempted to attenuate IRI with actions directed at the donor, the recipient, or the preservation solution. However, no unique approach has been found to be sufficient [3,4]. An association between organ quality before transplantation and long-term graft outcome has already been confirmed [5]. Particularly, the risk of DGF, as well as acute and chronic rejection,

Abbreviations: ATG, anti thymocyte immunoglobulin; DGF, Delayed graft function; IRI, Ischemia reperfusion injury; KIM-1, Kidney Injury Molecule 1; rATG, rabbit anti-rat thymocyte immunoglobulin.

☆ The authors declared no conflicting interests.

☆☆ Authorship: FC: designed the study, interpreted data, and wrote the paper; JR: wrote the paper and data presentation; NL: transplantation procedure; PG: immunohistochemical experiments; DG: transplantation procedure; PS: data interpretation and surgery; PC: monitored urea and creatinine levels; DV: designed protocols; IG: rtPCR; GP: wrote the paper; DI: analyzed data; CR: interpretation.

* Corresponding author at: Austria 2381 5D, 1425 Buenos Aires, Argentina. Tel./fax: +54 11 4774165.

E-mail addresses: fcicora5@gmail.com (F. Cicora), javierroberti@gmail.com (J. Roberti), nlausada@atlas.med.unlp.edu.ar (N. Lausada), vukogon@gmail.com (P. González), guerrieri_diego@hotmail.com (D. Guerrieri), pablo_stringa@hotmail.com (P. Stringa), paola_cicora@hotmail.com (P. Cicora), danielavasquez73@gmail.com (D. Vásquez), vanamgv@hotmail.com (I. González), gustavopalti@arnet.com.ar (G. Palti), danteintile@gmail.com (D. Intile), jcraimondi@gmail.com (C. Raimondi).

0966-3274/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2012.03.004

is increased if the quality of the organ before transplant is poor. Thus, a possible strategy is to ameliorate any initial damage to the organ that is to be transplanted. This may result in improved organ availability and the quality of transplantation [5,6]. Donor preconditioning between the diagnosis of brain death and organ recovery has several advantages, such as preserved cellular metabolic pathways, better membrane permeability, active transport mechanisms, and better possibilities for genetic modification of the organs [5,7]. Steroid treatment of brain-dead donors decreases the expression of proinflammatory factors in the tissue and serum as previously observed in humans and experimental animal models [8,9]. In a prospective randomized, controlled trial, methylprednisolone was administered to liver donors, resulting in a significant reduction in inflammation and outcome improvement [10]. Additionally, statin administration in donors has been shown to diminish the inflammatory response [11].

Anti-thymocyte immunoglobulin (Thymoglobulin®; Genzyme, Cambridge, MA, USA) is a polyclonal antibody preparation with multiple effects when administered to recipients. Polyclonal anti-thymocyte immunoglobulins (ATGs) are used to prevent and treat acute rejection episodes after transplantation and treat hematologic disorders such as graft-*versus*-host disease. The effects of ATG result from the depletion and induction of apoptosis of peripheral T lymphocytes. ATG can also modulate B lymphocytes and influence adhesion molecule expression (intercellular adhesion molecule 1 [ICAM1] and vascular cell adhesion molecule 1 [VCAM1]) [12]. ATG is thought to block cellular interactions and affects leukocyte rolling and adhesion levels on capillary endothelial surfaces by downregulating adhesion molecules and receptors responsible for said interactions (lymphocyte function-associated antigen 1 [LFA-1], very late antigen 4 [VLA-4], C–C chemokine receptor type 5 [CCR5], and C–C chemokine receptor type 7 [CCR7]). ATG may indirectly decrease inflammatory mediators and inhibit leukocyte-chemotaxis or chemokine receptor expression. Additionally, ATG may affect IRI through the inhibition of leukocyte homing and trafficking to the graft by binding to chemokine receptors [13,14].

1.1. Objective

The aim of this study has been to evaluate whether the administration of rATG to kidney donors in an experimental model of syngeneic rat transplantation ameliorates IRI and facilitates immediate graft function recovery [15].

2. Materials and methods

2.1. Animals

Wistar male rats, 280–350 g (Veterinary Faculty, Universidad Nacional de La Plata) were used as kidney donors and recipients. Animals were submitted to a 12 h day/night cycle with access to water and standard laboratory chow *ad libitum*. All experiments were performed in accordance to guidelines set by the National Institutes of Health (NIH publication No. 28 revised 1996).

2.2. Generation and characterization of rATG

Genzyme Corporation (Cambridge, MA, USA) provided rabbit anti-rat thymocyte immonuglobuline (rATG) produced analogously to commercial ATG (Thymoglobulin®). Briefly, rabbits were immunized with a mixture of thymocytes of four strains of rats (Sprague Dawley, F344 Fisher, Lewis and Long Evans). Thymocyte suspensions were prepared using thymi from several donor rats. Fifty New Zealand White rabbits were immunized twice, two weeks apart, and terminally bled two weeks after the last immunization. Total rabbit IgG obtained from the serum was pooled and purified similarly to Thymoglobulin® (Genzyme, Cambridge, MA, USA). Control rabbit IgG was purified from whole normal rabbit serum.

2.3. Experimental design

- rATG group (n = 6): rats received a kidney from donors that had been administered rATG (10 mg/kg intravenously) 12 h before starting the surgery to recover the kidneys. The surgical procedure is described below. Of note, recipient animals did not receive any immunosuppressive drug.
- Control group (n = 6): rats received a kidney from donors that had not been administered rATG. Surgical procedure to recover kidney as described below.
- Sham group (n = 6): rats were sham operated.

2.4. Kidney transplantation

Kidney donor rats were randomly divided into three groups: rATG treatment group was administered rATG (10 mg/kg intravenously, dose recommended by manufacturer, Genzyme, Cambridge, MA, USA) 12 h before starting the surgery; control group, and sham group. All donor animals were anesthetized with intraperitoneal atropine (0.01 mg/kg), buprenorphine (0.04 mg/kg), diazepam (10 mg/kg); and 10 min later, ketamine (100 mg/kg) was administered. The donor's blood vessels and the ureter were fully separated. Kidneys were flushed through the aorta with 3 ml of 4 °C cold Ringer lactate solution until homogeneously pale. Left kidneys were removed with vascular and ureteral pedicle and placed in cold Ringer lactate solution at 4 °C for 180 ± 15 min (cold ischemia).

Recipient rats were bilaterally nephrectomized. After flushing the grafts with 5 ml of normal Ringer solution, arterial and venous anastomoses were performed as end-to-side anastomoses to the aorta and inferior vena cava, respectively. Finally, the anastomosis of the ureter with the urinary bladder was constructed. Of note, no immunosuppressive drug was administered to recipient animals before or after transplantation. Body temperature was monitored and constantly kept between 35 °C and 37 °C. Animals were placed on a warm blanket with free access to water and standard laboratory chow *ad libitum*. Twenty four hours after transplantation, blood was drawn for analysis, animals were sacrificed and kidneys were recovered for histological analysis. Plasmatic urea and creatinine levels were measured at baseline and at 24 h post transplantation.

2.5. Blood measurements

BUN and creatinine tests were performed twice, 24 h before transplantation and 24 h after transplantation, using U.V. Kinetic and Colorimetric–Kinetic respectively (Mindray Bs 300; Mindray Corp, Nanshan, China).

2.6. Renal histopathology

Anatomopathological samples were analyzed by a pathologist blinded to group assignments. Kidneys were fixed in 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. Sections of 4 μm thick were cut, deparaffinized, hydrated and stained with hematoxylin eosin. Renal sections were examined on blind fashion for grade of cortical tubular epithelial necrosis. Counts were performed in 10 fields of 1 mm^2 and necrosis was assessed using scores from 1 to 5: 1 (<5%), 2 (5–25%), 3 (25–50%), 4 (50–75%) and 5 (>75%) [16].

2.7. TUNEL assay

TUNEL assay was performed according to the manufacturer's instructions (ApopTag, Oncor Inc., Gaithersburg, MD, USA). Deparaffinized

4 μm-thick tissue sections were pre-treated with 20 μl/ml Proteinase K (Dako, Glostrup, Denmark) for 30 min at 37 °C. After washing, sections were incubated with digoxigenin-labeled deoxyuridine triphosphate (dUTP) in the presence of terminal deoxynucleotidyl-transferase. Once the enzymatic reaction was blocked, sections were incubated with a specific peroxidase-labeled antidigoxin antibody. Peroxidase was then reduced by 0.05 diaminobenzidine (Sigma, St. Luis, MO, USA) in 0.1 ml/l phosphate buffered saline, pH 7.6 containing 1% H_2O_2. Sections were washed and lightly stained with hematoxylin. Negative control reactions were performed for each reaction step. They were obtained by omission of the terminal deoxynucleotidyl transferase, antidigoxin antibody and peroxidase substrate. Positive controls included sections of paraffin embedded lymphoma of human origin. The external medullar region was examined and the labeled nuclei were counted. Ten fields of 1 mm^2 were examined with a reticulated lens.

2.8. Genes expression analysis, RNA isolation and cDNA preparation

Twenty milligrams of frozen kidney tissue were weighed and homogenized with a rotor-stator homogenizer in presence of lysis buffer in the RNA extraction kit. Total RNA was extracted using the RNeasy Plus Mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol, and analyzed by denaturing agarose gel electrophoresis. Total RNA concentration was measured with the Qubit fluorescence detector (Invitrogen, Carlsbad, CA, USA) The first strand cDNA synthesis reaction was performed with RT2 First Strand kit (Qiagen, Valencia, CA, USA), and the resulting product was checked with PCR using beta-actin primers.

2.9. QPCR array

The expression of the following genes was analyzed with Real Time PCR: caspase 8, caspase 9, Cxcl1, Cxcl3, ICAM-1, IL-6, IL-21, IL-23a, KIM1, IL-17, TNF-α, BCL-2, HO-1, TGF-beta1 and Stat3. Genes expression was evaluated with RT2 Profiler PCR Array (SABiosciences, Qiagen, Frederick, MD, USA) in a Stragene Mx3000p (Agilent Technologies, Santa Clara, CA, USA) to detect changes in mRNA levels. Each condition was performed in triplicate. The beta-actin housekeeping gene was used for normalization and data were analyzed using threshold method with Stratagene MxPro (Agilent Technologies, Santa Clara, CA, USA) and Qbase Software (Biogazelle, Zwijnaarde, Belgium).

2.10. Statistical analysis

Continuous variables were analyzed using Student's *t* and Cochran's *t* tests according to homoskedasticity. To evaluate real time PCR genes, groups were compared with variance analysis (ANOVA). Statistic F was replaced with Brown–Forsyth when the Levene test result for homogeneity of variance was <0.05. Multiple comparison tests were performed to analyze the significant differences among means.

3. Results

In the sham group, delta urea and creatinine levels showed no difference when assessed pre procedure and post procedure (data not shown). Urea levels were significantly lower in the kidneys from donors that had received rATG (control: 211 ± 8 mg/dl *vs.* treatment: 110 ± 15 mg/dl, $p<0.001$). Similarly, creatinine levels were significantly lower in kidneys from donors treated with rATG (control kidneys: 4.6 ± 0.24 mg/dl *vs.* treatment kidneys: 2.6 ± 0.22 mg/dl, $p<0.001$) (Fig. 1).

For the histological analysis, we used median scores representing the severity of cortical tubular epithelial necrosis (control group 2.3 *vs.* treatment group 1.6, $p<0.05$) (Figs. 2 and 3). TUNEL staining showed 80 ± 13 positive cells in control group kidneys and 9 ± 3 ($p<0.001$) in kidneys from the treatment group (Figs. 2 and 4).

The expression changes of 15 genes were analyzed by semi-quantitative RT-PCR: TNFα, IL-6, IL-21, TGF-β1, KIM-1, caspase 8, Bcl-2, HO-1, caspase 9, IL17, IL-23a, CxCl3, Stat-3, and ICAM-1. The *in situ* expression of proinflammatory cytokines TNF-α, IL-6, IL-21 and TGF-β1 was reduced in the group treated with rATG ($p<0.01$). KIM-1 was significantly decreased in treatment group ($p<0.001$). Caspase 8, a gene related to apoptosis extrinsic pathway, was also significantly diminished in treatment group compared to control group ($p<0.01$). Cytoprotective genes Bcl-2 and HO-1 were upregulated *in situ* in the treatment group compared to control group ($p<0.001$). The *in situ* expression of IL-17, caspase 9, IL-23a, CxCl3, CxCl1Stat-3 and ICAM1 showed no difference between control and treatment groups ($p>0.05$). Figs. 5 and 6 show selected genes expression data.

Of note, animals treated with rATG did not show any adverse effects during infusion. The damage of epithelial cells was assessed by necrosis and apoptosis severity scores.

4. Discussion

Our findings suggest that administration of anti-rat thymocyte immunoglobulin (rATG) to donors may ameliorate renal damage associated with IRI. The significance of our results lies in identification of the following phenomena in kidneys from the treatment group: reduced urea and creatinine levels, reduced necrosis and apoptosis scores, decreased gene expression of interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), IL-21, transforming growth factor beta 1 (TGF-β1), kidney injury molecule 1 (KIM-1), and caspase 8, and upregulation of the cytoprotective genes B-cell CLL/lymphoma 2 (BCL-2) and heme oxygenase 1 (HO1).

Low urea and creatinine levels may be related to reduced histological damage of renal tubular epithelial cells and attenuated inflammatory mediators produced by rATG. The reduced level of plasma creatinine in the kidneys from the treatment group as compared with that of the control group could be the result of lower percentages of acute tubular necrosis and apoptosis, which could improve transplant outcome.

Histological damage was evaluated using necrosis and apoptosis severity scores in epithelial tubular cells. Both parameters were reduced in the treatment group. The diminished *in situ* expression of TNF-α and caspase 8 demonstrated that the apoptotic extrinsic pathway was affected. TNF-α is an inflammatory mediator related to

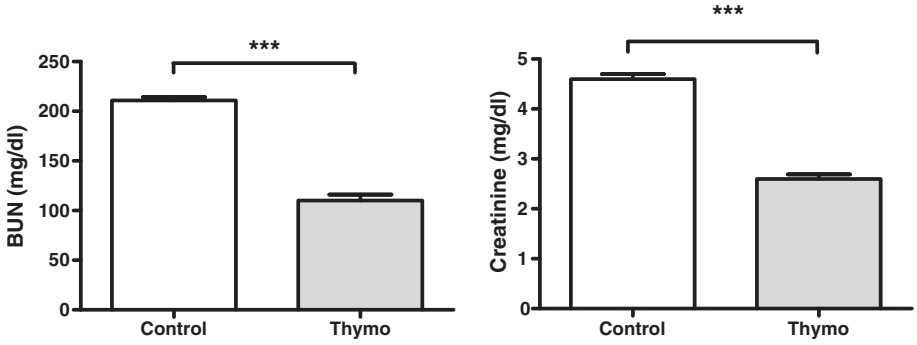

Fig. 1. Urea and creatinine levels were significantly lower in kidneys from group that had been administered rATG. Urea: control group kidneys: 211 ± 8 mg/dl *vs.* rATG treatment group kidneys: 110 ± 15 mg/dl ($p<0.001$). Creatinine: control kidneys: 4.6 ± 0.24 mg/dl *vs.* rATG treatment kidneys: 2.6 ± 0.22 mg/dl ($p<0.001$).

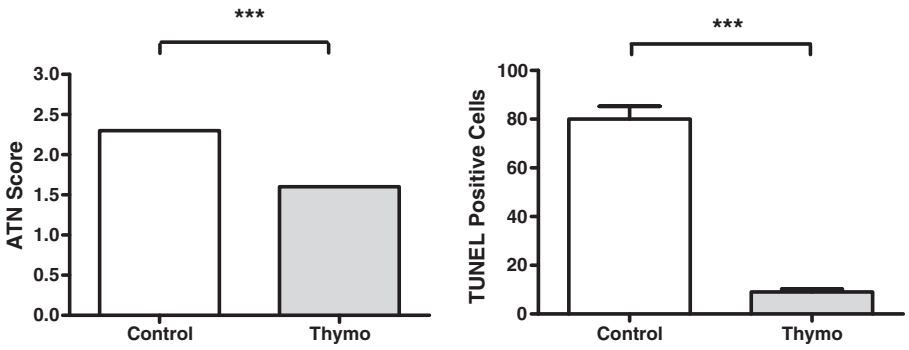

Fig. 2. For the histological analysis we used median scores representing necrosis severity: control group 2.3 *vs.* treatment group 1.6 ($p<0.05$). TUNEL staining demonstrated that kidneys from control group had a total 80 ± 13 positive cells whereas kidneys from treatment group had total 9 ± 3 ($p<0.001$).

the apoptosis of tubular epithelial cells and the apoptotic extrinsic pathway. Apoptosis is triggered by the interaction of TNF-α and its receptor (TNFR1 or Fas), leading to endonuclease activation that fragments DNA and triggers cell death through apoptosis. TNF-α promotes neutrophil and monocyte migration to the kidneys, increases adhesion molecules such as ICAM-1 and L-selectine, and is related to ICAM and VCAM adhesion molecules *via* nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) activation [17]. In previous studies, we observed the same effects when rapamycin was administered to donors [18]. Regarding the apoptotic intrinsic pathway, we found no difference in caspase 9 expression between the groups. This pathway was affected by upregulation of BCL-2, an antiapoptotic-related protein. HO1, another cytoprotective protein, was upregulated in the treatment group kidneys.

IRI produces acute tubular necrosis (ATN), which, in turn, causes graft dysfunction during the immediate post-transplant period [19,20]. In the kidneys from the donors that had received rATG, we observed a significantly reduced ATN severity score and diminished expression of KIM-1, a cell surface protein highly expressed in injured tubular epithelial cells that serves as a tubular dysfunction parameter [21–23]. Because the treatment group was sacrificed 24 h after transplantation, KIM-1 may not be considered to be a follow-up parameter for ATN recovery.

During IRI, leukocytes migrate to the graft by adhering to the endothelium, which is associated with tissue and vascular damage and organ dysfunction in the immediate post-transplant period [6,22]. Adhesion is produced by molecules such as ICAM1, the expression if which was not significantly different between groups. Blocking the leukocyte–endothelium interaction may reduce tissue and vascular damage in IRI. Beiras-Fernandez et al. evaluated the effects of ATG on this interaction [24]. They found that animals that were reperfused without ATG showed leukocyte rolling 5 min after reperfusion and increased leukocyte adhesion to the endothelium 30 min after reperfusion. Conversely, leukocyte rolling was reduced and leukocyte adhesion was inhibited when ATG was administered [24]. In a recent study, Aiello et al. reported that administration of ATG to recipients 2 h before transplantation was effective in preventing renal damage related to IRI. This effect was not observed when ATG was given at the time of reperfusion. The authors found that rATG pretransplant treatment decreased intragraft macrophages, CD8+ and CD4+ T cells, and the proportion of infiltrating LFA-1+ cells [14].

Cytokines cannot be classified exclusively as pro- or anti-inflammatory, and their actions are unpredictable because they depend on the presence of responsive cells, the combination of other cytokines, and their release timing. TGF-beta1 has many effects on the cell, tissues, and, specifically, on extracellular matrix (ECM) [25] homeostasis in the kidneys. TGF-β1 enhances the biosynthesis rate of ECM molecules, but also blocks the synthesis of enzymes that degrade the ECM. TGF-beta1, IL-6, and IL-21 cause differentiation of naïve T cells into Th17 cells through an acute rejection pathway caused by IRI [26–28]. Guan et al. examined the regulation of TGF-β1 expression in TECs and its role in the development of renal IRI. They concluded that TGF-β1 could act as a feedback survival factor in the processes of resisting kidney injury and maintaining epithelium homeostasis [29], which could diminish the fibrosis level in the post-transplant period. We observed that administering rATG to the donor diminished *in situ* levels of TGF-β1, IL-6, and IL-21. Thus, the administration of rATG to donors could create a favorable microenvironment for Tregs, but hostile for Th17.

Even though we do not present dendritic cells (DCs) phenotype-function markers in this study, rATG may have *in vivo* and *in vitro*

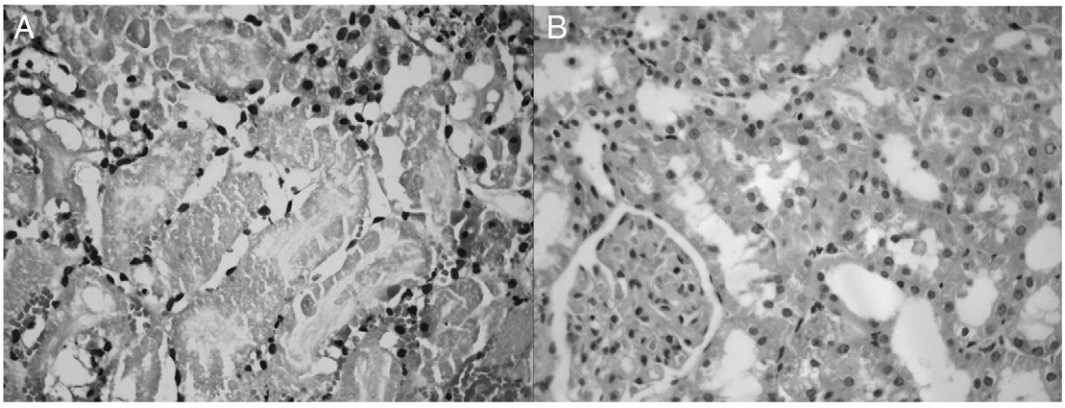

Fig. 3. Hematoxylin eosine stain for the evaluation of renal injury after 24 h of ischemia–reperfusion. Image (A) shows tubular cells damage in renal cortex and (B) shows reduced epithelial damage in rATG treated rats. (Original magnification 200×).

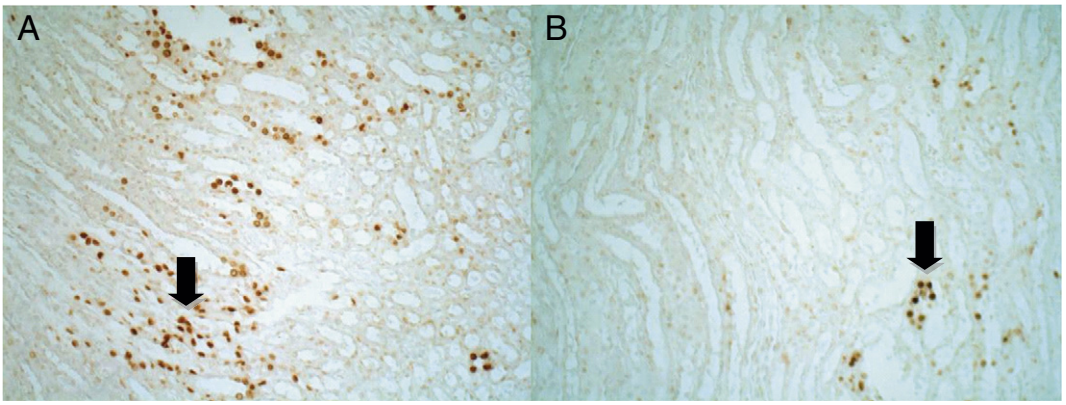

Fig. 4. Representative TUNEL staining of kidney sample after 24 h of reperfusion; (A) shows non-treated kidneys and (B) shows kidneys treated with rATG. Arrows indicate positive apoptotic nuclei. The number of positive apoptotic nuclei in rATG treatment group was significantly reduced (B). (Original magnification 100×).

effects on these cells [30–32]. Typically, DCs collect antigenic material in tissues and then migrate to lymph nodes to present antigen to naïve T cells [32]. The renal mononuclear phagocytic system has significant homeostatic and sentinel functions in the kidney and maintains immune tolerance against self- and innocuous antigens [32]. When the kidney is injured, the renal mononuclear phagocytic system changes considerably; resident and recruited rMoPh show functional heterogeneity and plasticity in defense against microbes, mediation of parenchymal injury, and support of tissue repair [32]. Womer et al. [30] demonstrated a discriminating binding of ATG to the mDC subset *in vivo*, suggesting that allo-transplantation and/or immunosuppressive therapy may have significant effects on DC levels. Further studies may increase our knowledge about DCs' role in this model including cold ischemia and ATG administered to the donor.

Our objective was to treat donors with rATG to prevent changes that may cause a post-reperfusion inflammatory state. We used a pure theoretical model to study IRI without the confounding effects that antigenic stimulation or the brain death process may have. It would be beneficial to include longer observation times and, in future studies assess the effects of rATG on recipients and donors and elucidate ATG precise mechanisms.

In conclusion, our findings suggest that ATG administered to donors may ameliorate the IRI process in kidney transplantation. IRI amelioration is expressed by the lower necrosis and apoptosis scores and the improvement of renal function, which may be explained

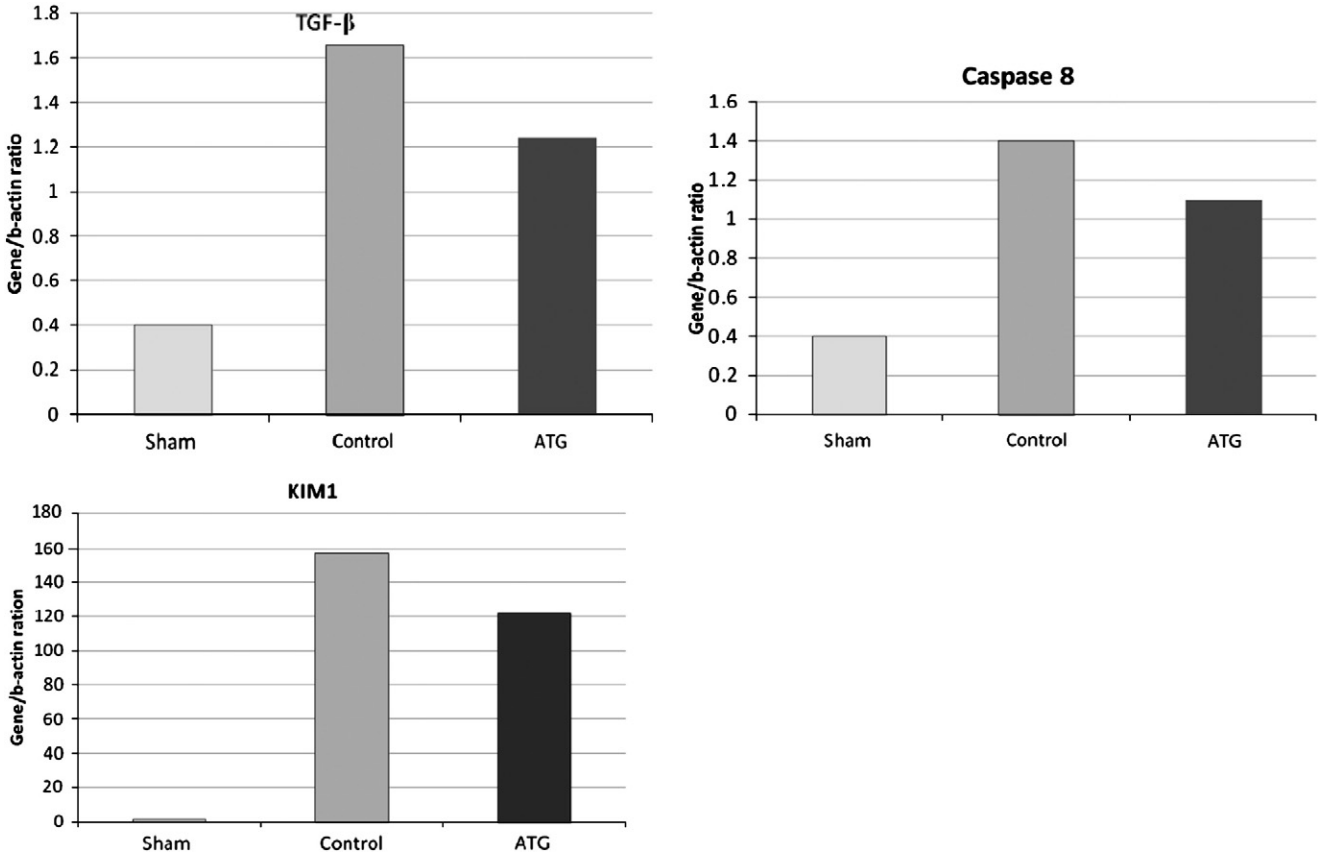

Fig. 5. Confirmation of expression of selected number of genes by RT-PCR: TFG-β ($p<0.01$), Caspase 8 ($p<0.01$) and KIM-1 ($p<0.001$) were significantly decreased in kidneys from donors that had been treated with rATG when compared to control group. The same was observed for TNF-α, IL-6, IL-21 ($p<0.01$, figures not shown). Bcl-2 and HO-1 were upregulated *in situ* in the treatment group compared to control group ($p<0.001$, figures not shown).

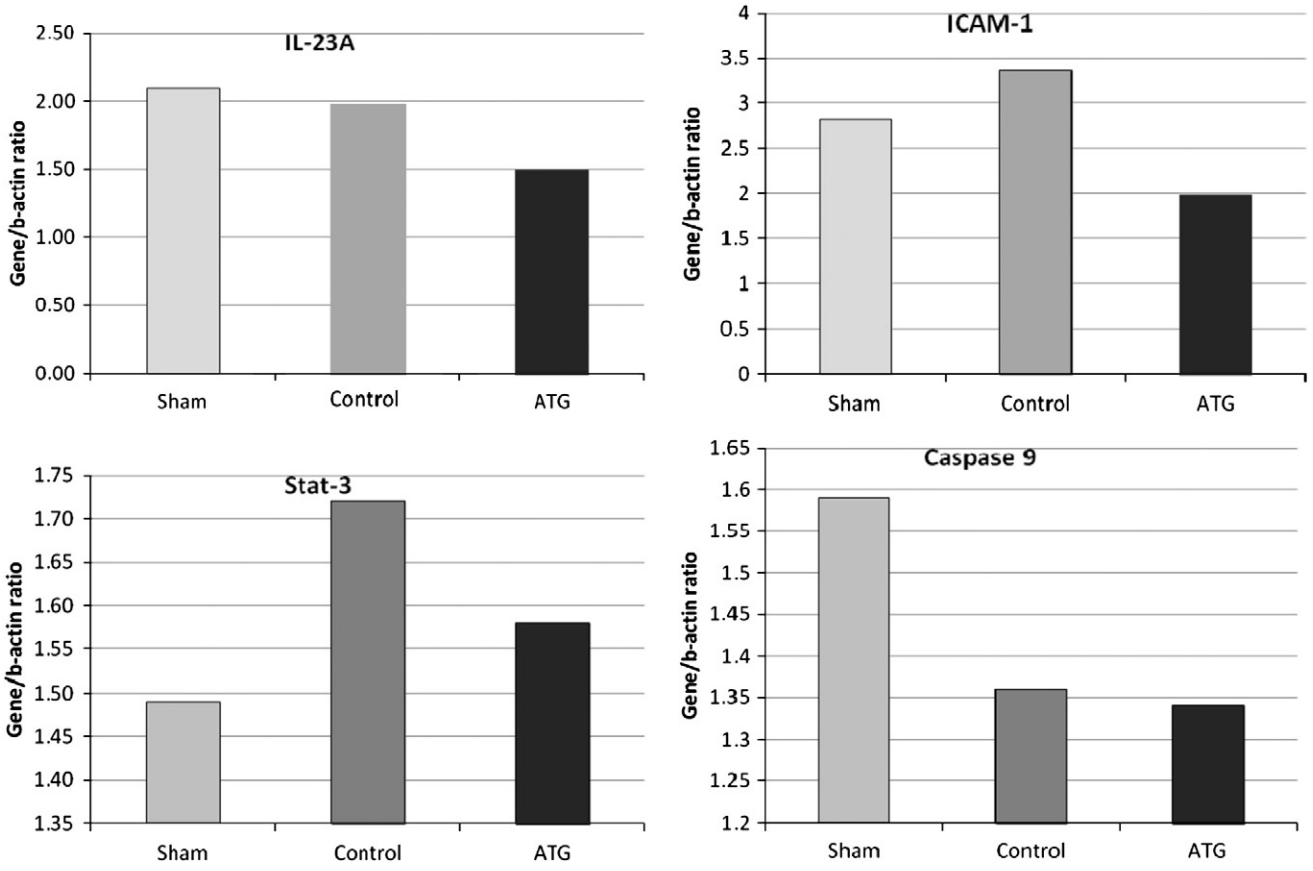

Fig. 6. Confirmation of expression of selected number of genes by RT-PCR: The *in situ* expression of IL-23a, ICAM-1, Stat-3 and caspase 9 showed no difference between control and treatment groups ($p > 0.05$). The same was observed for IL-17, CxCl1 and CxCl3 ($p > 0.05$, figures not shown).

through the diminished *in situ* expression of inflammatory mediators. In addition, we found decreased cytokine levels related to Th17 cell differentiation, which created a microenvironment that was favorable for Tregs, but hostile for Th17. Further studies regarding donor management, including brain death, are needed to continue evaluating the possible administration of ATG to donors as a novel strategy to improve outcomes in renal transplantation.

Funding

This paper was funded by FINAER, Argentina and Genzyme S.A., Argentina.

References

[1] Mehrabi A, Mood ZhA, Sadeghi M, Schmied BM, Müller SA, Welsch T, et al. Thymoglobulin and ischemia reperfusion injury in kidney and liver transplantation. Nephrol Dial Transplant 2007;22(Suppl. 8) viii54–viii60.
[2] Boros P, Bromberg JS. New cellular and molecular immune pathways in ischemia/reperfusion injury. Am J Transplant 2006;6(4):652–8.
[3] Sievert A. Leukocyte depletion as a mechanism for reducing neutrophil-mediated ischemic–reperfusion injury during transplantation. J Extra Corpor Technol 2003;35(1):48–52.
[4] Eum HA, Lee SM. Effect of Trolox on altered vasoregulatory gene expression in hepatic ischemia/reperfusion. Arch Pharm Res 2004;27(2):225–31.
[5] Martins P, Chandraker A, Tullius S. Modifying graft immunogenicity and immune response prior to transplantation: potential clinical applications of donor and graft treatment. Transp Int 2006;19:351–9.
[6] Schuurs TA, Gerbens F, van der Hoeven JA, Ottens PJ, Kooi KA, Leuvenink HG, et al. Distinct transcriptional changes in donor kidneys upon brain death induction in rats: insights in the processes of brain death. Am J Transplant 2004;4(12): 1972–81.
[7] Brasile L, Stubenitsky B, Booster M, Arenada D, Haisch C, Kootstra G. Transfection and transgene expression in a human kidney during *ex vivo* warm perfusion. Transplant Proc 2002;34:2624.
[8] Kuecuek O, Mantouvalou L, Klemz R, Kotsch K, Volk HD, Jonas S, et al. Significant reduction of proinflammatory cytokines by treatment of the brain-dead donor. Transplant Proc 2005;37(1):387–8.
[9] Pratschke J, Kofla G, Wilhelm MJ, Vergopoulos A, Laskowski I, Shaw GD, et al. Improvements in early behavior of rat kidney allografts after treatment of the brain-dead donor. Ann Surg 2001;234(6):732–40.
[10] Kotsch K, Ulrich F, Reutzel-Selke A, Pascher A, Faber W, Warnick P, et al. Methylprednisolone therapy in deceased donors reduces inflammation in the donor liver and improves outcome after liver transplantation: a prospective randomized controlled trial. Ann Surg 2008;248(6):1042–50.
[11] Gottmann U, Brinkkoetter PT, Hoeger S, Gutermann K, Coutinho QZM, Ruf T, et al. Atorvastatin donor pretreatment prevents ischemia/reperfusion injury in renal transplantation in rats: possible role for aldose-reductase inhibition. Transplantation 2007;84(6):755–62.
[12] Michallet MC, Preville X, Flacher M, Fournel S, Genestier L, Revillard JP. Functional antibodies to leukocyte adhesion molecules in antithymocyte globulins. Transplantation 2003;75(5):657–62 Epub 2003/03/18.
[13] Li SQ, Liang LJ. Protective mechanism of L-arginine against liver ischemic–reperfusion injury in rats. Hepatobiliary Pancreat Dis 2003;2(4):549–52.
[14] Aiello S, Cassis P, Mister M, Solini S, Rocchetta F, Abbate M, et al. Rabbit anti-rat thymocyte immunoglobulin preserves renal function during ischemia/reperfusion injury in rat kidney transplantation. Transpl Int 2011;24(8):829–38.
[15] Caumartin Y, Stephen J, Deng JP, Lian D, Lan Z, Liu W, et al. Carbon monoxide-releasing molecules protect against ischemia–reperfusion injury during kidney transplantation. Kidney Int 2011;79(10):1080–9.
[16] Patel NS, Chatterjee PK, Di Paola R, Mazzon E, Britti D, De Sarro A, et al. Endogenous interleukin-6 enhances the renal injury, dysfunction, and inflammation caused by ischemia/reperfusion. J Pharmacol Exp Ther 2005;312(3):1170–8.
[17] Donnahoo KK, Meldrum DR, Shenkar R, Chung CS, Abraham E, Harken AH. Early renal ischemia, with or without reperfusion, activates NFkappaB and increases TNF-alpha bioactivity in the kidney. J Urol 2000;163(4):1328–32.
[18] Cicora F, Lausada N, Vasquez DN, Cicora P, Zalazar G, Gonzalez P, et al. Sirolimus in kidney transplant donors and clinical and histologic improvement in recipients: rat model. Transplant Proc 2010;42(1):365–70.
[19] Bonventre JV, Zuk A. Ischemic acute renal failure: an inflammatory disease? Kidney Int 2004;66(2):480–5 Epub 2004/07/16.
[20] Land WG. The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. Transplantation 2005;79(5):505–14.
[21] Lane BR. Molecular markers of kidney injury. Urol Oncol in press.
[22] Harlan JM, Winn RK. Leukocyte–endothelial interactions: clinical trials of anti-adhesion therapy. Crit Care Med 2002;30(5 Suppl.):S214–9.

[23] Yeung MY, McGrath M, Najafian N. The emerging role of the TIM molecules in transplantation. Am J Transplant 2011;11(10):2012–9.
[24] Beiras-Fernandez A, Chappell D, Hammer C, Beiras A, Reichart B, Thein E. Impact of polyclonal anti-thymocyte globulins on the expression of adhesion and inflammation molecules after ischemia–reperfusion injury. Transpl Immunol 2009;20(4):224–8.
[25] Ozdemir BH, Ozdemir FN, Demirhan B, Haberal M. TGF-[beta]1 expression in renal allograft rejection and closporine A toxicity. Transplant 2005;80(12):1681–5.
[26] Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity 2008;28(4):454–67.
[27] Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol 2008;9(6):641–9.
[28] Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006;24: 99–146.
[29] Guan Q, Nguan CY, Du C. Expression of transforming growth factor-beta1 limits renal ischemia–reperfusion injury. Transplantation 2010;89(11):1320–7.
[30] Womer KL, Peng R, Patton PR, Murawski MR, Bucci M, Kaleem A, et al. The effects of renal transplantation on peripheral blood dendritic cells. Clin Transplant 2005;19:659–67.
[31] Nelson JN, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J. The renal mononuclear phagocytic system. J Am Soc Nephrol 2012;2012(23):194–203.
[32] Tadagavadi RK, Reeves WB. Renal dendritic cells ameliorate nephrotoxic acute kidney injury. J Am Soc Nephrol 2010;21:53–63.