1

General Introduction

D.K. de Vries, A.F.M. Schaapherder, M.E.J. Reinders

Adapted version of: Front Immunol. 2012;3:162

Introduction

Kidney transplantation is the preferred treatment of end-stage renal disease, because of the recipient's improved life expectancy, better quality of life, and the lower total healthcare costs, compared to dialysis treatment.[1,2] Since the first successful kidney transplantation in 1954, patient and graft survival increased tremendously.[3,4] Despite all the improvements in immunosuppressive drugs and surgical techniques, it appears that during the last decade a plateau in long term allograft survival has been reached.

As the demand for kidney grafts is ever increasing, the number of kidney transplantations is limited by the availability of organ donors. Almost half of all kidney transplantations are living donor kidney transplantations, whereas the others are transplantations with deceased donor kidney grafts.[5] Deceased donor kidney grafts are retrieved from either brain dead donors or cardiac dead donors. Graft survival for living unrelated donor kidney transplantation is superior compared to that of deceased donor kidney transplantation, even though the average human leukocyte antigen (HLA) matching is worse in living unrelated transplantation.[6] Therefore, the limited graft survival of deceased donor kidneys cannot be attributed exclusively to differences in immunogenicity; other causes of damage are probably more important. The most prominent of these causes is ischemia/reperfusion (I/R) injury, characterized by the exacerbation of tissue damage upon reestablishment of circulation after a period of ischemia. I/R injury is an important cause of delayed graft function, having a major influence on both graft function as well as graft survival.[7]

Renal ischemia/reperfusion injury

Renal I/R injury occurs in a multitude of clinical situations. Periods of hypotension with impaired blood flow to the kidney can cause renal I/R injury, whereas more acute ischemia occurs in renal arterial thrombosis. In kidney transplantation renal ischemia is inevitable, and the duration of ischemia is often beyond control. Preventive and therapeutic measures in I/R injury would be required to reduce the severity of graft dysfunction and failure thus allowing safe expansion of the donor pool with marginal donor kidneys that have suffered more initial injury before organ retrieval. Unfortunately, current treatment for renal I/R injury is still primarily supportive, and experimental therapies aimed at minimizing I/R injury have been applied in animal models generally, not clinical trials. In order to design better therapeutics for clinical renal I/R injury, detailed knowledge on the pathophysiological mechanisms leading to ischemic acute graft injury after transplantation is required.

Pathophysiology of renal ischemia/reperfusion injury

The pathophysiology of I/R injury is multifactorial and only partially understood. Inflammation however, is regarded the crucial event in the development of tissue injury and graft dysfunction in renal I/R injury. Based on animal experiments, many individual factors such as cytokines and complement have been suggested to be involved in the inflammatory response. However, intervention studies aimed at specific inhibition of a single factor have generally shown disappointing results.[8,9] Cooperation, redundancy and interactions make the involved mechanisms more complex than previously thought. Pharmacological inhibition of the entire inflammatory cascade would appear a logical intervention, however the negative side-effects appear larger than the anticipated beneficial effects.[10]

Although there may be differences in the exact pathophysiological mechanisms of I/R injury between different organs, some processes appear to play a universal role. The endothelium and microvasculature are very sensitive to hypoxia and are easily affected in I/R injury. Upon reperfusion, the vascular endothelial cell lining can undergo swelling which may lead to narrowing of the vascular lumen.[11,12] Moreover, vasorelaxation can be impaired, together contributing to the no-reflow phenomenon.[13] Endothelial injury can increase microvascular permeability which may lead to inflammatory cell trafficking into the reperfused kidney. There have been many reports of invading granulocytes, monocytes, dendritic cells (DC's) and lymphocytes into various tissues after reperfusion.[14-20] Together with leukocytes, platelets can be activated by injured endothelium. In myocardial infarction, platelets mediate thrombotic occlusion and increase damage by causing microvascular occlusions, contributing to the no-reflow phenomenon.[21] On the other hand, platelets are also able to invade tissue.[22] This is essential since platelets can contribute to the inflammatory response through release of cytokines, chemokines and growth factors from their granules.[23-25] In fact, platelets have been suggested to be involved in the inflammatory response of I/R injury in various organs. They are able to roll and adhere to post-reperfusion endothelium in a P-selectin dependent mechanism.[26-32] In mouse myocardial tissue, the first activated platelets are present within two minutes after reperfusion, and then accumulate in the infarcted myocardium.[33,34]

The ensuing inflammatory response which follows is considered to exacerbate damage. Both the innate as well as the adaptive immune system can be activated after reperfusion. Activation of the innate immune system is probably mediated by activation of pattern-recognition receptors such as toll-like receptors that recognize their endogenous ligands that are released upon tissue damage.[35] The complement system is part of the humoral immune response and can play a role both as first line innate defense, but may also contribute to the adaptive immune response.[36] In many animal experiments a role for

(terminal) complement activation in I/R injury has been suggested,[37-41] although recent animal experiments doubt the involvement of the complement system itself in the initiation of injury.[42] The role of complement activation in human I/R injury is even more complex. While a role of complement activation was suggested in human myocardial I/R injury,[43,44] diverse anti-complement intervention studies did not lead to major improvements.[45-50]

Ischemia-related metabolic adaptations and dysregulated mitochondrial homeostasis are thought to result in substantial release of reactive oxygen and nitrogen species (RONS) upon reintroduction of oxygen. The RONS overload can overwhelm the endogenous antioxidant system, resulting in oxidative damage. This may trigger secondary processes and contribute to the pro-inflammatory response upon reperfusion.[51-54] Numerous animal studies clearly demonstrate that antioxidant therapy ameliorates I/R injury.[55-57] Despite these findings, studies in humans consistently fail to show any clinically relevant effect.[55,58-61] The basis for this discrepancy between human and animal studies is still unclear. Yet, it may suggest that the contribution of RONS to I/R injury in humans is less than commonly thought.

Ultimately, when I/R injury to the cell is severe, various programs of cell death can be activated. Three major forms of cell death can be distinguished: necrosis, apoptosis, and autophagy. Besides acute cell death by necrosis or apoptosis during and directly after the ischemic period, cell death continues for several days following reperfusion. All three types of cell death can contribute to the continued loss of cells for days and even weeks in the reperfused tissue. In animal models, both necrosis and apoptosis continued after reperfusion with a maximum severity three days after reperfusion.[62,63] Autophagy during the ischemic episode appears to keep cells viable and might play a protective role. It can be suggested, however, that activation of autophagy after reperfusion is detrimental. Indeed, a mouse model of myocardial I/R illustrates that protein levels of the autophagic mediator beclin can be greatly upregulated during reperfusion. Mice with reduced beclin levels exhibited smaller myocardial infarct sizes.[64,65]

Long term impact

Although short term results of kidney transplantation are excellent, 5 year graft loss can be up to 30% in older recipients.[66] Protocol biopsies obtained in the first years after transplantation have shown rapid increase in the prevalence of interstitial fibrosis/tubular atrophy (IF/TA). This finding has been correlated with later graft dysfunction and graft loss, mostly in cases of concomitant interstitial inflammation and fibrosis.[67,68] Both allogen dependent and independent factors determine IF/TA. I/R injury is an important non-allogeneic factor and the duration of the cold ischemic period is directly correlated to delayed graft function and even graft failure.[69,70] Even without allogeneic transplantation, I/R injury itself has been

shown to cause interstitial fibrosis and glomerulosclerosis in experimental models (Figure 1).[71-73]

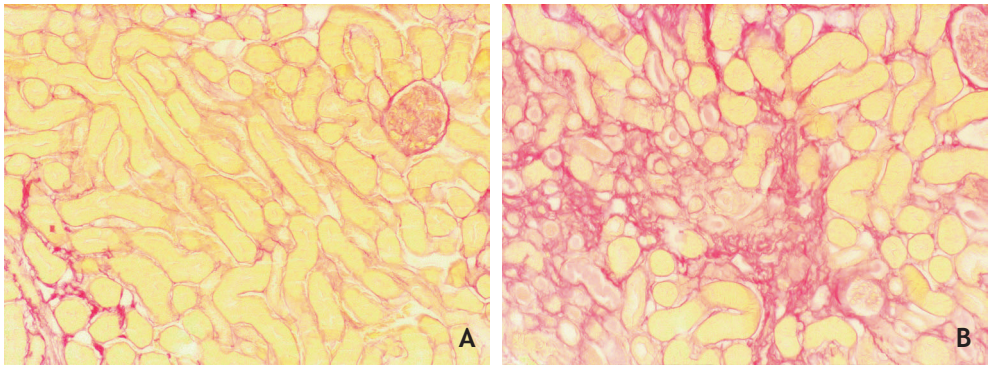

Figure 1: Experimental renal I/R injury induces severe patchy renal fibrosis, although kidney function partially recovers. Sirius red staining shows A) normal mouse kidney and B) severe fibrosis 3 weeks after mice underwent 25 minutes of warm renal I/R injury. (Non published data)

Opportunities in studying renal I/R injury

Until now, results of renal I/R experiments in small animal models have not been translatable into clinical kidney transplantation. The most probable reason is that the exact mechanism involved are probably different between species. Detailed knowledge on the pathophysiological mechanisms leading to I/R injury in human kidney transplantation is required to form a basis for experimental therapies.

Studying the pathophysiology of human renal I/R injury requires careful techniques that specifically assess the processes that occur in the kidney at the moment of reperfusion and thereafter. Two complementary approaches have been chosen in previous studies: assessment of processes in the intravascular compartment or assessment of changes in the renal tissue. Intravascular changes have been assessed frequently in renal I/R injury, and almost all studies measured changes in circulating factors by collecting sequential peripheral blood samples. In these peripheral blood samples however, the source of the released factors can never be ascertained to be the kidney. Even more since haemodynamics change upon reperfusion of the kidney graft, and the leg is reperfused simultaneously with the kidney upon removal of the iliac arterial clamp. Furthermore, release of factors into the circulation may be undetected because of their dilution in the total circulating volume. By collecting arteriovenous blood samples over the kidney during reperfusion, specific measurements can be done studying those factors that are released from the kidney, i.e. that have a higher concentration in renal venous blood compared to arterial blood. Moreover, the release of these markers can be assessed with higher sensitivity, since a small release will produce the

largest concentration difference in the efferent vein. Finally, by measuring time-series of these arteriovenous differences a dynamic and specific footprint of the processes occurring in the reperfused kidney can be reconstituted. In Figure 2 the technique of arteriovenous measurements over the transplanted kidney is illustrated. The renal artery and vein are selectively cannulated before reperfusion, and during the first half hour of reperfusion, timed and paired arterial and venous samples can be collected from the kidney. This technique is applied in many of the studies described in this thesis.

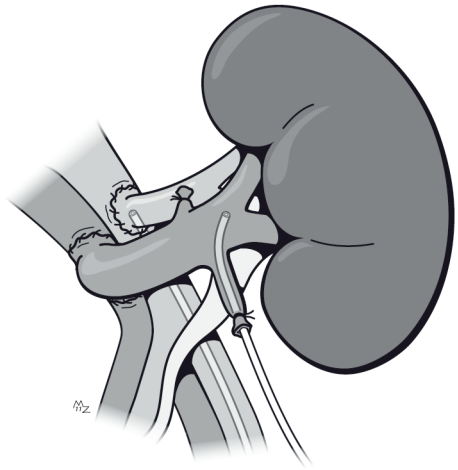

Figure 2: Schematic representation of the arteriovenous sampling method over the reperfused kidney by simultaneous blood collection from the renal artery (left) and renal vein (right). Illustration by Manon Zuurmond© (www.manonproject.com). Adapted from de Vries et al. [77]

Aims of this thesis

The aims of this thesis were to explore the factors and processes involved in the pathophysiology of renal I/R injury in clinical kidney transplantation, in order to establish a basis for the development of specific therapies preventing and limiting renal I/R injury in kidney transplantation. Exact knowledge on the sequence of events by which graft damage is initiated after reperfusion in human kidney transplantation was still lacking. In chapter 2, 3 and 4, the release of pro-inflammatory cytokines from the reperfused graft is assessed and compared between living and deceased donor kidney transplantations. In chapter 5, an important actor of the innate immune system, the complement system, is assessed in human kidney transplantations. Whether endothelial activation and concomitant platelet activation are present in early reperfusion of transplanted kidneys is investigated in chapters 6, 7 and 8. Finally, oxidative damage, the most commonly mentioned process in the pathophysiology of I/R injury is carefully investigated in human kidney transplantation in chapter 9. Chapter

10 shows new insights as I/R injury is approached from an unbiased, hypothesis generating angle, in which gene expression profiles are compared to assess changes upon reperfusion and baseline differences between different donor types. Chapter 11 and 12 summarize the findings in this thesis and review future perspectives in treatment of I/R injury.

References

1. Tonelli M, Wiebe N, Knoll G et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. Am J Transplant 2011; 11:2093-2109.
2. Wyld M, Morton RL, Hayen A et al. A systematic review and meta-analysis of utility-based quality of life in chronic kidney disease treatments. PLoS Med 2012; 9:e1001307.
3. Hariharan S, Johnson CP, Bresnahan BA et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342:605-612.
4. Stefoni S, Campieri C, Donati G et al. The history of clinical renal transplant. J Nephrol 2004; 17:475-478.
5. Horvat LD, Shariff SZ, Garg AX. Global trends in the rates of living kidney donation. Kidney Int 2009; 75:1088-1098.
6. Terasaki PI, Cecka JM, Gjertson DW et al. High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med 1995; 333:333-336.
7. Yarlagadda SG, Coca SG, Formica RN, Jr. et al. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. Nephrol Dial Transplant 2009; 24:1039-1047.
8. de Vries DK, Lindeman JH, Tsikas D et al. Early renal ischemia-reperfusion injury in humans is dominated by IL-6 release from the allograft. Am J Transplant 2009; 9:1574-1584.
9. Park P, Haas M, Cunningham PN et al. Inhibiting the complement system does not reduce injury in renal ischemia reperfusion. J Am Soc Nephrol 2001; 12:1383-1390.
10. Morariu AM, Loef BG, Aarts LPHJ et al. Dexamethasone: Benefit and prejudice for patients undergoing on-pump coronary artery bypass grafting - A study on myocardial, pulmonary, renal, intestinal, and hepatic injury. Chest 2005; 128:2677-2687.
11. Leaf A. Cell swelling. A factor in ischemic tissue injury. Circulation 1973; 48:455-458.
12. Summers WK, Jamison RL. The no reflow phenomenon in renal ischemia. Lab Invest 1971; 25:635-643.
13. Lieberthal W, Wolf EF, Rennke HG et al. Renal ischemia and reperfusion impair endothelium-dependent vascular relaxation. Am J Physiol 1989; 256:F894-F900.
14. Skjeldal S, Torvik A, Grogaard B et al. Histological studies on postischemic rat skeletal muscles. With emphasis on the time of leukocyte invasion. Eur Surg Res 1993; 25:348-357.
15. Clark RK, Lee EV, White RF et al. Reperfusion following focal stroke hastens inflammation and resolution of ischemic injured tissue. Brain Res Bull 1994; 35:387-392.
16. Shigematsu T, Wolf RE, Granger DN. T-lymphocytes modulate the microvascular and inflammatory responses to intestinal ischemia-reperfusion. Microcirculation 2002; 9:99-109.
17. Day YJ, Huang L, Ye H et al. Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: role of macrophages. Am J Physiol Renal Physiol 2005; 288:F722-F731.
18. Day YJ, Huang L, Ye H et al. Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: the role of CD4+ T cells and IFN-gamma. J Immunol 2006; 176:3108-3114.
19. Burne-Taney MJ, Ascon DB, Daniels F et al. B cell deficiency confers protection from renal ischemia reperfusion injury. J Immunol 2003; 171:3210-3215.
20. de Vries DK, Lindeman JH, Ringers J et al. Donor brain death predisposes human kidney grafts to a proinflammatory reaction after transplantation. Am J Transplant 2011; 11:1064-1070.
21. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovasc Res 2004; 61:498-511.
22. Weissmuller T, Campbell EL, Rosenberger P et al. PMNs facilitate translocation of platelets across human and mouse epithelium and together alter fluid homeostasis via epithelial cell-expressed ecto-NTPDases. J Clin Invest 2008; 118:3682-3692.
23. Reed GL. Platelet secretory mechanisms. Semin Thromb Hemost 2004; 30:441-450.

24. Lisman T, Porte RJ. The role of platelets in liver inflammation and regeneration. Semin Thromb Hemost 2010; 36:170-174.
25. Thornton P, McColl BW, Greenhalgh A et al. Platelet interleukin-1alpha drives cerebrovascular inflammation. Blood 2010; 115:3632-3639.
26. Yadav SS, Howell DN, Steeber DA et al. P-Selectin mediates reperfusion injury through neutrophil and platelet sequestration in the warm ischemic mouse liver. Hepatology 1999; 29:1494-1502.
27. Khandoga A, Biberthaler P, Enders G et al. P-selectin mediates platelet-endothelial cell interactions and reperfusion injury in the mouse liver in vivo. Shock 2002; 18:529-535.
28. Cywes R, Packham MA, Tietze L et al. Role of platelets in hepatic allograft preservation injury in the rat. Hepatology 1993; 18:635-647.
29. Sindram D, Porte RJ, Hoffman MR et al. Platelets induce sinusoidal endothelial cell apoptosis upon reperfusion of the cold ischemic rat liver. Gastroenterology 2000; 118:183-191.
30. am Esch JS, Tustas RY, Robson SC et al. Recipient levels and function of von Willebrand factor prior to liver transplantation and its consumption in the course of grafting correlate with hepatocellular damage and outcome. Transpl Int 2005; 18:1258-1265.
31. Massberg S, Enders G, Leiderer R et al. Platelet-endothelial cell interactions during ischemia/reperfusion: the role of P-selectin. Blood 1998; 92:507-515.
32. Chintala MS, Bernardino V, Chiu PJ. Cyclic GMP but not cyclic AMP prevents renal platelet accumulation after ischemia-reperfusion in anesthetized rats. J Pharmacol Exp Ther 1994; 271:1203-1208.
33. Xu Y, Huo Y, Toufektsian MC et al. Activated platelets contribute importantly to myocardial reperfusion injury. Am J Physiol Heart Circ Physiol 2006; 290:H692-H699.
34. Liu Y, Gao XM, Fang L et al. Novel role of platelets in mediating inflammatory responses and ventricular rupture or remodeling following myocardial infarction. Arterioscler Thromb Vasc Biol 2011; 31:834-841.
35. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010; 10:826-837.
36. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res 2010; 20:34-50.
37. de Vries B, Matthijsen RA, Wolfs TG et al. Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: inhibition of late apoptosis and inflammation. Transplantation 2003; 75:375-382.
38. Park P, Haas M, Cunningham PN et al. Inhibiting the complement system does not reduce injury in renal ischemia reperfusion. J Am Soc Nephrol 2001; 12:1383-1390.
39. Zheng X, Zhang X, Feng B et al. Gene silencing of complement C5a receptor using siRNA for preventing ischemia/reperfusion injury. Am J Pathol 2008; 173:973-980.
40. Zhang J, Hu W, Xing W et al. The protective role of CD59 and pathogenic role of complement in hepatic ischemia and reperfusion injury. Am J Pathol 2011; 179:2876-2884.
41. Zhou W, Farrar CA, Abe K et al. Predominant role for C5b-9 in renal ischemia/reperfusion injury. J Clin Invest 2000; 105:1363-1371.
42. van der Pol P, Schlagwein N, van Gijlswijk DJ et al. Mannan-Binding Lectin Mediates Renal Ischemia/Reperfusion Injury Independent of Complement Activation. Am J Transplant 2012.
43. Baldwin WM, III, Samaniego-Picota M, Kasper EK et al. Complement deposition in early cardiac transplant biopsies is associated with ischemic injury and subsequent rejection episodes. Transplantation 1999; 68:894-900.
44. Yasojima K, Schwab C, McGeer EG et al. Human heart generates complement proteins that are upregulated and activated after myocardial infarction. Circ Res 1998; 83:860-869.
45. Testa L, Van Gaal WJ, Bhindi R et al. Pexelizumab in ischemic heart disease: a systematic review and meta-analysis on 15,196 patients. J Thorac Cardiovasc Surg 2008; 136:884-893.
46. Fattouch K, Bianco G, Speziale G et al. Beneficial effects of C1 esterase inhibitor in ST-elevation myocardial infarction in patients who underwent surgical reperfusion: a randomised double-blind study. Eur J Cardiothorac Surg 2007; 32:326-332.
47. Verrier ED, Shernan SK, Taylor KM et al. Terminal complement blockade with pexelizumab during coronary artery bypass

graft surgery requiring cardiopulmonary bypass: a randomized trial. JAMA 2004; 291:2319-2327.

48. Mahaffey KW, Granger CB, Nicolau JC et al. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction: the COMPlement inhibition in myocardial infarction treated with thromboLYtics (COMPLY) trial. Circulation 2003; 108:1176-1183.

49. Granger CB, Mahaffey KW, Weaver WD et al. Pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to primary percutaneous coronary intervention in acute myocardial infarction: the COMplement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial. Circulation 2003; 108:1184-1190.

50. Lazar HL, Bokesch PM, van LF et al. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. Circulation 2004; 110:II274-II279.

51. Valko M, Leibfritz D, Moncol J et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39:44-84.

52. Misra MK, Sarwat M, Bhakuni P et al. Oxidative stress and ischemic myocardial syndromes. Med Sci Monit 2009; 15:RA209-RA219.

53. Gourdin MJ, Bree B, De KM. The impact of ischaemia-reperfusion on the blood vessel. Eur J Anaesthesiol 2009; 26:537-547.

54. Crimi E, Sica V, Williams-Ignarro S et al. The role of oxidative stress in adult critical care. Free Radic Biol Med 2006; 40:398-406.

55. Yellon DM, Hausenloy DJ. Mechanisms of disease: Myocardial reperfusion injury. New England Journal of Medicine 2007; 357:1121-1135.

56. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med 2009; 7:97.

57. Ambrosio G, Flaherty JT, Duilio C et al. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts. J Clin Invest 1991; 87:2056-2066.

58. Bath PM, Iddenden R, Bath FJ et al. Tirilazad for acute ischaemic stroke. Cochrane Database Syst Rev 2001;CD002087.

59. Suzuki K. Anti-oxidants for therapeutic use: why are only a few drugs in clinical use? Adv Drug Deliv Rev 2009; 61:287-289.

60. El-Hamamsy I, Stevens LM, Carrier M et al. Effect of intravenous N-acetylcysteine on outcomes after coronary artery bypass surgery: a randomized, double-blind, placebo-controlled clinical trial. J Thorac Cardiovasc Surg 2007; 133:7-12.

61. Land W, Zweler JL. Prevention of reperfusion-induced, free radical-mediated acute endothelial injury by superoxide dismutase as an effective tool to delay/prevent chronic renal allograft failure: a review. Transplant Proc 1997; 29:2567-2568.

62. Zhao ZQ, Nakamura M, Wang NP et al. Reperfusion induces myocardial apoptotic cell death. Cardiovasc Res 2000; 45:651-660.

63. Zhao ZQ, Velez DA, Wang NP et al. Progressively developed myocardial apoptotic cell death during late phase of reperfusion. Apoptosis 2001; 6:279-290.

64. Hariharan N, Zhai P, Sadoshima J. Oxidative stress stimulates autophagic flux during ischemia/reperfusion. Antioxid Redox Signal 2011; 14:2179-2190.

65. Matsui Y, Takagi H, Qu X et al. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res 2007; 100:914-922.

66. Keith DS, Cantarovich M, Paraskevas S et al. Recipient age and risk of chronic allograft nephropathy in primary deceased donor kidney transplant. Transpl Int 2006; 19:649-656.

67. Park WD, Griffin MD, Cornell LD et al. Fibrosis with inflammation at one year predicts transplant functional decline. J Am Soc Nephrol 2010; 21:1987-1997.

68. Nankivell BJ, Borrows RJ, Fung CL et al. The natural history of chronic allograft nephropathy. N Engl J Med 2003; 349:2326-2333.

69. Salahudeen AK, Haider N, May W. Cold ischemia and the reduced long-term survival of cadaveric renal allografts. Kidney Int 2004; 65:713-718.

70. Ojo AO, Wolfe RA, Held PJ et al. Delayed graft function: risk factors and implications for renal allograft survival. Transplantation 1997; 63:968-974.

71. Herrero-Fresneda I, Torras J, Lloberas N et al. Cold ischemia in the absence of alloreactivity induces chronic transplant nephropathy through a process mediated by the platelet-activating factor. Transplantation 2000; 70:1624-1631.

72. Tullius SG, Heemann U, Hancock WW et al. Long-term kidney isografts develop functional and morphologic changes that mimic those of chronic allograft rejection. Ann Surg 1994; 220:425-432.

73. Basile DP, Donohoe D, Roethe K et al. Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. Am J Physiol Renal Physiol 2001; 281:F887-F899.

74. Sagrinati C, Netti GS, Mazzinghi B et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol 2006; 17:2443-2456.

75. Gupta S, Verfaillie C, Chmielewski D et al. A role for extrarenal cells in the regeneration following acute renal failure. Kidney Int 2002; 62:1285-1290.

76. Poulsom R, Forbes SJ, Hodivala-Dilke K et al. Bone marrow contributes to renal parenchymal turnover and regeneration. J Pathol 2001; 195:229-235.

77. De Vries DK, Kortekaas KA, Tsikas D et al. Oxidative damage in clinical ischemia/reperfusion injury: a reappraisal. Antioxid Redox Signal. 2013;19:535-45