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adults. Chest 1991; 100(6): 1619.
5. Afessa B, Tefferi A, Hoagland HC, et al. Outcome of recipients of bone marrow transplants who require intensive-care unit support. Mayo Clin Proc 1992; 67(2): 117.
6. Huaringa AJ, Leyva FJ, Giralt SA, et al. Outcome of bone marrow transplantation patients requiring mechanical ventilation. Crit Care Med 2000; 28(4): 1014.
7. Hollmig KA, Soehngen D, Leschke M, et al. Long-term survival of recipients of allogeneic bone-marrow transplantation after mechanical ventilation. Eur J Med Res 1997; 2(2): 62.
8. Paz HL, Crilley P, Weinar M, et al. Outcome of patients requiring medical ICU admission following bone marrow transplantation. Chest 1993; 104(2): 527.
9. Rubenfeld GD, Crawford SW. Withdrawing life support from mechanically ventilated recipients of bone marrow transplants: a case for evidence-based guidelines. Ann Intern Med 1996; 125(8): 625.
10. Staudinger T, Stoiser B, Mullner M, et al. Outcome and prognostic factors in critically ill cancer patients admitted to the intensive care unit. Crit Care Med 2000; 28(5): 1322.
11. Berger MM, Marazzi A, Freeman J, et al. Evaluation of the consistency of Acute Physiology and Chronic Health Evaluation (APACHE II) scoring in a surgical intensive care unit. Crit Care Med 1992; 20(12): 1681.
12. Weiner RS, Horowitz MM, Gale RP, et al. Risk factors for interstitial pneumonia following bone marrow transplantation for severe aplastic anaemia. Br J Haematol 1989; 71(4): 535.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995; 15(6): 825.

LIVING KIDNEY DONORS AND HYPOXIA-INDUCIBLE FACTOR-1α

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Background. **Hypoxia inducible factor (HIF)-1, a heterodimeric transcription factor composed of α and β subunits, is induced in the adaptive response to hypoxia and is critical for initiating the transcriptional activation of growth factors. We speculate that prolonged ischemia and hypoxia time leads to the production of HIF-1α, which in turn induces the production of fibrogenic cytokines in the graft.**

Methods. **To investigate our hypothesis, we measured the expression of HIF-1α in time-zero biopsy specimens from living-donor kidneys (≤2.5 hr of ischemia) and cadaveric donor kidneys (12–32 hr of ischemia).**

Results. **By real time reverse-transcriptase polymerase chain reaction analysis, the mRNA expression level of HIF-1α was fivefold lower in time-zero biopsy specimens from living-donor kidneys than in specimens from cadaveric donor kidneys. In these time-zero biopsy specimens, the mRNA expression level of the fibrogenic cytokine transforming growth factor-β was also significantly lower (twofold).**

Conclusions. **Low HIF-1α mRNA expression levels correlate with short ischemia times and prevent the transcription of fibrogenic cytokines that initiate the irreversible process of graft fibrosis.**

Living-donor kidney transplantation results in superior graft survival compared with cadaveric donor kidney transplantation. For instance, the half-life of spousal living-unrelated transplants is 14 years versus 9 years for cadaveric donor grafts (*1*). This success cannot be easily explained by donor or acceptor selection, and therefore other factors must play a role. Several studies have reported that even the long-term outcome of transplantation is largely dependent on factors at the time of transplantation; in this respect, cold ischemia time is a dominating factor (*2*,*3*). Ischemia induces the release of various cytokines with fibrogenic properties and remodeling activities (transforming growth factor [TGF]-β, platelet-derived growth factor, and vascular endothelial growth factor [VEGF]). These growth factors play a significant role in the development of chronic allograft nephropathy (CAN), a process characterized by interstitial fibrosis and extracellular matrix deposition (*3*). Data from the UNOS registry showed that graft failure caused by chronic allograft rejection is more often seen in cadaver donor kidneys than in living-donor kidneys (40% vs. 27%, respectively) (*4*).

One of the first genes up-regulated by ischemia is the gene encoding for hypoxia inducible factor (HIF)-1, a heterodimeric transcription factor consisting of two basic helix-loop-helix proteins of the PAS family, termed HIF-1α and aryl hydrocarbon nuclear translator HIF-1β (*5*,*6*). Functional HIF-1 sites were defined in genes involved in angiogenesis (VEGF), glycolysis (lactate dehydrogenase A), erythropoiesis (erythropoietin), and vasomotor control (endothelin-1). We postulate that differences in ischemia between living-donor kidneys and cadaveric donor kidneys are correlated with HIF-1α mRNA expression levels early after transplantation and determine the development of CAN in the long term.

Time-zero biopsy specimens of living-(un)related (n=13, ≤2.5 hr of ischemia) and of cadaveric donor kidneys (n=9, 12–32 hr of ischemia) were taken before reperfusion. Messenger RNA was isolated from these time-zero biopsy specimens by the method of Chomczynski and Sacchi method. We used the real time reverse-transcriptase polymerase chain reaction technique to quantify the amount of HIF-1α and TGF-β mRNA expression. Nucleotide sequences for the oligo primers and probes for HIF-1α were as follows: sense 5' AAC.ATG.ATG GTT CAC TTT TTC AAG C 3', antisense 5' GTC AGC TGT GGT AAT CCA CTT TCA T 3', and probe 5' TAG GAA TTG GAA CAT TAT TAC AGC AGC CAG ACG 3'. Predeveloped Taqman PDAR assays were used to measure TGF-β mRNA and 18S ribosomal RNA (18S rRNA) concen-

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trations (Applied Biosystems, Norwalk, CT).18S rRNA was used in each sample to control for sample-to-sample variations. HIF-1α and TGF-β mRNA expression levels were normalized to 18S rRNA concentrations.

HIF-1α mRNA expression was present in all time zero specimens (n=22). The level of HIF-1α mRNA expression was strongly associated with the type of transplant. The HIF-1α mRNA expression levels in time-zero biopsy specimens from living-(un)related donor kidneys were significantly lower (fivefold) than in time-zero biopsy specimens from cadaveric donor kidneys (P=0.0011, Mann-Whitney test, Fig. 1A). In these living-donor kidneys the TGF-β mRNA expression levels were also low compared with cadaveric donor kidneys (P=0.05, Fig. 1B). The mRNA expression level of TGF-β was correlated with HIF-1α mRNA levels: with rising HIF-1α mRNA expression levels, the TGF-β mRNA levels were also increased (Fig. 2, P=0.0016, Spearman's test).

In kidney transplantation, the most prevalent cause of long-term graft dysfunction is CAN, an irreversible nontreatable process of fibrosis (*3*). Currently, kidneys from living donors are frequently used for transplantation, and it is widely recognized that their long-term survival is significantly better compared with cadaveric donor grafts (*1*). These findings are compatible with the hypothesis that in kidneys from living donors, ischemia-related damage is reduced because of the significantly shorter cold ischemia time or alternatively because of the absence of the ischemic consequences of brain death caused by vasoconstriction.

When cells are deprived of oxygen, a general oxygen-sensing mechanism is activated in which HIF-1 plays a pivotal role (*5*,*6*). HIF-1 is a potent transcriptional regulator of oxygen-dependent genes such as VEGF and TGF-β. Our results demonstrate a significant association between HIF-1α mRNA expression levels and the exposure to ischemia in kidney grafts. It has been shown that HIF-1 activity is regulated at various levels, whereas both mRNA and protein expression levels are induced by hypoxia and by acute ischemia in vivo (*5*). Our findings suggest that ischemia at the transplantation procedure results in up-regulation of the oxygen-sensitive transcription factor HIF-1α, which triggers the production of fibrogenic cytokines such as TGF-β (Fig. 2).

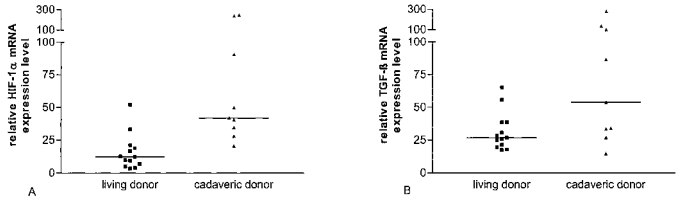

FIGURE 1. HIF-1α mRNA expression (A) and TGF-β (B) mRNA expression level in time-zero biopsy specimens from living-donor kidneys (n=13) and cadaveric donor kidneys (n=9). Significant differences were found in mRNA expression levels for HIF-1α and TGF-β between living-donor kidneys and cadaveric donor kidneys (P=0.0011 and P=0.05, respectively).

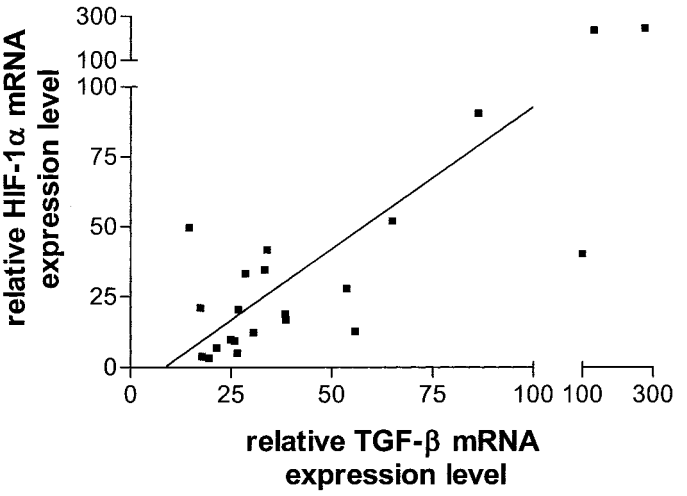

FIGURE 2. Correlation between HIF-1α and TGF-β mRNA expression levels in time-zero donor kidneys (n=22).

In vitro studies support that this can indeed be the case. High TGF-β mRNA expression levels were measured in renal cells cultured under hypoxic conditions (*7*,*8*). The low HIF-1α mRNA expression levels may explain the superior graft outcomes of living kidney donors. Because of the relatively short cold ischemia time, the activation of the hypoxia-dependent transcription factor HIF-1α is diminished and consequently the activation of fibrogenic cytokines may be delayed or prevented. Understanding the molecular mechanisms by which ischemic conditions early after transplantation induce graft fibrosis in the long term might provide a tool for therapeutic strategies and may have consequences for clinical management.

In conclusion, having demonstrated that ischemia is correlated with mRNA expression levels of HIF-1α, we postulate that this transcription factor may be pivotal in the modulation of fibrogenic cytokines in the initiation of the irreversible process of graft fibrosis and graft loss.

REFERENCES

1. Gjertson DW, Cecka JM. Living unrelated donor kidney transplantation. Kidney Int 2000; 58: 491–499.
2. Halloran PF, Homik J, Goes N, et al. The "injury response": a concept linking nonspecific injury, acute rejection, and long-term transplant outcomes [review]. Transplant Proc 1997; 29: 79.
3. Paul LC. Chronic allograft nephropathy: an update [review]. Kidney Int 1999; 56: 783.
4. Cecka JM. The UNOS scientific renal transplant registry. In: Cecka JM, Terasaki PL, eds. Clinical Transplants 1999, chap. 1. Los Angeles, UCLA Immunogenetics Center 1999, pp 1–21.
5. Ratcliffe PJ, O'Rourke JF, Maxwell PH, et al. Oxygen sensing, hypoxia inducible factor-1 and the regulation of mammalian gene expression. J Exp Biol 1998; 201: 1153–1162.
6. Semenza GL. HIF-1 and human disease: one highly involved factor [review]. Genes Dev 2000; 14: 955.
7. Norman JT, Clark, IM, Garcia PL. Hypoxia promotes fibrogenesis in human renal fibroblasts. Kidney Int 2000; 58: 2351–2366.
8. Sahai A, Mei C, Schrier RW, et al. Mechanisms of chronic hypoxia-induced renal cell growth. Kidney Int 1999; 56: 1277–1281.