Analysis of dendritic cells and ischemia-reperfusion changes in postimplantation renal allograft biopsies may serve as predictors of subsequent rejection episodes

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Ischemia-reperfusion injury increases allograft immunogenicity and enhances myeloid dendritic cell maturation and trafficking to recipient's secondary lymphoid tissue. Here, we used postreperfusion biopsies from patients who received kidney allografts from deceased donors between 2006 and 2009 to assess the impact of ischemia-reperfusion damage and myeloid dendritic cell density on subsequent allograft rejection episodes. Histologic changes of severe ischemia-reperfusion damage in postreperfusion biopsies were found to be associated with subsequent rejection episodes and suboptimal allograft survival. Using BDCA-1 as a marker of myeloid dendritic cells, postreperfusion biopsies from deceased donors had lower dendritic cell density compared to postreperfusion biopsies from living donors or normal controls. This suggests a rapid emigration of donor dendritic cells out of the allograft. In our cohort, low dendritic cell density was associated with a subsequent increase in rejection episodes. However, it appears that the donor's cause of death also influenced dendritic cell density. Therefore, we assessed the additive impact of severe ischemia-reperfusion changes and low dendritic cell density on subsequent rejection. The aforementioned combination was a powerful and independent predictor of allograft rejection. Thus, our data highlight the prognostic value of histopathologic changes associated with ischemia-reperfusion in postreperfusion biopsies and suggest a rapid posttransplant emigration of myeloid dendritic cells out of the allograft to enhance alloimmunity. These findings may provide a rationale for minimizing ischemia-reperfusion injury and therapeutic targeting of donor-derived dendritic cells to promote rejection-free allograft survival.

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While immunologically mediated injury is known to have a major impact on allograft survival,[1,2] ischemia-reperfusion injury (IRI) has been recognized as a powerful activator of the inflammatory immune cascade.[3] Several solid organ transplantation models have confirmed the critical role of IRI in initiating cellular and humoral rejection.[4–7] In kidney transplantation, IRI can cause damage to the tubular epithelial cells and microvascular endothelium, which manifests histologically as acute tubular necrosis (ATN)[8] and sometimes as microvascular thrombosis.[9] In addition to the ischemia-reperfusion (IR) changes visible morphologically, IRI can enhance the maturation, allo-stimulation, and migration capabilities of donor-derived dendritic cells (DCs), which can further increase allograft immunogenicity.[10–12] Despite the overwhelming experimental evidence of the harmful effect of IRI on the allograft in nonimmunosuppressed animals, our knowledge of the impact of IRI and donor DCs on kidney allograft rejection in humans remains limited. In addition to the differences of the immune system in humans compared with rodents, human kidney allograft recipients receive potent induction

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immunotherapy, and donor kidneys are preserved in solutions that can decrease ischemia effects. ATN and intraglomerular fibrin thrombi are considered reliable markers for the severity of IRI.[13–16] Studying the impact of severe IRI and donor DCs on subsequent rejection may provide the rationale to develop and investigate novel preimplantation therapy to ameliorate IRI, decrease early rejection, and improve long-term allograft function.

At Columbia University Medical Center (CUMC), postreperfusion biopsies are performed routinely approximately 1 hour after reperfusion. In addition, CUMC performs a large volume of kidney transplantation from deceased donors with unusually long cold ischemia time (CIT; average 31 hours in our cohort). The above factors would enable us to investigate any potential link between severe IRI and alloimmunity. For this, we assessed postreperfusion biopsies from adult patients who received allografts from deceased donors at CUMC between January 2006 and December 2009 ($n = 363$). We aimed to evaluate the association of severe IRI (as manifested histologically by diffuse ATN and/or intraglomerular fibrin thrombi) and the density of donor DCs (using BDCA-1 immunostain) on subsequent rejection episodes.

RESULTS

Patient characteristics and follow-up

Between 2006 and 2009, 382 adult recipients of deceased donor kidney allografts with available postreperfusion biopsies were identified. To ensure adequate follow-up, we excluded 19 patients who had <30 days of follow-up (either because of early graft failure or loss of follow-up) or who had primary graft nonfunction. Demographic, clinical, and pathologic characteristics of the remaining 363 patients are presented in Table 1. During 61 ± 28 months of follow-up, 148 patients (41%) had 1 or more rejection episodes (borderline changes or above). The first rejection episodes were classified as borderline changes ($n = 53$, 16 of which subsequently developed overt rejection despite anti-rejection treatment), overt acute T-cell–mediated rejection (grade 1A or above, $n = 53$), antibody-mediated rejection ($n = 31$), or mixed rejection ($n = 11$). Graft failure occurred in 105 patients (43 ± 26 months after transplantation) and was attributed to alloimmunity (33 of 105 [31%]; 13 acute and 20 chronic rejection), noncompliance (11 of 105 [11%]), and nonalloimmune causes (61 of 105 [58%]; 3 thrombosis and/or infarction, 10 recurrent disease, 18 infection [including polyomavirus nephropathy], 16 attributed to chronic allograft nephropathy, and 14 others). Notably, 39 of 61 patients (64%) whose graft failure was classified as nonalloimmune had 1 or more rejection episodes.

Severe IR changes in postreperfusion allograft biopsies were associated with rejection episodes and suboptimal outcome

To assess the impact of IR changes on subsequent rejection episodes and allograft outcome in patients who received deceased donor kidney allografts, postreperfusion biopsies were retrospectively classified as having severe IR changes ($n = 150$) versus nonsevere IR changes ($n = 213$) depending on the presence of diffuse ATN or intraglomerular fibrin thrombi (Figure 1). Compared with kidneys with nonsevere IR changes, kidneys with severe IR changes had longer CIT, more tubulointerstitial scarring, and more vascular sclerosis. Patients with severe IR changes also tended to have a more frequent history of previous kidney transplantation. With regard to prognosis, patients with severe IR changes had more frequent delayed graft function (DGF) and lower estimated glomerular filtration rate at 1, 2, and 3 years after transplantation (Table 1). Moreover, patients with severe IR changes had worse allograft survival and earlier rejection episodes (Figure 2). We explored whether a similar pattern was observed in patients who were excluded from the study due to primary graft nonfunction but were maintained for >30 days in hope of improvement ($n = 6$). Five of these patients developed rejection episodes (4 overt acute T-cell–mediated rejection and 1 mixed; 17–89 days after transplantation), of whom 4 (80%) showed severe IR changes on their postreperfusion biopsies.

We then assessed whether severe IR changes could independently predict rejection episode–free survival. Univariate analyses of demographic, clinical, and histologic data identified recipient's African American race, preformed donor-specific antibody (DSA), history of previous transplantation, percentage of glomerulosclerosis, and severe IR changes as significantly associated with subsequent rejection episodes (Table 2). We subsequently conducted a multivariate analysis including variables with $P \leq 0.1$ on univariate analyses in addition to variables that were different between patients with versus without severe IR changes. Severe IR changes on postreperfusion biopsy were an independent predictor of subsequent rejection episodes (Table 2). Because IR changes, but not CIT, appeared to be associated with subsequent rejection episodes, we further evaluated the impact of CIT on prognosis. Although CIT was significantly longer in patients with DGF (DGF: 33 ± 10 hours vs. no DGF: 30 ± 11 hours; $P = 0.005$), the association of CIT with estimated glomerular filtration rate became weakly significant only at the second year after transplantation (year 1: $r = –0.09$, $P = 0.11$; year 2: $r = –0.16$, $P = 0.003$; year 3: $r = –0.12$, $P = 0.03$). Moreover, CIT could not predict graft failure (hazard ratio: 0.99, 95% confidence interval: 0.98–1.01; $P = 0.38$). The above findings suggest that histologic evaluation for the presence of IR changes is a better prognostic predictor of subsequent allograft function, rejection, and survival than is duration of CIT.

Postreperfusion allograft biopsies of deceased donor kidneys contained low BDCA-1$^+$ cell density, probably due to early emigration out of the allograft

To asses DC density, immunostaining for BDCA-1 (labels myeloid DCs), CD123 (labels plasmacytoid DCs), and HLA-DR (labels myeloid and plasmacytoid DCs) were optimized on formalin-fixed, paraffin-embedded tissue (see Supplementary Material, including Supplementary Figure S1). DC subtypes