Necroptosis and parthanatos are involved in remote lung injury after receiving ischemic renal allografts in rats

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Early renal graft injury could result in remote pulmonary injury due to kidney–lung cross talk. Here we studied the possible role of regulated necrosis in remote lung injury in a rat allogeneic transplantation model. *In vitro*, human lung epithelial cell A549 was challenged with TNF-α and conditioned medium from human kidney proximal tubular cells (HK-2) after hypothermia-hypoxia insults. *In vivo*, the Brown–Norway rat renal grafts were extracted and stored in 4 °C Soltran preserving solution for up to 24 h and transplanted into Lewis rat recipients, and the lungs were harvested on day 1 and day 4 after grafting for further analysis. Ischemia-reperfusion injury in the renal allograft caused pulmonary injury following engraftment. PARP-1 (marker for parthanatos) and receptor interacting protein kinase 1 (Rip1) and Rip3 (markers for necroptosis) expression was significantly enhanced in the lung. TUNEL assays showed increased cell death of lung cells. This was significantly reduced after treatment with necrostatin-1 (nec-1) or/and 3-aminobenzamide (3-AB). Acute immune rejection exacerbated the remote lung injury and 3-AB or/and Nec-1 combined with cyclosporine A conferred optimal lung protection. Thus, renal graft injury triggered remote lung injury, likely through regulated necrosis. This study could provide the molecular basis for combination therapy targeting both pathways of regulated necrosis to treat such complications after renal transplantation.

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Renal transplantation is inevitably associated with ischemic injury due to cold storage in transportation and histocompatibility tests. This injury can be exacerbated upon grafting, by ischemia-reperfusion injury (IRI), which involves a cascade of events, including acute tubular necrosis, inflammation, and oxidative damage to renal parenchyma,[1] and also remains as one of the most formidable obstacles in the success of renal transplantation.[2] In addition, IRI in allografts promotes the development of acute immune rejection and negatively affects long-term graft survival.[3] Early acute renal graft injury due to IRI and its associated acute rejection contribute substantially to high morbidity in transplant patients.[4] Recently, accumulating laboratory and clinical evidence have highlighted that acute ischemic renal injury induces remote lung injury;[5] likewise, early renal graft injury after transplant surgery might be also associated with the rapid development of pulmonary insufficiency.

Remote lung injury is a recognized complication, which has been reported in the models of intestinal,[6] hepatic,[7] and limb IRI.[8] Remote lung injury after acute kidney injury remains partially understood; putative mechanisms include the release of pro-inflammatory cytokines such as IL-1β, IL-6, and particularly tumor necrosis factor-α (TNF)-α from injury renal tissue into the systemic and pulmonary circulation, causing pulmonary tissue injury and inflammation.[9,10] However, the precise molecular interactions between ischemic renal allografts and remote lung injury have not been sufficiently explored.

Regulated necrosis is a form of genetically programed necrotic cell death; it encompasses a range of pathways. Necroptosis is one form of regulated necrosis,[11] and its execution requires the assembly of the necroptosome containing the receptor-interacting protein kinase 1 (Rip1) and 3 (Rip3).[12,13]It has already been shown to be associated with TNF-α[14] and occurs in injured organs such as the kidney,[15] brain,[16] and heart.[17] In addition, Poly (ADP-ribose) polymerase 1(PARP-1)-mediated regulated necrosis, referred to as parthanatos, has been shown to be a distinct pathway in regulated necrosis,[18] although there may be shared molecular

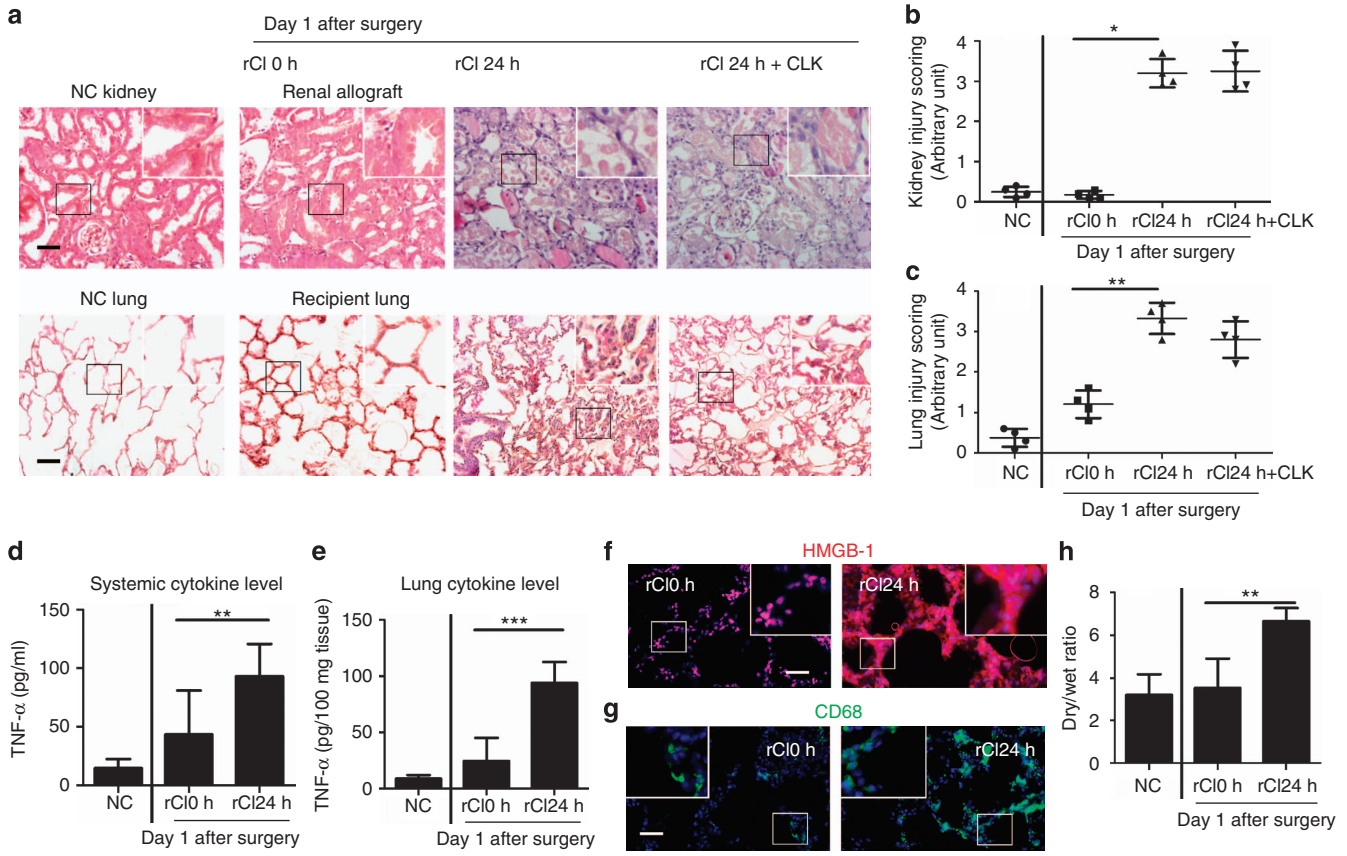

Figure 1 | Renal graft ischemia-reperfusion injury led to remote lung injury. Brown–Norway renal graft was stored in 4 °C Soltran preserving solution for 0 (live transplantation) or 24 h (renal graft cold ischemia rCl0 or 24 h) and then transplanted into Lewis recipient; the renal graft and lung were harvested on day 1 after transplantation. The contralateral kidney was removed or kept (CLK group). (**a**) Histology (hematoxylin and eosin staining) of renal grafts and lung. (**b**) Injury scores of kidney morphology. (**c**) Injury scores of lung morphology, (**d**) serum concentration of tumor necrosis factor-α (TNF-α), and (**e**) lung tissue concentration of TNF-α assessed by enzyme-linked immunosorbent assay. (**f**) Nuclear release of high-mobility group protein B1 (HMGB-1) in lung. (**g**) CD 68$^+$ cell infiltration in lung. (**h**) Dry/wet ratio of lung samples. Scale bar = 50 μm. Data are expressed as mean ± s.d. ($n = 4$), (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). CLK, (with) contralateral kidney; NC, naive control; rCI, renal graft cold ischemia.

components.[19] The present study was undertaken to investigate the remote lung injury associated with renal allograft IRI and acute immune rejection using the Brown–Norway to Lewis allograft transplantation model. We explore the role of different pathways of regulated necrosis in the lungs due to cross talk with injured renal allografts.

RESULTS

Ischemic renal allograft led to remote lung injury after engraftment

To examine whether acute lung injury was induced by ischemic renal grafts, a histological analysis was performed on both renal grafts and lung on day 1 after transplant surgery. Ischemic renal allografts displayed significant renal graft injury due to IRI (Figure 1a and b). Lung injury was more prominent in recipients receiving ischemic renal grafts, which displayed as alveolar septal thickening, interstitial edema, and vascular congestion, as well as leukocyte infiltration and margination. No pathological changes were observed in the lung tissue from rats of live transplantation (Figure 1a and c). Both serum level and lung tissue level TNF-α in rats receiving ischemic renal allografts were found to be increased 24 h after grafting, whereas that in rats receiving non-ischemic renal grafts remained comparably low (Figure 1d and e). In addition, nuclear release of high-mobility group protein B1, a classical damage-associated molecular pattern molecule,[20] was observed in the lung tissue (Figure 1f), and CD68$^+$ monocyte infiltration was significantly higher in the lung with remote injury than the lung with live transplantation (Figure 1g). Consistent with the histological findings, the wet-to-dry ratios were significantly higher in the ischemia group than in the non-ischemia group (Figure 1h), indicating development of lung tissue edema in ischemic kidney graft recipients.

PARP-1-mediated regulated necrosis (parthanatos) and inflammation was found in lung tissues of recipients receiving ischemic allografts

PARP-1 activities were notably increased, demonstrated through enhanced PARP-1 expression and production of poly-ADP ribose (PAR) in lung tissue after transplantation with ischemic renal graft (Figure 2a–e). The fluorescence