Nonapoptotic cell death in acute kidney injury and transplantation

Andreas Linkermann[1]

[1]Clinic for Nephrology and Hypertension and Georges-Köhler-Haus for Biomedical Research and Transplantation, Christian-Albrechts-University, Kiel, Germany

Acute tubular necrosis causes a loss of renal function, which clinically presents as acute kidney failure (AKI). The biochemical signaling pathways that trigger necrosis have been investigated in detail over the past 5 years. It is now clear that necrosis (regulated necrosis, RN) represents a genetically driven process that contributes to the pathophysiology of AKI. RN pathways such as necroptosis, ferroptosis, parthanatos, and mitochondrial permeability transition-induced regulated necrosis (MPT-RN) may be mechanistically distinct, and the relative contributions to overall organ damage during AKI in living organisms largely remain elusive. In a synchronized manner, some necrotic programs induce the breakdown of tubular segments and multicellular functional units, whereas others are limited to killing single cells in the tubular compartment. Importantly, the means by which a renal cell dies may have implications for the subsequent inflammatory response. In this review, the recent advances in the field of renal cell death in AKI and key enzymes that might serve as novel therapeutic targets will be discussed. As a consequence of the interference with RN, the immunogenicity of dying cells in AKI in renal transplants will be diminished, rendering inhibitors of RN indirect immunosuppressive agents.

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Correspondence: *Andreas Linkermann, Clinic for Nephrology and Hypertension and Georges-Köhler-Haus for Biomedical Research and Transplantation, Christian-Albrechts-University, Kiel, Germany. E-mail: andreas.linkermann@uksh.de*

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The kidney represents the best organ to study cell death *in vivo*. Unlike the brain or the heart, in which investigations of ischemic injury are hampered by the presence of collateral vessels, renal ischemia-reperfusion injury (IRI) affects the whole organ, resulting in a significantly lower SD with regard to histologic injury scores and more reliable markers of organ function than in the heart or brain. Therefore, in recent years, renal investigations have disproportionately contributed to our understanding of cell death, notwithstanding the tremendous clinical need to analyze stroke and myocardial infarction.

In addition, intravital microscopy can be easily used to investigate the kidney, and movies obtained from such analyses have improved our understanding of renal dynamics.[1–3] With a beating heart and the skull around the brain, this technique requires additional strategies to evaluate progression and intercellular development, such as synchronized cell death and necrosis-induced necrosis in heart and brain models of injury that are more simply monitored in kidneys and isolated renal tubules.[4] Understanding the cellular mechanisms of death in the kidney and developing small molecules to successfully interfere with regulated necrosis (RN) in such models are likely to illuminate renal injury as well as provide benefits in neurology and cardiology.

In addition, the clinical presentation of acute kidney injury (AKI) often manifests with easily accessible changes in the urine sediment that can be confirmed by kidney biopsy (especially in animal models) and that can facilitate translation of animal research to patients.

THE PHYSIOLOGICAL ROLE OF RN

Extrinsic death receptor-mediated apoptosis depends on caspase-8, 1 of the most prominent initiator caspases in mice described more than 2 decades ago.[5–7] Because of the embryonic lethality of caspase-8–deficient mice, researchers misinterpreted apoptosis as a process required for vertebrate viability. Therefore, the reversal of the lethal phenotype of caspase-8–deficient mice on a receptor-interacting protein kinase 3 (RIPK3)-deficient background was both striking and surprising,[8–11] and other functions of caspase-8 also appear to be involved in the control of RIPK3.[12] This led to the conclusion that the single most important function of caspase-8 is the prevention of necroptosis,[13–15] mediated by RIPK3,[16–18] rather than promoting extrinsic apoptosis, the loss of which appears to be far less dramatic than its regulatory effects on RIPK3, because caspase-8/RIPK3–double deficient are viable and

fertile.[8–10] C8/RIPK3-double knockout mice exhibit a phenotype similar to those previously recognized from mice that carry mutations in the death receptor Fas or Fas ligand, referred to as *lpr* and *gld* point mutations, respectively.[8,10] Such phenotypes result in accumulation of $B220^+$ cells, an enlarged spleen and lymph nodes, and an overall reduced life span, but these mutations are not as striking as those described for caspase-8–deficient mice.[19] Importantly, loss of RIPK3, apart from a slight failure to gain weight,[4] does not lead to any obvious phenotype.[20] Therefore, the physiological nonredundant role for extrinsic apoptosis appears to be limited to the immune system, whereas RIPK3 deficiency is not essential for normal murine development. Currently, it has been suggested that the phylogenic preservation of the dangerous RIPK3 molecule is intended to defend against microbes.

REGULATED NECROSIS FIGHTS MICROBES

The default process for the clearance of virally infected cells mostly involves extrinsic apoptosis through Fas (on virally infected cells)-Fas ligand (on $CD8^+$ effector T cells), cells that are triggered by presenting MHC II to the T-cell receptor,[21] and malfunction of this system has been associated with renal disease.[22,23] This apoptotic cascade involves caspase-8 activation that then transduces the deadly signal though effector caspase-3, caspase-6, and caspase-7, resulting in clearance of these cells in an "immunologically silent manner."[24,25] Some viruses, however, express proteins that are inhibitors of caspases, such as vaccinia virus, cytomegalovirus, cowpox virus, and so on.[11,26,27] In these viral infections, RIPK3 is no longer inhibited by caspase-8, and the effect is spontaneous initiation of necroptosis[11,28] to clear the virally infected cell in an immunogenic manner. In a simple experiment, RIPK3-ko mice were shown to die after infection with murine vaccinia virus, whereas all wild-type littermates survived.[16] Therefore, necroptosis is considered a "backup defense" mechanism against caspase-inhibitor–expressing viruses.[29–34] Apart from viruses, bacteria such as *Listeria monocytogenes* are recognized by hepatic Kupffer cells that respond by undergoing necroptosis and thereby trigger (i) an antibacterial immune response and (ii) the release of the "necroptotic" cytokine IL-33 to actively initiate the replacement of Kupffer cells to restore hepatic homeostasis.[35,36] In fact, it is plausible that bacteria are capable of inducing necroptosis because virtually every Toll-like receptor that binds to the intracellular adapter protein TIR-domain–containing adapter-inducing interferon beta (TRIF) is capable of inducing necroptosis by directly interacting with RIPK3 through a RIP homotypic interacting motif (RHIM) domain.[33,34,37] In summary, bacteria (through Toll-like receptors), viruses (through caspase inhibition and other mechanisms), and cytokines (through death receptors) are capable of inducing necroptosis.

SIGNALING PATHWAYS OF REGULATED NECROSIS

Necrosis is a common feature of diverse disorders, including tissue infarction, cancer, atherosclerosis, pancreatitis, trauma, and the vitality of organ transplants.[3,32,38] Unfortunately, even the possibility of interfering with necrosis has been neglected by clinicians over several centuries, because necrosis was interpreted as "good given" and therefore beyond therapeutic intervention. Currently, as it has become clear that necrosis is a regulated process (RN), the opportunity to prevent it has fascinated researchers, rendering rapid movement in the field of RN. In the transplanted organ, it is now appreciated that each additional hour of cold ischemia time increases the risk for graft failure and mortality,[39] the pathophysiological basis of which may likely be the total amount of necrotic debris in a transplant. However, further development in this field requires that we clearly define RN subroutines, as recently suggested by the current version of the recommendation of the interpretation of cell death pathways.[40–42]

Necroptosis—"the leviathan of RN"

Necroptosis signaling. Necroptosis is the best-studied RN pathway. The backbone of this cell death pathway is the receptor-interacting protein kinase 3 (RIPK3). RIPK3-dependent phosphorylation of mixed lineage kinase domain-like (MLKL), a pseudokinase that mediates the deadly signal to the plasma membrane by unknown means, is central to this form of killing. Only 1 of the 3 known phosphorylation sites, Ser 345 in the MLKL activation loop, is critical for the membrane to burst,[43] and 2 other phosphorylation sites are of minor relevance.[43] During this process, a 4-helical bundle domain is unleashed from the closed conformation of MLKL to target the plasma membrane.[44–48] RIPK3 activation, however, is not sufficient to signal necroptosis without stabilization of the so-called necrosome, a structure that consists of RIPK3 proteins that may associate in amyloid-like structures that are stabilized by heat shock protein 90 and its co-chaperone CDC37.[49,50] The phosphorylation of MLKL is antagonized by 1 of the most prominent intracellular phosphatases, Ppm1b,[51] which regulates necroptosis downstream of necrosome formation (Figure 1). Three major physiological patterns result in necrosome assembly and activation. As pointed out earlier, and probably most important *in vivo*, every TRIF-binding Toll-like receptor functions as a necroptosis receptor, and therefore bacteria are inducers of necroptosis, as exemplified in the case of *Listeria monocytogenes* infection.[35] TRIF binds directly to RIPK3 through its RHIM domain.[33,52] Only 4 proteins in the human genome contain such a RHIM domain: TRIF, RIPK1, RIPK3, and DNA-dependent activator of interferon, a DNA sensor for viral proteins that engages RIPK1 and RIPK3[34,37] and thereby enables viruses to pull the necrotic trigger in a necrosome-dependent manner (Figure 1). The default pathway, which requires caspase inhibition in the presence of death receptor ligation (e.g., tumor necrosis factor receptor 1 [TNFR1]), results in deubiquitination of RIPK1, a process that allows translocation from the plasma membrane to the cytosolic kinase target RIPK3, again through the RHIM domains.[16,17] TNFR family members are also capable of inducing apoptosis. The molecular switch that regulates cell