Cell Death Pathways Drive Necroinflammation during Acute Kidney Injury

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Abstract

Renal tubules represent an intercellular unit and function as a syncytium. When acute tubular necrosis was first visualized to occur through a process of synchronized regulated necrosis (SRN) in handpicked primary renal tubules, it became obvious that SRN actually promotes nephron loss. This realization adds to our current understanding of acute kidney injury (AKI)-chronic kidney disease (CKD) transition and argues for the prevention of AKI episodes to prevent CKD progression. Because SRN is triggered by necroptosis and executed by ferroptosis, 2 recently identified signaling pathways of regulated necrosis, a combination therapy employing necrostatins and ferrostatins may be beneficial for protection against nephron loss. Clinical trials in AKI and during the pro-

cess of kidney transplantation are now required to prevent SRN. Additionally, necrotic cell death drives autoimmunity and necroinflammation and therefore represents a therapeutic target even for the prevention of antibody-mediated rejection of allografts years after the transplantation process.

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The Caspase-Controlled Cell Death System

Apoptosis

It is beyond the scope of this review to refer to the data published on apoptosis in acute kidney injury (AKI). The interested reader is referred to a recent review in which

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Box 1. Open questions on regulated cell death in AKI*How does the type of cell death relate to the intensity of AKI?*

For a single cell, it is hard to reason that it matters how exactly it dies. However, it does matter for the surrounding tissue and the inflammatory response. In the concept of necroinflammation, different cell death pathways are immunogenic to a different extent. Whereas apoptosis does not cause an immune response, pathways of regulated necrosis may initiate a detrimental immune cell infiltrate that further deteriorates the primary damage [8]. The second wave of death may also be dependent on necrosis and thereby fuel a vicious circle of necroinflammation [13].

Is there a role for apoptosis in AKI?

Despite the work of decades, it is still unclear if apoptosis has a role in human AKI. Clearly, Bax/BAK-dko mice are protected from ischemic [14] AKI and unilateral ureteric obstruction [15]. However, the pan-caspase inhibitor zVAD-fmk did not protect mice in a model of ischemia-reperfusion injury in our hands [16]. In theory, the presence of an immune cell infiltration that contains NK cells, T cells and neutrophils might argue towards a necroinflammatory component.

Can autoimmunity be explained by failure to remove necrotic cellular debris?

Failure to remove necrotic debris by a process referred to as LC3-associated phagocytosis (LAP) continuously stimulates the immune system, results in the generation of anti-nuclear antibodies (ANAs) and antibodies against double-stranded DNA (ds-DNA antibodies) and drives autoimmunity. [17, 18]. This may not exclusively be the case for systemic lupus erythematosus (SLE), but also for other autoimmune diseases. One such example that causes AKI may be ANCA-associated vasculitis [19].

we discussed this topic in detail [1]. Along these lines, the caspase-controlled cell death system also includes pyroptosis, a pathway that results in necrotic cell death downstream of inflammasomes activation. This format does not allow us to review this pathway in detail, as it has not been investigated in AKI yet. However, recent data suggest that it might be very important in AKI-chronic kidney disease (CKD) progression [2].

Necroptosis

Necroptosis is an evolutionary conserved pathway of regulated necrosis (RN) to defend viruses that express caspase inhibitors. Caspase-8 controls the protein receptor-interacting protein kinase 3 (RIPK3), the only known serine-threonine kinase that can phosphorylate the necroptosis executing pseudokinase MLKL (mixed lineage kinase domain-like). pMLKL, by unknown means that involve the ESCRT-III complex [3], results in plasma membrane rupture. With respect to AKI, RIPK3-deficient mice [4] and MLKL-deficient mice [5] are protected from renal ischemia-reperfusion injury. Inhibition of necroptosis, therefore, appears to be a promising therapeutic target, but highly specific kinase inhibitors of RIPK1 (Nec-1s, ponatinib) did not protect from IRI in our hands (A.L., unpublished data). Obviously, there is a combination of several pathways of RN and its relative contribution to the overall organ damage of AKI is unclear today.

It has recently been confirmed that the active production of cytokines occurs during necroptosis progression

[6], but precise mechanisms are incompletely understood. However, the prevention of necroptosis signaling by necrostatins should prevent the release of immune modifying cytokines, but it remains unclear if necrostatins affect CD8⁺ T-cell cross priming [7]. If RN-pathways specifically shape the immune response, a novel hypothesis emerges and we refer to it as necroinflammation [8]. Box 1 summarizes the most important open questions with respect to necroinflammation during AKI.

The Lipid Peroxidation/Autooxidation-Controlled Necrosis System*Ferroptosis*

Ferroptosis is an iron-dependent cell death pathway that involves specific lipid peroxidation patterns that are involved in plasma membrane rupture [9]. However, the precise mechanisms of how lipid peroxidation actually causes the membrane to disintegrate are elusive. Importantly, the glutathione (GSH) peroxidase 4 (GPX4) routinely prevents ferroptosis and lipid peroxidation, specifically of a recently identified phosphatidylethanolamine residue (see below).

Ferroptosis has first been described in renal tubules that no longer underwent necrotic transformation upon addition of a second generation ferrostatins [10]. This led to the investigation of third-generation ferrostatins that were identified in screening approaches on small molecules. These small molecules are stable in vivo and there-

fore facilitated the assessment of ferroptosis-interference in vivo in models of AKI. In our hands, the ferrostatins 16–86 was the most potent single compound to preserve the renal structure and function in a model of severe ischemia-reperfusion injury [11]. Strikingly, the mode of necrotic cell death strongly mimics the cellular changes observed in urine sediments upon acute tubular necrosis. In handpicked isolated perfused renal tubules, the addition of ferroptosis-inducing agents (erastin) resulted in an intercellular chain reaction that we referred to as synchronized RN (SRN) [11]. It is now clear that ferroptosis drives this reaction, and in vitro models are being developed. In parallel, the importance of ferroptosis in AKI was confirmed by the genetic depletion of GPX4 in renal tubules that resulted in lethal tubular necrosis that was sensitive to the application of a ferrostatins [12]. GPX4 is a selenocysteine-containing protein (a selenoprotein) and the prevention of ferroptosis may explain the need for selenium uptake of humans. Clearly, the renal tubular system is among the most ferroptosis-sensitive parenchymal structures in the human body. However, as tempting as ferroptosis may exhibit a model for nephron loss, from an evolutionary perspective, it is not immediately clear why humans should conserve an RN-pathway of synchronized necrosis (SRN) as an evolutionary benefit. In this respect, ferroptosis may better be interpreted as a fail-safe rather than an RN pathway. Importantly, life on earth requires a system to inactivate highly reactive oxygen and reactive oxygen species, especially in cells with high ATP turnover. In this sense, failure to appropriately cope with the ROS load may result in ferroptosis.

Hardly anything is known about the immunogenicity of ferroptosis. As short lived lipid peroxides are highly toxic (possibly explaining the phenomenon of SRN), they might even kill infiltrating lymphocytes or other immune cells. In addition, it is not clear how a wave of SRN might

be terminated mechanistically. However, ferroptosis was only recently described and is certainly under intense investigation.

Conclusions and Outlook

It remains a major task to unravel the relative contribution of RN pathway to the overall organ damage during AKI. Several complicating factors need to be overcome. First, the in vivo models of AKI used today hardly reflect the situation of AKI on an ICU or in multimorbid patients. Second, our biomarkers of AKI and necroinflammation are insufficient. A biomarker panel is needed – not only a damage marker or only a functional marker. Third, inhibitors of necroptosis are in clinical trials for autoimmune conditions, but clear AKI trials remain to be initiated. Ferrostatins, to the best of our knowledge, have not passed phase 1 clinical trials so far. Finally, the clinically used AKIN and/or KDIGO classifications were not correlated with long-term outcome of AKI patients and hardly represent reliable gold standards for clinical comparison.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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