The fire within: pyroptosis in the kidney

Stefan Krautwald and Andreas Linkermann

Division of Nephrology and Hypertension, Christian-Albrechts-University, Kiel, Germany

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THE HISTOPATHOLOGICAL CHANGES that typically occur upon ischemia-reperfusion injury (IRI) have long been referred to as acute tubular necrosis. With the detection of apoptosis as a prototype mechanism for programmed cell death, researchers have focused on this elegant caspase-dependent and nonimmunogenic cell death mechanism also in IRI—with very limited clinical success. Despite hundreds of reports that have claimed to have detected a pathophysiological role for apoptosis in renal IRI, often detected by nonspecific TUNEL assays, and despite the availability of potent apoptosis inhibitors, no cell death targeting therapy has ever made it into the clinic. Recently, with the detection of mitochondrial permeability transition (3) and necrosis (8) as crucial pathomechanisms in kidney IRI, pathways of regulated necrosis (RN) have attracted a lot of attention because potent inhibitors of these pathways have proven beneficial in vivo in a preclinical study (7) and are currently being prepared for clinical trials. Importantly, RN pathways are not restricted to mitochondrial permeability transition and necroptosis but involve several others, such as poly(ADP-ribose) polymerase-mediated RN, ferroptosis, pyroptosis, pyronecrosis, and others. However, the complex web of potential interconnections of RN pathways remains to be untangled more thoroughly. But what do we conserve so many RN pathways for? The most convincing answer is in the different immunogenicity of these necrotic cell death modalities (Fig. 1). Whereas apoptosis is by far the least immunogenic form of cell death, all RN pathways release unprocessed intracellular contents into the extracellular space (4), and only this allows the clearance of microbes that express caspase inhibitors to block apoptosis (1, 5). Among RN pathways, pyroptosis is special. Whereas inflammasomes trigger the activation of both caspase-1 and caspase-11 in this RN pathway, and caspase-11 takes over the task to ultimately let the cell “explode,” within the unique process of pyroptotic cell death, caspase-1 additionally cleaves pro-IL-1β and pro-IL-18 to attract even more components of the immune system than those that are activated by the sole release of the intracellular contents in the cell death process itself. Therefore, it is hard to imagine a stronger trigger of inflammation than pyroptosis).

As published in a recent issue of the American Journal of Physiology-Renal Physiology, Yang et al. (10) demonstrated the existence of pyroptosis in a rat model of IRI and provided corroborating in vitro experiments using hypoxia/reoxygenation. Mechanistically, the authors found yet another cell death-independent cellular response mechanism to stress, referred to as endoplasmic reticulum (ER) stress, to regulate the initiation of pyroptosis. This was demonstrated by the upregulation of two ER stress-associated proteins, referred to as C/EBP homologous protein (CHOP) and glucose-related protein 78 in both rat IRI and hypoxia/reoxygenation by Western blot analysis and, in the case of CHOP, by immunohistochemistry as well. In parallel, expression levels of caspase-1 and caspase-11 were shown to be increased in Western blots from whole kidney lysates. These findings suggested the involvement of both ER stress and pyroptosis in IRI. The authors further demonstrated a reduction of lactate dehydrogenase release in the hypoxia/reoxygenation setting, almost to baseline levels, in cells pretreated with small interfering (si)RNA against CHOP. In parallel, plasma membrane pore formation, a hallmark of pyroptosis, was reduced by >50% upon treatment with CHOP siRNA, as evaluated by ethidium bromide staining. Again, at the very same time, caspase-1 and caspase-11 expression levels markedly declined, and caspase-11 and caspase-1 mRNA expression levels were reduced upon knockdown of CHOP, whereas IL-1β mRNA remained unchanged. Finally, ER stress can be induced by tunicamycin, an agent that triggers a characteristic ER stress feature, the unfolded protein response. Pretreatment with tunicamycin mitigated the renal organ damage and elevation of serum urea and serum creatinine levels after rat IRI. Taken together, these results suggest that ER stress-regulated pyroptosis significantly contributes to the pathogenesis of rat IRI. However, these experiments, besides providing a set of answers, raise several questions. Why, if caspases are involved centrally in this response, does the pan-caspase inhibitor zVAD not provide protection from IRI, as has been previously shown (8)? What is the significance of ER stress and pyroptosis in other models of acute kidney injury, e.g., cisplatin-induced acute kidney injury or contrast-induced acute kidney injury? What are the mechanisms that
initiate the ER stress signal in the IRI model, and how is the cross-talk between ER stress and caspase activation mediated intracellularly? Are inflammasomes involved at all, or does ER stress provide this cross-activation by directly targeting caspases? Most importantly, what are the different components of the immune repertoire that are recruited by pyroptosis compared with other RN pathways? Obviously, more and stronger evidence is required to understand the relative contribution of pyroptosis to the overall organ damage in IRI, but future work will certainly address these questions and hopefully clarify these issues for the sake of our patients.

In the current opinion, pyroptosis is thought to be triggered by inflammasomes, high-molecular-weight protein complexes that act as microbial and cellular stress sensors. Pyroptosis was initially described as a macrophage death upon infection with Salmonella typhimurium (2) and has been ascribed typical morphological features of necrosis, such as an osmotic pressure-driven increase in overall cellular volume (swelling) and the rapid release of cellular contents. However, many of the data available from caspase-1-deficient mice need to be reevaluated as these animals carry a defined passenger mutation that renders them functionally caspase-1/caspase-11 double deficient (6). However, necrotic cell death in IRI, especially highly immunogenic pyroptosis, may be an attractive therapeutic target for transplantation. Currently, IRI-induced necrosis is not at all treated. Instead, clinical practice entirely relies on immunosuppression after the transplantation of kidneys, which are full of dead cell corpses, many of which may have undergone RN long before reperfusion. Most certainly, these dead cells serve as severe immunological targets; however, if it is possible to pharmacologically prevent this necrotic proinflammatory cell death, a situation with significant similarity to living organ donation, inhibition of regulated necrosis in the first place might be very efficient in the prevention of organ rejection and might preserve organ function (9).

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