Necroinflammation in Kidney Disease

Shrikant R. Mulay,* Andreas Linkermann,† and Hans-Joachim Anders*

*Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany; and †Clinic for Nephrology and Hypertension, Christian-Albrechts-University Kiel, Kiel, Germany

ABSTRACT

The bidirectional causality between kidney injury and inflammation remains an area of unexpected discoveries. The last decade unraveled the molecular mechanisms of sterile inflammation, which established danger signaling via pattern recognition receptors as a new concept of kidney injury–related inflammation. In contrast, renal cell necrosis remained considered a passive process executed either by the complement-related membrane attack complex, exotoxins, or cytotoxic T cells. Accumulating data now suggest that renal cell necrosis is a genetically determined and regulated process involving specific outside-in signaling pathways. These findings support a unifying theory in which kidney injury and inflammation are reciprocally enhanced in an autoamplification loop, referred to here as necroinflammation. This integrated concept is of potential clinical importance because it offers numerous innovative molecular targets for limiting kidney injury by blocking cell death, inflammation, or both. Here, the contribution of necroinflammation to AKI is discussed in thrombotic microangiopathies, necrotizing and crescentic GN, acute tubular necrosis, and infective pyelonephritis or sepsis. Potential new avenues are further discussed for abrogating necroinflammation-related kidney injury, and questions and strategies are listed for further exploration in this evolving field.


Tissue injury and inflammation are tightly linked and can cause each other.1 Infectious, toxic, or traumatic injuries usually result in tissue inflammation, and in turn, autoimmune inflammation causes tissue injury. However, what are the molecular mechanisms underlying these bidirectional causalities? Immunity-related cell death involves the complement system or cytotoxic T cells as effector mechanisms of infection and autoimmunity.2,3 Vice versa, cell death–related immunity is best explained by the recently discovered paradigm of dying cells releasing danger-associated molecular patterns (DAMPs) that trigger innate immunity via pattern recognition receptors (PRR) (Supplemental Material).4 However, somehow these two concepts coexist in parallel and still lack a unifying theory on injury and inflammation.5–9 The very recent discovery of the numerous forms of regulated necrosis gives rise to such a theory, herein referred to as necroinflammation.1,6,10 Necroinflammation integrates several concepts on the causal links between tissue injury and necrosis. In this review, we discuss the definition of necroinflammation and its functions, molecular pathways involved, contribution to kidney disease, and strategies to therapeutically disrupt necroinflammation to limit kidney injury and failure.

WHAT IS NECROINFLAMMATION?

Necroinflammation describes an autoamplification loop driven by necrosis (defined by cell death involving rupture of the plasma membrane) and inflammation (defined by cytokine release, increased vascular permeability, and recruitment of immune effector cells).10 The autoamplification loop of necroinflammation is accelerated by immunity-related cellular necrosis and necrosis-related immune activation. Necroinflammation can be initiated by a few necrotic cells that activate the innate immune system, which subsequently leads to necrosis of more cells triggering more inflammation in a process that eventually may lead to organ failure.10 The same process can be initiated by inflammation but often the initial insult is not as obvious as in a gout attack. Monosodium urate crystals trigger IL-1β secretion and induce death in neutrophils.11,12 Neutrophil death implies the release of proteases, DNA, and histones that trigger inflammation of the joint structures, which recruits more neutrophils that die and so on.11,12 Clinically, this process presents as a sudden onset of arthritis and sometimes even as fever and acute illness, when inflammation reaches systemic dimensions.11 Similarly, in stroke, myocardial infarction, or acute tubular necrosis, the number of cells dying from the initial insult may be few, whereas the subsequent inflammatory response contributes to further cell death.

Published online ahead of print. Publication date available at www.jasn.org

Correspondence: Dr. Hans-Joachim Anders, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ziemssenstr. 1, D-80336 München, Germany. Email: hjanders@med.uni-muenchen.de

Copyright © 2016 by the American Society of Nephrology
However, why did evolution favor such a devastating mechanism? Janeway and Medzhitov proposed the concept that pathogens activate innate immunity, which was subsequently confirmed on the discovery of the various types of PRRs and their pathogen-associated molecular patterns (PAMPs). From this example it is obvious that the danger control program of inflammation was selected during evolution to at first combat pathogens. Pathogen entry implies a disrupted barrier to the outside (e.g., wounded skin or a corneal, oral, or intestinal ulceration). In this setting, inflammation not only kills invaded pathogens but also provides a functional barrier to prevent further pathogen entry until re-epithelialization regenerates a structural barrier to the outside. Inflammation kills host cells at the site of infection to attack intracellular pathogens, which despite some collateral tissue injury, as a net impact, usually helps host survival. Matzinger insisted that also sterile dangers alert the innate immune system, which was confirmed by the discovery of dying cell-released DAMPs during sterile injuries (Table 1). PAMPs and DAMPs are integrated at the level of the same PRRs that translate danger recognition into innate immune activation. This explains why, for example, gouty arthritis is clinically indistinguishable from bacterial arthritis. Together, this suggests that necroinflammation is an autoamplification loop of necrosis and inflammation that evolved as a life-saving mechanism of host defense but causes unnecessary tissue damage in sterile diseases.

NECROINFLAMMATION INVOLVES MULTIPLE MOLECULAR PATHWAYS

**Categories of Regulated Necrosis**

Both apoptosis and necrosis are executed in a regulated manner; however, necrosis especially alerts innate immunity because the ruptured plasma membrane involves massive DAMP release into the extracellular space. Many pathways of regulated necrosis have been implicated, but the following six appear as the most appropriate to be distinguished for the purpose of this review. It is of note that in failing organs, a single one of these pathways may predominate, but often they overlap and trigger each other. For more detailed information on the different categories of regulated cell death, we refer the reader to excellent recent reviews.

**Necroptosis** is defined by the receptor-interacting protein kinase 3 (RIPK3)–mediated phosphorylation of mixed-lineage kinase domain-like (MLKL), which by unknown means leads to plasma membrane rupture. Numerous signaling events can trigger RIPK3 phosphorylation, especially a change of the RIPK1 polyubiquitination pattern that may be initiated downstream of several cell surface receptors. Necroptosis has been well described to kill parenchymal cells of all tissues, including renal tubular epithelial cells.

<table>
<thead>
<tr>
<th>DAMPs Released by</th>
<th>Receptors</th>
<th>References</th>
<th>Alarmins</th>
<th>Receptors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>HSPs</td>
<td>TLR2/4</td>
<td>IL-1α</td>
<td>ST2</td>
<td>112,113</td>
</tr>
<tr>
<td></td>
<td>Uric acid</td>
<td>NLRP3, CLEC12a</td>
<td>IL-33</td>
<td>TLR4</td>
<td>112,116</td>
</tr>
<tr>
<td></td>
<td>S100A, S100B</td>
<td>RAGE</td>
<td>TNF-α</td>
<td>NLRP3</td>
<td>119,120</td>
</tr>
<tr>
<td></td>
<td>F-actin</td>
<td>CLEC9A</td>
<td>Nucleophosmin</td>
<td>TLR4</td>
<td>122</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>mtDNA</td>
<td>TLR9</td>
<td>SAP-130</td>
<td>MINCLE</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>NLRP3</td>
<td>Granulysin, Defensin cathelicidin, EDN</td>
<td>Additional receptors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-formyl peptide</td>
<td>FPR-1</td>
<td>Neuropeptide</td>
<td>Additional receptors</td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>DNA, RNA, U1snRNP</td>
<td>TLR7/8/9</td>
<td>IL-1R</td>
<td>112,113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HMGB1</td>
<td>TLR2/4</td>
<td>TNFR1/2</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histones</td>
<td>TLR2/4</td>
<td>NLRP3</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TFAM</td>
<td>RAGE</td>
<td>PAR2</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uromodulin</td>
<td>TLR4, NLRP3</td>
<td>Trypsin</td>
<td>130</td>
<td></td>
</tr>
</tbody>
</table>

HSP, heat shock protein; mtDNA, mitochondrial DNA; U1snRNP, U1 small nuclear ribonucleoprotein; HMGB1, high-mobility group box protein 1; TFAM, mitochondrial transcription factor A; CLEC12a, C-type lectin 12a; FPR-1, n-formyl peptide receptor-1; RAGE, receptor for advanced glycation end products; AIM2, absent in melanoma 2; EDN, eosinophil-derived neurotoxin; MINCLE, macrophage inducible Ca++-dependent C-type lectin; PAR2, protease-activated receptor.

---

(i.e., unnecessary collateral tissue damage).
Ferroptosis is characterized by a defined lipid peroxidation signature in oxylipidomics analysis caused by a failure of glutathione-peroxidase 4 function.\textsuperscript{26,27} Ferroptosis involves ER stress and dysfunction caused by a lack of glutathione.\textsuperscript{28,29} which may be induced by inhibition of the cellular Cys/Glu-antipporter System Xc-, which may be controlled by p53 via SLC7A11 or heat shock protein beta 1.\textsuperscript{30,31} Ferroptosis can occur in any glutathione-depleted cell and has also been described in renal tubular epithelial cells.\textsuperscript{27,32}

Mitochondrial-permeability transition-mediated regulated necrosis (MPT-RN) is independent of ferroptosis or necroptosis because it depends on cyclophilin D and may partially overlap with a poly (ADP-ribose) polymerase 1–mediated pathway of regulated necrosis, previously referred to as parthanatos. Both MPT-RN\textsuperscript{32-35} and parthanatos\textsuperscript{36-39} have been particularly worked out in renal cells and have been reviewed elsewhere.\textsuperscript{40,41}

Pyroptosis is a consequence of inflammasome-driven caspase-11 activation.\textsuperscript{42,43} Concomitant caspase-1 activity induces mature IL-1β and IL-18 secretion, rendering pyroptosis particularly inflammatory.\textsuperscript{42} Pyroptosis has been clearly documented in infected macrophages and dendritic cells, and if pyroptosis can occur in renal cells is under debate.\textsuperscript{44,45}

NETosis is a controlled and often suicidal act of activated neutrophils, which results in the formation of neutrophil extracellular traps (NETs), consisting of expelled chromatin loaded with lysosomal and cytotoxic proteases.\textsuperscript{46} The involved signaling pathways have not yet been fully understood but include NADPH-dependent ROS production and RIPK1 signaling.\textsuperscript{47}

Another avenue of cell death is mitotic catastrophe. When cells are forced to overcome the G2/M arrest of the cell cycle despite significant DNA damage, aberrant division of chromosomes (\textit{i.e.}, aneuploidy) renders the cell to death (\textit{i.e.}, often necrosis).\textsuperscript{48-53} This is obvious in podocytes that impair their capacity to maintain foot processes and to adhere to the filtration barrier once forced to retract their cytoskeleton from the foot processes to form the mitotic spindle.\textsuperscript{49-52} Another example is the necessity to delete cells with significant cell damage in the early injury phase of AKI.\textsuperscript{53}

### How Necrosis Induces Inflammation

Necrotic cells release DAMPs and alarmins from several intracellular compartments (Figure 1, Table 1). Alarmins are a heterogeneous group of preformed proinflammatory molecules that are released by cell death from stores inside the cell.\textsuperscript{54,55} By contrast, DAMPs are molecules with other proinflammatory functions under normal conditions that turn into danger signals only once being released by cell death and by alerting the innate immune system via a group of PRRs on the surface or inside other cells.

#### Toll-like Receptors

Toll-like receptors (TLRs) are single transmembrane receptors containing a cytosolic Toll/IL-1 receptor interaction domain. On DAMP binding, TLR homo- or heterodimerization supports the recruitment of specific cytosolic adapter molecules, such as myeloid differentiation primary response gene 88, TRIF, TRAM, or TIRAP, to the Toll/IL-1 receptor domain, which activates a set of kinases and transcription factors, including NF-κB, activating protein-1, or IFN-related factors.\textsuperscript{56}

#### NOD-like Receptors

NOD-like receptors (NLRs) are intracellular PRRs detecting danger signals in the cytosol. Among all NLRs, NOD-like receptor protein 3 (NLRP3) is specifically involved in DAMP recognition. For example, uric acid and ATP activate NLRP3 to bind to its adapter molecule, adapter protein apoptosis associated speck-like protein containing a CARD, which both form a caspase-1–activating complex, named the inflammasome.\textsuperscript{57} Caspase-1 activation cleaves pro–IL-1β and pro–IL-18 and induces the secretion of the mature cytokines. Both cytokines further induce innate immunity via their respective cytokine receptors (\textit{i.e.}, IL-1R type 1, IL-18Rs).\textsuperscript{56}

### How Inflammation Induces Necrosis

DAMPs released by dying cells activate the PRRs on infiltrating immune cells, such as dendritic cells, macrophages, neutrophils, and lymphocytes, and intrinsic renal parenchymal cells, and induce the expression and local release of numerous proinflammatory mediators (Figure 1).\textsuperscript{60} In particular, TNF-α and IFN-γ can induce necroptosis via two distinct pathways. TNFR1 activation by TNF-α or TRIF activation via TLRs affects the polyubiquitination of RIP1, which activates the kinase domain and induces the phosphorylation of RIPK3 and subsequently of MLKL\textsuperscript{61-63} whereas IFN-γ signals use a STAT3-protein kinase R-dependent pathway to induce necroptosis.\textsuperscript{22} In this process RIPK1 serves as a negative regulator to TNF-α–mediated cell death via caspase-8.\textsuperscript{61} TNF-α and IL-8 can also induce NETosis in neutrophils.\textsuperscript{46,64} NET formation involves the release of histones, which elicit cytotoxic impact on endothelial cells, mesangial cells, and podocytes, a process that is poorly defined at the molecular level.\textsuperscript{65} Extracellular histones do not seem to kill cells via a specific surface
receptor but rather in a charge-dependent manner. Furthermore, PAMP- or DAMP-induced NLRP3 activation not only triggers cytokine release but also pyroptosis. An inactive pro-caspase-1 is synthesized on TLR activation, and activated NLR in inflammasome complexes cleave pro-caspase-1 into its active form. Hence, it is considered as an infection-induced cell death. Together, several proinflammatory signals induce regulated necrosis.

**NECROINFLAMMATION IN KIDNEY DISEASE**

**Sepsis Urosepsis**

Local immune responses disrupting epithelial and mesenchymal barriers can facilitate pathogen spreading into the circulation (i.e., sepsis). For example, when ascending urinary tract infection reaches the kidney, infective pyelonephritis triggers a potent inflammatory response for host defense. Pathogen entry into the renal parenchyma involves LPS-/TLR4-mediated pathogen recognition and chemokine (C-X-C motif) ligand 12-driven neutrophil recruitment, which can cause abscess formation and massive renal cell necrosis. Necrosis disrupts internal barriers and promotes pathogen spreading and urosepsis. Circulating bacterial endotoxin is a potent stimulus for cytokine release from monocytes and other immune and nonimmune cells, which has been referred to as a cytokine storm. In this process, cytokine-induced NETosis and histone release can occur everywhere in the microvasculature, which is indicated by increased levels of histones in the circulation of septic patients. Circulating histones cause cytotoxic impact to endothelial cells and activates platelets, which imply disseminated intravascular coagulation, tissue hypoperfusion, and organ dysfunction (e.g., of the lung, intestine, heart, kidneys). These mechanisms of remote disease exacerbation apply to any source of sepsis or trauma. Massive local DAMP or histone release can cause remote microvascular injuries and clotting (e.g., in the lung), which serves as a pathophysiologic explanation to acute respiratory distress syndrome in such settings.

**Acute Tubular Necrosis**

*Ex vivo* microscopy (high-resolution microscopy of freshly isolated primary kidney tubules) revealed how single-cell necrosis can spread on neighboring cells so that the entire tubule segment undergoes a synchronized cell death. Whether synchronized necrosis occurs in vivo has yet to be demonstrated. Regulated tubule necrosis is not limited to...
post-ischemic AKI\textsuperscript{35,79} or delayed graft function,\textsuperscript{77,80} but cisplatin nephrotoxicity involves necroptosis of tubular cells, triggering necroinflammation\textsuperscript{35,81} (Table 2). The same process causes tubular necrosis in rhabdomyolysis.\textsuperscript{82} In addition, contrast media–related renal dysfunction is entirely reversible by necrostatin-1.\textsuperscript{83} Moderate-dose adriamycin-induced tubular necrosis and survival is attenuated in \textit{Ripk3}–deficient mice,\textsuperscript{84} but whether tubular cells themselves directly die via necroptosis, or succumb to some other cell death secondary to initial RIPK3-mediated hypoperfusion, is still a matter of debate. First, tubules cannot be sensitized to necroptosis by the loss of Fas-associated protein with death domain or caspase-8\textsuperscript{35}; second, necrostatins do not protect isolated renal tubules from ischemic injury. In contrast, inhibition of ferroptosis is very effective in protection from ischemia-reperfusion injury \textit{in vivo} and hydroxychloroquine–iron–induced tubular damage \textit{ex vivo}.\textsuperscript{32} Therefore, the primary mode of tubular cell death in these models is still uncertain (Figure 2). Even MPT-RN appears to contribute to tubular injury, either directly or indirectly. Combined deficiency of cyclophilin D and RIPK3 led to highly significant survival benefits, even in severe models of post-ischemic tubular necrosis, suggesting that MPT-RN and necroptosis additively contribute to tubular necrosis.\textsuperscript{33,34}

In turn, inflammation is an important accelerator of tubular injury\textsuperscript{15,85} and involves potential triggers of necroptosis such as TNF-\textalpha.\textsuperscript{86,87} Oxalate crystal formation inside tubules induces RIPK3- and MLKL-dependent tubular cell death (S.R. Mulay and H.-J. Anders, unpublished data), and the associated ATP release from dying tubular cells triggers NLRP3

---

**Table 2. Regulated necrosis pathways tested in different models of acute tubular necrosis**

<table>
<thead>
<tr>
<th>ATN</th>
<th>RN Pathway</th>
<th>In Vivo/Ex Vivo</th>
<th>Protection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic renal injury</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>34,78</td>
</tr>
<tr>
<td></td>
<td>Ferroptosis</td>
<td>Ex vivo</td>
<td>No</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>MPT</td>
<td>In vivo</td>
<td>Yes</td>
<td>33,34</td>
</tr>
<tr>
<td>Delayed graft function</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>77</td>
</tr>
<tr>
<td>Cisplatin nephrotoxicity</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>36,81</td>
</tr>
<tr>
<td>Rhabdomyolysis</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>82</td>
</tr>
<tr>
<td>Contrast media related</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>83</td>
</tr>
<tr>
<td>Adriamycin nephrotoxicity</td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>84</td>
</tr>
<tr>
<td>Hydroxychloroquine/iron induced</td>
<td>Ferroptosis</td>
<td>Ex vivo</td>
<td>Yes</td>
<td>32</td>
</tr>
<tr>
<td>Calcium oxalate crystals related</td>
<td>Ferroptosis</td>
<td>In vivo</td>
<td>Yes</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Necroptosis</td>
<td>In vivo</td>
<td>Yes Unpublished data</td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 2.** Necroinflammation in glomerular and tubulointerstitial inflammation. (A) Neutrophil extracellular trap formation in glomerular capillaries (e.g., in ANCA vasculitis) causes histone-related endothelial cell death and subsequent capillary loop necrosis. DAMP release activates other glomerular cells to produce cytokines and chemokines, which recruit more cytokine-producing leukocytes, including neutrophils undergoing NETosis. TNF-\textalpha and histones drive further necrosis of glomerular cells. The associated rupture of the glomerular basement membrane implies not only podocyte injury but also plasma leakage into Bowman’s space. Plasma components such as fibrinogen activate parietal epithelial cell hyperplasia and crescent formation. (B) In tubule injury, regulated necrosis of any kind may be the initial event. Histones released by tubular cells and netting neutrophils elicit direct cytotoxicity. The associated release of DAMPs and alarmins induces inflammation, which implies the recruitment of cytokine-producing leukocytes into the peritubular interstitium. Also, TNF-\textalpha and possibly other cytokines drive necroptosis as a secondary cell death category contributing to tubular necrosis and renal dysfunction. This sets up the auto-amplification loop of necroinflammation.
inflammasome activation, IL-1β–dependent renal inflammation, and renal failure in mice.88 Blocking either necroptosis with necrostatin-1 or IL-1β with anakinra can abrogate kidney injury and dysfunction88 (S.R. Mulay and H.-J. Anders unpublished data). ATN-associated release of proinflammatory cytokines, DAMPs, and other components may also cause remote tissue injury.80,89 These authors detected parthanatos, necroptosis, and MPT-RN in the transplanted organ after reperfusion and in the lungs of the recipients.

**Rapidly Progressive GN**

Rapidly progressive GN usually presents as necrotizing and crescentic GN, which are classic presentations of ANCA-associated vasculitis, lupus nephritis, anti-glomerular basement membrane disease, or Henoch–Schonlein purpura.90 Mechanistically, necrotizing GN develops from glomerular endothelial cell necrosis (Figure 2).91 For example, in experimental antilongular basement membrane disease, neutrophils undergo NETosis inside the glomerular capillaries. It is the release of histones that induces glomerular endothelial cell necrosis.92 This became obvious because either the specific inhibition of NETosis or the neutralization of extracellular histones abrogated crescentic GN.92 Similarly, NETs are also found in

### Table 3. Some molecular targets to therapeutically interrupt necroinflammation

<table>
<thead>
<tr>
<th>Molecular Target</th>
<th>Primary Effect</th>
<th>Compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated necrosis</td>
<td>Stabilizing RIPK1</td>
<td>Necrostatins, ponatinib</td>
<td>143</td>
</tr>
<tr>
<td>RIPK3</td>
<td>Blocking phosphorylation of MLKL</td>
<td>GSK872, GSK840, Dabrafenib</td>
<td>145</td>
</tr>
<tr>
<td>pMLKL</td>
<td>Unknown</td>
<td>Necrosulfonamide</td>
<td>146</td>
</tr>
<tr>
<td>Cyclophilin D</td>
<td>Preventing mitochondrial membrane potential–related necrosis</td>
<td>Sanglifehrin A, cyclosporin A</td>
<td>35</td>
</tr>
<tr>
<td>PARP1</td>
<td>Inhibiting PARP-1–driven parthanatos</td>
<td>Olaparib</td>
<td>147</td>
</tr>
<tr>
<td>GPX4</td>
<td>GPX4 blocks ferroptosis</td>
<td>Ferrostatins</td>
<td>32</td>
</tr>
<tr>
<td>Complement C5a</td>
<td>Blocking complement-mediated inflammation and injury</td>
<td>Eculizumab plus others</td>
<td>148–151</td>
</tr>
<tr>
<td>Properdin</td>
<td>Blocking alternative complement pathway–related inflammation</td>
<td>MAb 1340</td>
<td>152</td>
</tr>
<tr>
<td>Chemokines/CCRs</td>
<td>Blocking leukocyte recruitment</td>
<td>NOX-E36, R504393</td>
<td>153,154</td>
</tr>
<tr>
<td>CCL2/CCR2</td>
<td>Blocking leukocyte recruitment</td>
<td>VUF10085</td>
<td>155</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Blocking leukocyte recruitment</td>
<td>TAK-779, NR58–3.14.3</td>
<td>155–158</td>
</tr>
<tr>
<td>Numerous CK</td>
<td>Blocking leukocyte recruitment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokines TNF-α</td>
<td>Blocking TNF-mediated cell death and inflammation</td>
<td>Infliximab plus others</td>
<td>110,159–161</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Blocking IL-1R-mediated immunity</td>
<td>Anakinra plus others</td>
<td>88,162–166</td>
</tr>
<tr>
<td>IL-6</td>
<td>Blocking IL-6–mediated immunity</td>
<td>Tocilizumab plus others</td>
<td>167–170</td>
</tr>
<tr>
<td>IL-17</td>
<td>Blocking IL-17–mediated immunity</td>
<td>Secukinumab plus others</td>
<td>171–173</td>
</tr>
<tr>
<td>Anti-TWEAK</td>
<td>Blocking TWEAK-mediated immunity</td>
<td>RG7212, BIIB023</td>
<td>174</td>
</tr>
<tr>
<td>Jak-STAT</td>
<td>Blocking cytokine-driven immunity</td>
<td>AG490, S3I-201, ruxolitinib, tofacitinib</td>
<td>179,180</td>
</tr>
<tr>
<td>DAMPs Histones</td>
<td>Blocking histone cytotoxicity and TLR2/TLR4/NLRP3 activation</td>
<td>Antihistone IgG, Activated protein C Heparin</td>
<td>74,92,103</td>
</tr>
<tr>
<td>PRRs TLR2, TLR4, TLR7, TLR9</td>
<td>Blocking TLR-driven immunity</td>
<td>OPN-305, IIMO-8400 Glyburide</td>
<td>181–183</td>
</tr>
<tr>
<td>NLRP3</td>
<td>Blocking inflammasome-driven IL-1β/IL-18 release and caspase-1–driven pyroptosis</td>
<td>VX-765, MCC950 β-hydroxybutyrate</td>
<td>184,185,186</td>
</tr>
</tbody>
</table>

RIPK1, receptor-activating protein kinase 1; pMLKL, phospho-mixed lineage kinase domain-like protein; PARP-1, poly (ADP-ribose) polymerase 1; GPX4, glutathione peroxidase 4; CCR, chemokine (C-C motif) receptor; CCL, chemokine (C-C motif) ligand; CXCR3, chemokine (C-X-C motif) receptor 3; CK, chemokines, TWEAK, TNF-like weak inducer of apoptosis; Jak-STAT, Janus kinase and Signal Transducer and Activator of Transcription; PRR, pattern recognition receptors; MAb, monoclonal antibody.
necrotic glomerular lesions in human renal vasculitis.93 Complement-mediated cytotoxicity is another trigger for glomerular necroinflammation.94 Glomerular cell necrosis induces DAMP release that further drives cytokine and chemokine release, leukocyte recruitment, and inflammation.92 Infiltrating immune cells, in turn, further contribute to necroinflammation by NETosis (neutrophil),92 cytokine-induced cell death (all proinflammatory leukocytes),95 or direct T cell–related cytotoxicity.96 Vascular wall rupture not only facilitates hemorrhage but also plasma leakage as a major driver of parietal epithelial cell hyperplasia and crescent formation.97 Therefore, NETosis and histone or complement cytotoxicity triggering glomerular necroinflammation are a central pathomechanism of rapidly progressive GN related to ANCA-associated vasculitis and the other forms of necrotizing GN.98,99

**Thrombotic Microangiopathy**

Thrombotic microangiopathy (TMA) can be initiated by various triggers, which all share endothelial cell injury-related microvascular thrombosis and organ dysfunction.100 In some disorders, endothelial cell injury is the primary event (e.g., Shiga toxin–induced in hemolytic uremic syndrome, uncontrolled complement activation–induced atypical hemolytic uremic syndrome, preeclampsia, systemic sclerosis, malignant hypertension).100 In other forms of TMA, an inappropriate activation of clotting initiates the process (e.g., antiphospholipid syndrome, ADAMTS13 deficiency).100 Independent of the triggering mechanism, TMA involves an autoamplification loop of tissue necrosis and inflammation.100 Thrombosis involves a massive release of cytokines and chemokines from platelets that activate endothelial cells and recruit leukocytes to the site of injury, a process referred to as immunothrombosis.101 Circulating neutrophils get increasingly involved and contribute to thrombus formation via NET formation,102 which is associated with local histone release. Extracellular histones elicit three different impacts: they kill microvascular endothelial cells,74,92,103 trigger platelet activation and further thrombosis,104 and activate innate immunity via TLRs and the NLRP3 inflammasome.103,103 As a proof of principle, histone injection into the renal artery induces widespread TMA and renal cell necrosis.103 Currently, this autoamplification loop of microvascular injury, thrombosis, and inflammation can only be terminated by abrogating the initial trigger of TMA.100 Long-term outcomes of renal TMA depend on each cell type’s capacity to repair from intrinsic or extrinsic progenitor reservoirs.106

**TARGETING RENAL NECROINFLAMMATION FOR PROPHYLAXIS AND THERAPY**

If necroinflammation is an autoamplification loop, blockade of either component should be sufficient to decelerate and potentially abrogate it. This is well documented by numerous experimental studies of inhibiting different molecular targets that still fully abrogate experimental AKI (Table 3).107 For example, prophylactic therapy with a combination of three different cell death inhibitors (ferrostatins, necrostatin-1, sanglifehrin A) prevents kidney injury, inflammation, and dysfunction in models of ultra–severe duration of ischemia before reperfusion.35 Along these lines, preventing NETosis with a PAD4 inhibitor or extracellular histone neutralization abrogates crescentic GN.92 Autoacceleration of necroinflammation implies that the time point of intervention is of critical importance. Only preemptive blockade of necroinflammation can be maximally effective.
effective, and *vice versa*, most experimental studies reporting prophylactic treatments hardly predict efficacy of delayed therapy. Currently, it remains unknown if delayed cell death inhibition still can prevent kidney injury. In general, few studies report the protective impact of late-onset interventions, such as in histone neutralization preventing crescent formation in overt GN.\textsuperscript{92} GS-K-3 inhibition and recombinant pentraxin-3 can both limit necroinflammation in AKI.\textsuperscript{108,109} A single-dose application of the GS-K-3 inhibitor lithium was reported to attenuate post-ischemic AKI up to 3 hours after renal pedicle clamping\textsuperscript{108} and recombinant pentraxin-3 injection for up to 6 hours.\textsuperscript{109} Timing of interventions is essential for the design of clinical trials, and an earlier-the-better strategy is generally recommended. However, the current definition of AKI that is on the basis of functional markers (urinary output, serum creatinine) and not on injury markers (e.g., KIM-1) remains a major conceptual drawback. Any early therapy may arrive way too late to affect necroinflammation as a mechanism of AKI. However, late onset of therapy may still affect long-term outcomes of AKI. For example, TNF-α blockade starting 5 days after AKI or endothelin blockade up to 12 days after AKI prevents nephron loss and subsequent kidney atrophy in experimental AKI.\textsuperscript{110,111}

**SUMMARY**

Necroinflammation is a theory that combines previously established, coexisting concepts of kidney injury. Necroinflammation is a serial event of regulated necrosis triggering inflammation, secondary-regulated necrosis, and so forth. If not opposed at an early stage, necroinflammation can lead to organ failure or even systemic inflammation and remote organ injury. Following this concept, either anti-inflammatory agents or cell death inhibitors can abrogate this process. However, numerous questions remain, some of which are listed in Table 4. We conclude that the various aspects of necroinflammation offer great opportunities for novel discoveries and eventually also for novel treatment options for patients with kidney disease.

**ACKNOWLEDGMENTS**

The authors are supported by the Deutsche Forschungsgemeinschaft (MU3906/1-1, AN372/21-1, AN372/14-3, EXC 306, project W TP1).

**REFERENCES**

exchange induces endoplasmic reticulum stress and fibrosis. eLife 3, e02523, 2014


Keary CJ, Cullen SP, Clancy D, Martin SJ: RIPK1 can function as an inhibitor rather than an initiator of RIPK3-dependent necroptosis. FEBS J 281: 4921–4934, 2014


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2015040457/-/DCSupplemental.