REFERENCES

1. Sakai T, Kriz W: The structural relationship between mesangial cells and basement membrane of the renal glomerulus. *Anat Embryol (Berl)* 176: 373–386, 1987
2. Kikkawa Y, Virtanen I, Miner JH: Mesangial cells organize the glomerular capillaries by adhering to the G domain of laminin alpha5 in the glomerular basement membrane. *J Cell Biol* 161: 187–196, 2003
3. Zimmerman SE, Hiremath C, Tsunezumi J, Yang Z, Finney B, Marciano DK: Nephronectin regulates mesangial cell adhesion and behavior in glomeruli. *J Am Soc Nephrol* 29: 1128–1140, 2018
4. Elger M, Sakai T, Kriz W: Role of mesangial cell contraction in adaptation of the glomerular tuft to changes in extracellular volume. *Pflugers Arch* 415: 598–605, 1990
5. Hakroush S, Moeller MJ, Theilig F, Kaissling B, Sijmonsma TP, Jugold M, et al.: Effects of increased renal tubular vascular endothelial growth factor (VEGF) on fibrosis, cyst formation, and glomerular disease. *Am J Pathol* 175: 1883–1895, 2009
6. Lemley KV, Elger M, Koeppen-Hagemann I, Kretzler M, Nagata M, Sakai T, et al.: The glomerular mesangium: Capillary support function and its failure under experimental conditions. *Clin Investig* 70: 843–856, 1992
7. Nagata M, Schärer K, Kriz W: Glomerular damage after uninephrectomy in young rats. I. Hypertrophy and distortion of capillary architecture. *Kidney Int* 42: 136–147, 1992
8. Vaughan MR, Quaggin SE: How do mesangial and endothelial cells form the glomerular tuft? *J Am Soc Nephrol* 19: 24–33, 2008
9. Shirato I, Sakai T, Kimura K, Tomino Y, Kriz W: Cytoskeletal changes in podocytes associated with foot process effacement in Masugi nephritis. *Am J Pathol* 148: 1283–1296, 1996
10. Kriz W, Lemley KV: Potential relevance of shear stress for slit diaphragm and podocyte function. *Kidney Int* 91: 1283–1286, 2017
11. Kriz W, Lemley KV: Mechanical challenges to the glomerular filtration barrier: Adaptations and pathway to sclerosis. *Pediatr Nephrol* 32: 405–417, 2017
12. Janmey PA, Miller RT: Mechanisms of mechanical signaling in development and disease. *J Cell Sci* 124: 9–18, 2011
13. Rennke HG, Anderson S, Brenner BM: Structural and functional correlations in the progression of kidney disease. In: *Renal Pathology*, edited by Tisher CC, Brenner BM, Philadelphia, Lippincott, 1989, pp 43–66
14. Kretzler M, Koeppen-Hagemann I, Kriz W: Podocyte damage is a critical step in the development of glomerulosclerosis in the uninephrectomised-desoxycorticosterone hypertensive rat. *Virchows Arch* 425: 181–193, 1994
15. Kriz W, Hosser H, Hähnel B, Simons JL, Provoost AP: Development of vascular pole-associated glomerulosclerosis in the Fawn-hooded rat. *J Am Soc Nephrol* 9: 381–396, 1998
16. Dworkin LD, Feiner HD: Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest* 77: 797–809, 1986
17. Friedrich C, Endlich N, Kriz W, Endlich K: Podocytes are sensitive to fluid shear stress in vitro. *Am J Physiol Renal Physiol* 291: F856–F865, 2006
18. Kriz W, Lemley KV: A potential role for mechanical forces in the detachment of podocytes and the progression of CKD. *J Am Soc Nephrol* 26: 258–269, 2015
19. Le Hir M, Keller C, Eschmann V, Hähnel B, Hosser H, Kriz W: Podocyte bridges between the tuft and Bowman's capsule: An early event in experimental crescentic glomerulonephritis. *J Am Soc Nephrol* 12: 2060–2071, 2001
20. Smeets B, Moeller MJ: Parietal epithelial cells and podocytes in glomerular diseases. *Semin Nephrol* 32: 357–367, 2012
21. D'Agati VD: The spectrum of focal segmental glomerulosclerosis: New insights. *Curr Opin Nephrol Hypertens* 17: 271–281, 2008

See related article, "Nephronectin Regulates Mesangial Cell Adhesion and Behavior in Glomeruli," on pages 1128–1140.

We AIM2 Inflame

Andreas Linkermann, Simon P. Parmentier, and Christian Hugo

Division of Nephrology, Department of Internal Medicine III, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany

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The AIM2 inflammasome senses DNA released by necrotic renal cells and translates a deadly signal into a proinflammatory trigger, thereby providing a prototype example of necroinflammation. Loss of cellular membrane integrity (necrosis) represents a genetically determined and highly regulated process during AKI.[1] During recent years, a discussion on the role of regulated necrosis as a novel therapeutic target during transplantation has emerged, but interestingly not because interference with necrotic cell death might preserve renal cell survival. Prevention of necrosis rather prevents the immunogenicity of necrotic debris. When any cell loses its membrane integrity, two steps follow. First, this cell loses most of its function. In the case of a tubular cell, this may represent features of AKI. Second, intracellular debris (often referred to as damage-associated molecular patterns[2,3]) becomes accessible to surveilling immune cells. In the case of kidney transplantation, such cells become strongly activated and primed (*e.g.*, in the case of B cells).[4] On immunosuppression during the transplant process, immediate immunogenic responses (rejections) are prevented. When immunosuppression is tapered months after transplantation, antibody-mediated rejections may then be triggered, *e.g.*, after otherwise trivial viral infection.[4] But how can necrotic debris conceptually be explained as the stimulating trigger of macrophages on a molecular level? An important set of well controlled data regarding this long-standing obstacle now comes from the group of Daniel Muruve.[5]

In this issue of the *Journal of the American Society of Nephrology*, Komada *et al.*[5] detected expression levels of the inflammasomal protein absent in melanoma 2 (AIM2) in human kidney sections obtained from nondiseased margins of nephrectomies in which AIM2 is predominantly present in the glomeruli. However, in samples obtained from patients with diabetic nephropathy or hypertensive sclerosis, a strong upregulation of AIM2 expression in infiltrating CD45-positive leukocytes and in the tubular compartment was demonstrated. These human findings were in line with data generated from mice that

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Correspondence: Dr. Andreas Linkermann, Division of Nephrology, Department of Internal Medicine III, University Hospital Carl Gustav Carus at Technische Universität Dresden, Fetscherstr. 74, 01307 Dresden, Germany. Email: andreas.linkermann@uniklinikum-dresden.de

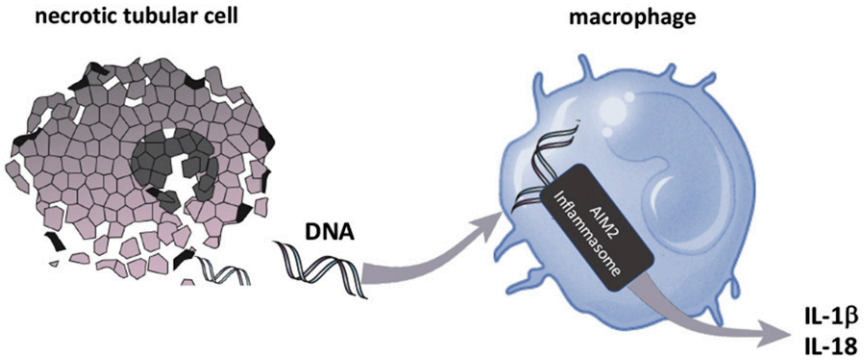

Figure 1. AIM2-mediated sensing of DNA released from necrotic cells. Upon acute tubular necrosis, tubular cells release intracellular content. This necrotic debris contains damage-associated molecular patterns (DAMPs) such as DNA. Macrophages sense DNA released from necrotic cells through the AIM2 inflammasome and subsequently produce proinflammatory cytokines, such as IL-1β and IL-18. The amplification of the immunogenicity of a necrotic DAMP into a stronger proinflammatory signal is referred to as necroinflammation.

underwent unilateral ureteral obstruction (UUO). In those samples of either whole kidney lysates or microdissected tubular and glomerular compartments, AIM2 mRNA and AIM2-knockout (AIM2-ko) controlled protein expressions were highly upregulated. The AIM2 data are particularly interesting because previous work on inflammasomes in the kidney demonstrated the involvement of another type of inflammasomes, the so-called NLRP3 inflammasome.[6] Consequently, the authors compared NLRP3-deficient and AIM2-deficient mice alongside with NLRP3/AIM2 double knockout (NLRP3/AIM2-dko) mice in the UUO model. Interestingly, after 7 days of UUO treatment, similar levels of protection were reported for all three genetic models in tubular injury scores and KIM1 protein expression, suggesting a partially redundant role for the inflammasomes during renal damage. However, whereas KIM1 gene expression appeared to be remarkably reduced in the NLRP3/AIM2-dko mice compared with AIM2-ko mice or wild-type controls, these knockout mice did not show a significant difference in renal fibrosis, as measured by picrosirius red staining or collagen I expression. Such a faint interplay between NLRP3 and AIM2 has recently been described during *Aspergillus* infection.[7]

The authors next investigated the role of F4/80-positive macrophages. Whereas an almost six-fold higher F4/80-positive area was found in UUO-treated wild-type mice compared with untreated controls, this increase was reduced by approximately 50% in AIM2-ko and NLRP3/AIM2-dko mice, highlighting the role of macrophages in this model. Importantly, effector functions of inflammasomes are mediated though caspases (caspase-1, -4, -5, and -11) through proteolytic cleavage of pro–IL-1β and pro–IL-18. Clearly, the IL1β-to-pro–IL-1β and the IL-18-to-pro–IL-18 ratios were decreased in both AIM2-ko and NLRP3/AIM2-dko UUO kidney samples compared with wild-type UUO. This finding suggests the proinflammatory role of inflammasomal signaling to be significantly reduced in the knockout mice. In keeping with the hypothesis that the AIM2 inflammasome in macrophages contributes to renal damage during UUO, bone marrow transfer experiments in which wild-type bone marrow was transplanted to AIM2-deficient mice brought the dimension of injury back to wild-type control levels. This experiment excluded a significant pathophysiologic role of AIM2 in the tubular cell compartment.

Elegant FACS studies employing CD45/F4/80 double-positive gating of kidney-derived samples revealed high levels of proinflammatory chemokines, such as CX_3CR1 and CCR2 only in cells derived from wild-type mice, but not in AIM2-ko or NLRP3/AIM2-dko mice. The presence of these cells and their activity suggested AIM2 stimulation in this tissue by DNA,[8] the source of which most likely were necrotic tubular cells. To this end, the authors used macrophage lineage specific LysM;cre mice that were transfected with a green fluorescent protein (*gfp*). Additional staining for DNA by SYTOX orange allowed for direct detection of DNA released from tubular cells to be taken up by LysM-gfp–positive cells with two-photon intravital microscopy. These experiments clearly demonstrate the uptake of necrotic cell released DNA by renal macrophages in living animals. However, the authors decided to add yet another line of evidence by treating THP1 cells (a macrophage cell line) with DNA generated from necrotic human tubular epithelial cells. Within 2 hours, the DNA released by the necrotic cell was taken up by the THP1 cells and IL-1β release was demonstrated to be highly increased in a caspase-sensitive manner. As expected, addition of DNase to the media destroyed the human tubular epithelial cell DNA and prevented the increased release of IL-1β.

Necrosis of renal tubular cells has recently been identified as a major driver of acute kidney injury in models of cisplatin-induced nephrotoxicity, folic acid–induced nephropathy, ischemia–reperfusion injury, and during kidney transplantation.[1] As now demonstrated by Komada *et al.*,[5] necrotic DNA is sensed, at least partially, by the AIM2 inflammasome to drive macrophage IL-1β release (Figure 1). These experiments support the model of necroinflammation (necrosis-driven potentiation of the initial damage by infiltrating immune cells).[9,10] Two therapeutic options evolve from these findings. First, it should be possible to prevent the necrotic cell death *per se*. Second, if necrosis cannot be prevented, targeting inflammasomes provides an attractive

alternative, but until now, no promising drug candidates are broadly available, to the best of our knowledge.

DISCLOSURES

None.

REFERENCES

1. Linkermann A: Nonapoptotic cell death in acute kidney injury and transplantation. *Kidney Int* 89: 46–57, 2016
2. Land WG, Agostinis P, Gasser S, Garg AD, Linkermann A: DAMP-induced allograft and tumor rejection: The circle is closing. *Am J Transplant* 16: 3322–3337, 2016
3. Land WG, Agostinis P, Gasser S, Garg AD, Linkermann A: Transplantation and Damage-Associated Molecular Patterns (DAMPs). *Am J Transplant* 16: 3338–3361, 2016
4. Sarhan M, von Mässenhausen A, Hugo C, Oberbauer R, Linkermann A: Immunological consequences of kidney cell death. *Cell Death Dis* 9: 114, 2018
5. Komada T, Chung H, Lau A, Platnich JM, Beck PL, Benediktsson H, et al: Macrophage uptake of necrotic cell DNA activates the AIM2 inflammasome to regulate a proinflammatory phenotype in CKD. *J Am Soc Nephrol* 29: 1165–1181, 2018
6. Anders HJ, Muruve DA: The inflammasomes in kidney disease. *J Am Soc Nephrol* 22: 1007–1018, 2011
7. Karki R, Man SM, Malireddi RK, Gurung P, Vogel P, Lamkanfi M, et al.: Concerted activation of the AIM2 and NLRP3 inflammasomes orchestrates host protection against Aspergillus infection. *Cell Host Microbe* 17: 357–368, 2015
8. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES: AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458: 509–513, 2009
9. Linkermann A, Stockwell BR, Krautwald S, Anders HJ: Regulated cell death and inflammation: An auto-amplification loop causes organ failure. *Nat Rev Immunol* 14: 759–767, 2014
10. Mulay SR, Linkermann A, Anders HJ: Necroinflammation in kidney disease. *J Am Soc Nephrol* 27: 27–39, 2016

See related article, "Macrophage Uptake of Necrotic Cell DNA Activates the AIM2 Inflammasome to Regulate a Proinflammatory Phenotype in CKD," on pages 1165–1181.

Evaluation of Potential Living Kidney Donors in the *APOL1* Era

Barry I. Freedman[1] and Bruce A. Julian[2]

[1]Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina; and [2]Department of Medicine, Division of Nephrology; University of Alabama at Birmingham School of Medicine, Birmingham, Alabama

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Correspondence: Prof. Barry I. Freedman, Wake Forest School of Medicine, Section on Nephrology, Medical Center Boulevard, Winston-Salem, NC 27157-1053. Email: bfreedma@wakehealth.edu

Inheriting two apolipoprotein L1 gene (*APOL1*) renal risk variants accounts for the majority of the excess risk for nondiabetic ESRD in individuals with recent African ancestry.[1] *APOL1* renal risk variants are common in blacks in the United States, with about 13% carrying two variants (defining the high-risk genotype), whereas 39% have one variant and 48% have no variant. In contrast, *APOL1* renal risk variants are virtually absent in nonadmixed European, Asian, and Hispanic populations. Approximately 20% of those with the *APOL1* high-risk genotype ultimately develop CKD, supporting the postulate that modifying factors are necessary to trigger development of nephropathy.[2]

The effect of having two *APOL1* renal risk variants extends beyond native kidney disease. Kidney transplants from deceased black donors with the *APOL1* high-risk genotype fail more quickly than allografts from donors with zero or one *APOL1* renal risk variant.[3] The outcomes of kidneys from deceased black donors with zero or one *APOL1* renal risk variant approximate those of kidneys from white donors.[3,4] Furthermore, serum creatinine concentrations are higher in recipients with functioning kidneys from donors with the *APOL1* high-risk genotype, raising concerns that additional allografts will also fail over longer intervals.[3,5] The poorer outcome of these allografts is independent of the ethnicity of the recipient, indicating that the effect of the genotype travels with the kidney.[6] Nonetheless, such as is the case for native kidney disease, the association of the *APOL1* high-risk genotype with a poor outcome is far from universal. Indeed, most allografts from deceased black donors with the *APOL1* high-risk genotype function well for prolonged intervals, again suggesting that modifying factors initiate or accentuate renal damage.

More recently, the kidney transplant community has begun to examine a possible effect of *APOL1* renal risk variants on outcomes in living donor transplantation. Living black kidney donors more often develop ESRD than donors from other racial groups, with frequencies about 3.5- to 5.3-fold higher than in age- and sex-matched whites[7] (Figure 1). Patient reports have described the loss of kidneys from living black donors with the *APOL1* high-risk genotype several years after transplantation due to proteinuric disease, with subsequent development of ESRD in the donor.[8] These reports raise the question: does the presence of the *APOL1* high-risk genotype adversely affect postdonation renal function in black living kidney donors?

In this issue of the *Journal of the American Society of Nephrology*, Doshi *et al.*[9] addressed that question by studying a cohort of 136 black living kidney donors with mean age of 37 years and mean follow-up of 12 years. Nineteen (14%) patients had the *APOL1* high-risk genotype, a frequency similar to that in the general black population. They found that the mean eGFR before nephrectomy was significantly lower in *APOL1* high-risk donors than in donors with zero or one *APOL1* renal risk variant. This difference suggests that deterioration of renal clearance function started before donation, although without clinical manifestations that would preclude acceptance as a kidney donor. Nephrectomy decreased eGFR by 25–30 ml/min per 1.73 m^2 in both donor subgroups, but the decline in