Ischemia-reperfusion induces renal tubule pyroptosis via the CHOP-caspase-11 pathway

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Yang J, Yao F, Zhang J, Ji Z, Li K, Zhan J, Tong Y, Lin L, He Y. Ischemia-reperfusion induces renal tubule pyroptosis via the CHOP-caspase-11 pathway. Am J Physiol Renal Physiol 306:F75–F84, 2014; First published October 16, 2013; doi:10.1152/ajprenal.00117.2013.---The apoptotic or necrotic death of renal tubule epithelial cells is the main pathogenesis of renal ischemia-reperfusion-induced acute kidney injury (AKI). Pyroptosis is a programmed cell death pathway that depends on the activation of the caspase cascade and IL-1 cytokine family members. However, the role of pyroptosis in AKI induced by ischemia-reperfusion remains unclear. In this study, we found that the levels of the pyroptosis-related proteins, including caspase-1, caspase-11, and IL-1β, were significantly increased after 6 h of renal ischemia-reperfusion injury (IRI) and peaked at 12 h after IRI. Enhanced pyroptosis was accompanied by elevated renal structural and functional injury. Similarly, hypoxia-reoxygenation injury (HRI) also induced pyroptosis in renal tubule epithelial NRK-52E cells, which was characterized by increased pore formation and elevated lactate dehydrogenase release. In addition, obvious upregulation of the endoplasmic reticulum (ER) stress biomarkers glucose-regulated protein 78 and C/EBP homologous protein (CHOP) preceded the incidence of pyroptosis in cells treated with IRI or HRI. Pretreatment with a low dose of tunicamycin, an inducer of ER stress, relieved IRI-induced pyroptosis and renal tissue injury. Silencing of CHOP by small interfering RNA significantly decreased HRI-induced pyroptosis of NRK-52E cells, as evidenced by reduced caspase-11 activity and IL-1β generation. Therefore, we conclude that pyroptosis of renal tubule epithelial cells is a key event during IRI and that CHOP-caspase-11 triggered by overactivated ER stress may be an essential pathway involved in pyroptosis.

pyroptosis; endoplasmic reticulum stress; C/EBP homologous protein; caspase-11; renal ischemia-reperfusion injury

ACUTE KIDNEY INJURY (AKI) is a common and severe condition induced by various stimuli, such as trauma, infection, and medications. AKI may lead to acute renal failure and result in high mortality (40–60%) (10, 28, 46). Ischemia-reperfusion injury (IRI) is the main cause of AKI. IRI causes structural damage of renal tubules by directly inducing death of renal tubule epithelial cells, and the dying cells may release signals that trigger renal inflammatory responses. The cross-talk of these complex pathways eventually initiates the development and progression of AKI (9, 21, 23, 31, 47).

Pyroptosis is a unique type of programmed cell death that is distinct from apoptosis and necrosis (6, 34). Caspase-1 is a key molecule that leads to the induction of pyroptosis. Proinflammatory factors, such as pro-IL-1β and pro-IL-18, can be activated by caspase-1 cleavage and trigger or aggravate inflammatory responses (34, 40). Therefore, pyroptosis may not only lead to cell death but also play an important role in the cascade of reactions that lead to damaged tissues (34). Several studies (2, 17, 33, 34) have indicated that pyroptosis contributes to infectious disease, nervous system disorders, and atherosclerosis. During the development and progression of AKI, renal cell death and inflammation may influence the severity and progression of AKI (47). Thus, upregulated levels of IL-1β family members, such as IL-1β and IL-18, in kidney tissues and urine may serve as a predictor for the progression of AKI (4, 11). Nevertheless, whether pyroptosis is involved in renal tubule epithelial cell death upon IRI and how pyroptosis and inflammatory cytokines are related remain unknown. The underlying mechanism by which pyroptosis acts during the development of AKI needs to be clarified.

Endoplasmic reticulum (ER) stress is an early and essential biological event during tissue injury (15). Moderate ER stress acts as a self-defense system and protects cells from injury, whereas overactivated ER stress may lead to cell death by activating C/EBP homologous protein (CHOP), JNK, and other signaling pathways (18, 35, 37, 44). CHOP mediates the mRNA expression of caspase-1 and initiates the activation of its effector, molecular caspase-1 (16). Caspase-1 is a key regulator for cell apoptosis and is responsible for the maturation of proinflammatory cytokine IL-1β family members by cleavage. However, the involvement of CHOP-caspase-11-mediated caspase-1-dependent pyroptosis and related inflammatory responses in renal IRI is still unclear. Therefore, we investigated the pyroptosis-related alterations that occur in renal tissues and renal tubule epithelial cells after IRI. The actual role of pyroptosis-related caspase-11 and caspase-1 activation upon renal injury was examined by pretreatment of cells with an ER stress inducer and CHOP silencing. Here, we report, for the first time, that pyroptosis is a key event during renal IRI and that it is regulated via the CHOP-caspase-11 signaling pathway.

MATERIALS AND METHODS

Reagents. Rat renal tubule epithelial (NRK-52E) cells were purchased from the American Type Culture Collection (Manassas, VA). DMEM, FBS, and trypsin were purchased from HyClone (Logan, UT). Polyclonal rabbit anti-rat glucose-regulated protein 78 (GRP78), mouse anti-rat CHOP antibodies, and control rabbit and mouse IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal rabbit anti-rat caspase-1 antibodies were purchased from Upstate (Billerica, MA). Tunicamycin (TM) and polyclonal anti-
anti-caspase-11 antibody were purchased from Sigma (St. Louis, MO). Polyclonal rabbit anti-rat anti-IL-1 antibody was obtained from Abcam (Cambridge, MA). FITC-labeled goat anti-mouse IgG and control goal IgG were from Jackson ImmunoResearch (West Grove, PA). Cy3-labeled goat anti-rabbit IgG was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibodies were provided by Zhongshan Goldbridge Biotechnology (Beijing, China).

**Animals.** Male Sprague-Dawley rats at 4–5 wk of age and weighing 150–200 g were obtained from the Laboratory Animal Center of the Third Military Medical University (Chongqing, China). The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Third Military Medical University.

**Induction of IRI.** The rat model of renal IRI was established as previously described by Yokota et al. (50). Briefly, rats were fasted overnight, anesthetized by an intraperitoneal injection of 3% pentobarbital sodium (0.1 ml/100 g body wt), and subjected to an abdominal incision. Their rectal temperatures were maintained at 37°C using a thermistor connected to a servo-controlled heating pad (model D1-L, Haake, Tokyo, Japan). Renal pedicles of the IRI group of rats were dissected and clamped with nontraumatic clamps for 40 min. Renal pedicles were then reperfused in situ for 1, 6, 12, or 24 h, after which six rats from each group at each time point were euthanized by decapitation, and their renal tissues were dissected carefully for the following experiments. Sham-operated control rats were subjected to an abdominal incision without clamping of the renal pedicles. Blood samples were collected, and rats were euthanized at these time points after IRI. A: kidney tissue sections were subjected to histological examination by periodic acid-Schiff (PAS) staining to evaluate renal tubule injury. Damaged tubules are indicated by red arrows. Renal tubule injury (B), serum creatinine (C), and blood urea nitrogen (BUN; D) levels were measured. Data are expressed as means ± SD of each group of rats (n = 6 rats/group at each time point). Control rats received sham surgery, and measurements were made when experimental rats were at 12 h after reperfusion. *P < 0.05 vs. sham control rats.

**Fig. 1.** Renal injury induced by ischemia-reperfusion. Renal pedicles of individual rats were reperfused for 1, 6, 12, or 24 h, respectively. Control rats received sham surgery (sham group) without ischemia-reperfusion injury (IRI). Blood samples were collected, and rats were euthanized at these time points after IRI.

**Fig. 2.** Renal IRI altered the levels of proteins associated with pyroptosis. Levels of pro-IL-1β, IL-1β, pro-caspase-1, caspase-1, pro-caspase-11, caspase-11, and control β-actin in renal tissues from rats euthanized at individual time points after IRI were characterized by Western blot assays (A). B and C: quantitative analysis of relative levels of protein expression. Data shown are representative images or are expressed as means ± SD of individual groups (n = 6 rats/group at each time point). *P < 0.05 vs. sham control rats.
samples were collected from individual rats at each time point after reperfusion to measure concentrations of serum creatinine and blood urea nitrogen (BUN). Control (n = 6) and IRI rats (n = 6) were randomized and treated intraperitoneally with 0.6 mg/kg TM (dissolved in DMSO) or the same amount of DMSO 2 days before surgery to generate the following four groups: control TM, control DMSO, IRI + TM, and IRI + DMSO. Individual rats were subjected to blood sampling at 12 h after reperfusion and euthanized.

**Histological examination.** Kidney tissue sections (4 μm) were stained with periodic acid-Schiff and analyzed under an Olympus microscope. At least 10 fields selected randomly from each sample were evaluated for renal tubule injury by at least two pathologists in a blinded manner, as previously described by Sharfuddin et al. (42). Briefly, the percentage of tubules in the outer medulla that showed epithelial necrosis or had necrotic debris was scored as follows: 0, <10%; 1, 10%; 2, 10–25%; 3, 26–75%; and 4, >75%.

**Cell model of hypoxia-reoxygenation injury.** NRK-52E cells were cultured in DMEM supplemented with 5% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). Subsequently, cells were cultured under hypoxic conditions (1% O2, 94% N2, and 5% CO2) in FBS- and antibiotic-free medium for 4 h at 37°C to induce hypoxic injury; they then were returned to 5% CO2 and 95% air for reoxygenation. Cells and their supernatants were harvested at 0, 1, 3, 6, 12, or 24 h after reoxygenation.

Fig. 3. Renal IRI-induced endoplasmic reticulum (ER) stress. Expression of C/EBP homologous protein (CHOP) in the S3 segment of proximal tubule tissues from rats euthanized at individual time points after IRI was characterized by immunohistochemistry (A). Protein expression of CHOP and glucose-regulated protein 78 (GRP78) in renal tissues was examined by Western blot analysis (B). C: quantitative analysis of relative levels of protein expression. Data shown are representative images or are expressed as means ± SD of individual groups (n = 6 rats/group at each time point). *P < 0.05 vs. sham control rats.

Fig. 4. Pretreatment with tunicamycin (TM) mitigated IRI-induced renal injury in rats. Groups of rats were treated with 0.6 mg/kg TM or vehicle (DMSO) and subjected to ischemia and reperfusion for 12 h or sham surgery. A: renal tissues were collected and subjected to PAS staining. Damaged tubules are indicated by red arrows. Renal tubule injury (B), serum creatinine (C), and BUN (D) levels were measured. Data are expressed as means ± SD of each group of rats (n = 6 rats/group at each time point). Control rats received sham surgery, and measurements were made when the experimental rats were at 12 h after reperfusion. *P < 0.05 vs. sham control rats; #P < 0.05 vs. vehicle-treated control rats after IRI.
Transfection. NRK-52E cells were transfected with CHOP-specific or control small interfering (si)RNA (sc-35438, Santa Cruz Biotechnology) using Lipofectamine 2000 (Invitrogen, Grand Island, NY), as previously described by Yang et al. (48). Cells incubated with Lipofectamine 2000 alone were also used as the negative control.

Pore formation test. Pore formation was analyzed by ethidium bromide staining as previously described (26). NRK-52E cells were seeded onto 24-well plates at a density of 5 × 10^5 cells/well. Cells were treated with PBS containing 25 μg/ml ethidium bromide and 5 μg/ml acridine orange. All cells were stained with acridine orange, whereas only cells with membrane pores allowed diffusion of ethidium bromide into the cell. Pore-forming activity was measured and expressed as the percentage of NRK-52E cells that stained positively with ethidium bromide. Images were acquired using a Leica microscope (DMI4000B, Leica Microsystems, Wetzlar, Germany) with ×10 and ×10 objectives and were analyzed using ImageJ software.

Lactate dehydrogenase release assay. As previously described (27), lactate dehydrogenase (LDH) release was measured by a colorimetric assay following the manufacturer’s instructions (27), lactate dehydrogenase (LDH) release was measured by a colorimetric assay following the manufacturer’s instructions (Shanghai Westang Biotech, Shanghai, China).

Western blot analysis. After protein concentrations were determined using bicinchoninic acid, equal amounts of protein lysates were separated by SDS-PAGE and electrotransferred onto nitrocellulose membranes followed by blockade with 3% BSA. Subsequently, membranes were incubated with anti-GRP78 (1:1,000), anti-CHOP (1:200), anti-caspase-11 (1:200), anti-caspase-1 (1:1,000), anti-IL-1β (1:200), or anti-β-actin (1:2,000, loading control) at 4°C overnight. After membranes were washed, bound antibodies were detected with horseradish peroxidase-conjugated secondary antibody (1:1,000) at room temperature for 1 h and visualized using enhanced chemiluminescence. Relative levels of each protein to control β-actin were quantified using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

ELISA. Concentrations of IL-1β in cell supernatants were assessed by ELISA using a rat IL-1β ELISA kit according to the manufacturer’s instructions (Shanghai Westang Biotech, Shanghai, China).

Immunofluorescence analysis. NRK-52E cells were fixed with 4% paraformaldehyde for 30 min and treated with 0.25% Triton X-100 at room temperature for 10 h. Subsequently, cells were incubated with primary antibodies against CHOP (1:300) or caspase-11 (1:200) overnight at 4°C. After being washed, samples were incubated with FITC-conjugated anti-rabbit IgG (1:20) or Cy3-conjugated anti-mouse IgG (1:50) at 37°C for 1 h and costained with 4',6-diamidino-2-phenylindole. Cells were imaged under a confocal laser scanning biological microscope (Olympus, Tokyo, Japan).

Statistical analysis. All values are presented as means ± SD. Differences among different groups were analyzed by one-way ANOVA followed by multiple pair-wise comparisons by the New-
man-Keuls test using SPSS 13.0 software (SPSS, Chicago, IL). *P* values of <0.05 were considered to be statistically significant.

**RESULTS**

**IRI induces renal pyroptosis and inflammation.** To investigate the effects of IRI on renal pyroptosis and inflammation, a rat model of renal IRI was used. Groups of rats were subjected to sham surgery or ischemia-reperfusion surgery, and the morphology of their kidneys was histologically examined (Fig. 1A). Quantitative analysis revealed that the scores for renal tubule damage increased from 1 to 12 h after IRI and then decreased at 24 h after IRI (Fig. 1B). Concentrations of serum creatinine and BUN were significantly elevated after IRI in rats (Fig. 1, C and D). These data indicated that IRI induced renal tubule injury and impaired renal function in rats. Western blot analysis revealed that the levels of pro-caspase-1, caspase-1, pro-caspase-11, caspase-11, pro-IL-1β, and IL-1β were markedly elevated at 6 h and peaked at 12 h after injury (Fig. 2). The levels of these proteins decreased at 24 h after IRI. These results suggest that IRI induces renal pyroptosis and inflammation.

**IRI induces renal pyroptosis by triggering ER stress.** Recent reports have demonstrated that ER stress can trigger cell death and inflammation during IRI. We found that the levels of GRP78 and CHOP significantly increased in the rat kidney as early as 1 h after IRI and peaked at 12 h after injury (Fig. 3). A previous study (38) has shown that pretreatment of mice with TM induces moderate levels of ER stress and inhibits renal IRI in mice. However, the mechanisms that underlie this effect remain unclear. We found that pretreatment with TM greatly mitigated ischemia-reperfusion-induced renal damage by reducing the scores of renal injury and improved renal function by reducing concentrations of serum creatinine and BUN in rats after IRI (Fig. 4). Western blot analysis revealed that the relative levels of CHOP in the kidneys from TM-treated rats were significantly reduced compared with those in vehicle-treated rats at 12 h after IRI (Fig. 5, A and B). There were no significant differences in the levels of CHOP between TM-treated and vehicle-treated sham-operated groups of rats. In contrast, the relative levels of GRP78 expression in the kidneys from TM-treated rats were significantly higher than those in vehicle-treated rats that had been subjected to a sham surgery or after ischemia-reperfusion induction (Fig. 5, A and B). Consistent with the downregulation of CHOP expression and inflammatory caspase activity, TM treatment significantly reduced the levels of pro-caspase-11, caspase-11, pro-caspase-1, caspase-1, pro-IL-1β, and IL-1β expression in the kidneys from the IRI group of rats but not from the sham-operated group of rats (Fig. 5, C–E). Together, these observations suggest that the induction of renal IRI activates ER stress pathway-related CHOP and induces renal pyroptosis.

The **CHOP-caspase-11 pathway is involved in hypoxic stress-induced pyroptosis of NRK-52E cells.** We next investigated the association between CHOP and pyroptosis during hypoxia-reoxygenation injury (HRI) using an in vitro cell model that has been widely used to investigate IRI in the kidney or other organs for many years and is still used by other research groups (13, 24, 49, 51). As shown in Figs. 6 and 7, GRP78, CHOP, pro-caspase-1, caspase-1, pro-caspase-11, caspase-11, pro-IL-1β, and IL-1β were expressed at low levels in NRK-52E cells. The induction of HRI significantly upregulated levels of CHOP, caspase-11, pro-IL-1β, and IL-1β expression at 1 h after HRI (*P* < 0.05 vs. control) and further increased levels of GRP78, pro-caspase-11, and caspase-1 expression at 3 h after HRI. Expression levels of these molecules peaked at 12 h after HRI and then decreased at 24 h after HRI. A similar pattern of IL-1β secretion was observed in cell supernatants after HRI (Fig. 7D). Pyroptosis is characterized by the insertion of pores into the plasma membrane of cells, which can be detected by the pore formation assay and LDH release assay. A significant increase in the percentage of pore formation and LDH level was seen in NRK-52E cells after HRI (Fig. 7, E and F). These results reveal that HRI can induce pyroptosis in renal tubule epithelial cells by activating the CHOP-caspase-11 pathway.

**Knockdown of CHOP expression by siRNA mitigates HRI-induced pyroptosis in NRK-52E cells.** To further validate the significance of CHOP-mediated pyroptosis after HRI, we investigated the effects of CHOP silencing on pyroptosis in NRK-52E cells. We found that knockdown of CHOP did not significantly alter the levels of GRP78, pro-caspase-1, caspase-1, pro-caspase-11, caspase-11, or IL-1β expression in control cells (Fig. 8, A–D). In agreement with our in vivo
Pyroptosis is characterized by rapid plasma membrane rupture and release of pro-inflammatory intracellular contents, which is morphologically and mechanistically distinct from other forms of cell death. Caspase-1 dependence is a defining feature of pyroptosis, as caspase-1 is the enzyme that mediates this process of cell death (6). Caspase-1 was first recognized as a protease that processes the inactive precursors of IL-1β and IL-18 into mature inflammatory cytokines, and it was initially called IL-1β-converting enzyme (3). Activation of caspase-1 induces pore formation on the cell membrane, which leads to the generation and release of abundant inflammatory factors and subsequently contributes to pyroptosis (5, 14). Our in vivo study has shown that pyroptosis is characterized by increased caspase-1 expression and IL-1β generation appeared at 6 h after IRI. The altered levels of caspase-1 and IL-1β in renal tissues were correlated with the structural and functional changes that occurred in renal tissues. Our in vitro study revealed that HRI caused an upregulation of caspase-1 in renal tubule epithelial cells, which was accompanied by increased pore formation, LDH release, and IL-1β generation. These results suggest that when cells undergo pyroptosis, they may lose membrane integrity, which leads to the release of the cellular contents of LDH and inflammatory factor IL-1β. These results together imply that pyroptosis and inflammatory responses are closely associated with the development and progression of AKI in renal tubule epithelial cells after IRI.

A variety of data have shown that by activating ER stress, IRI can induce several types of cell death, including autophagy, apoptosis, necroptosis, and mitochondria-mediated programmed necrosis (22, 29, 43). Recent studies (32, 36) have demonstrated that ER stress can activate the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome via protein kinase RNA-like ER kinase and inositol-requiring enzyme 1 signaling pathways, trigger caspase-1 activity, and lead to the...
death of pancreatic β-cells and to IL-1β release. These results suggest that overactivated ER stress may be an important cause of pyroptosis in certain cell types. The upregulation of GRP78 is a hallmark for the initiation of ER stress, and an elevated level of the downstream molecule CHOP indicates the activation of the ER-stress-mediated cell death signaling pathway. Hence, the upregulation of GRP78 and CHOP is the result of overactivated ER stress (18). In the present study, we found
that upregulation of GRP78 and CHOP preceded the incidence of pyroptosis, which was characterized by increased pore formation, LDH release, and elevated caspase-1 and IL-1β production in cells treated with IRI or HRI. Our in vitro study demonstrated that silencing of CHOP significantly suppressed HRI-induced renal tubule epithelial cell pyroptosis, which suggests that pyroptosis might be a downstream effector of overactivated ER stress. Pretreatment with a low dose of TM, an inducer of ER stress, relieved IRI-induced pyroptosis and renal tissue injury, as evidenced by decreased caspase-1 and IL-1β generation and reduced renal structural and functional damage compared with the IRI treatment group. These findings indicate that pretreatment with an ER stress inducer can protect against renal tubule epithelial pyroptosis induced by IRI and confirm that pyroptosis is a downstream effector of overactivated ER stress.

Linkermann et al. (30) previously reported that the pan-caspase inhibitor z-VAD-fmk (10 mg/kg) had no influence on renal injury. However, such an observation does not rule out the possibility that inflammatory caspases may play a critical role in renal IRI, and we need to reevaluate the value of z-VAD-fmk as a pan-caspase inhibitor used in the blockage of inflammatory caspases. Daemen et al. (8) previously reported that z-VAD-fmk (1 mg/kg) can inhibit inflammatory caspases and relieved renal IRI by inhibiting cell apoptosis and inflammation. This result is consistent with our present findings. Herzog et al. (20) showed that a high dose of z-VAD-fmk (10 mg/kg) blocked inflammatory caspases and induced lysosomal dysfunction. As the autophagic-lysosomal degradative process plays a protective role in IRI, z-VAD-fmk application may cause renal injury. Hence, the different effects of z-VAD-fmk on inflammatory caspases reported in different studies might be due to the different dosages applied.

Caspase-1 is a key regulatory molecule that leads to the induction of pyroptosis upon stimulus with various factors (34). Understanding the mechanism of caspase-1-mediated cell death may provide valuable insights for preventing tissue damage under pathological conditions. In injured cells, inactivated pro-caspase-1 is recruited in the cytoplasm under the regulation of inflammasomes and is subsequently hydrolyzed to form a tetramer (i.e., activated caspase-1) (41). Caspase-11, which is the upstream regulator of caspase-1, mediates the activity of caspase-1 by direct cleavage of pro-caspase-1 (45). Recent studies (25, 39) have shown that caspase-11 can activate caspase-1 under the coordination of NLRP3 inflammasomes and induce caspase-1-independent pyroptosis. These findings demonstrate that caspase-11 mediates pyroptosis in a caspase-1-dependent or -independent manner; thus, caspase-11 plays a critical role in pyroptosis (1). Being a key transcription factor in ER stress, CHOP also regulates caspase-11 (12). Lipopolysaccharide stimulation completely inhibits caspase-11 expression and activity in lung tissue and macrophages in CHOP knockout mice (12). Moreover, apoptosis of pulmonary epithelial cells and IL-1β generation are reduced and the inflammatory responses in lung tissues are relieved in CHOP-deficient mice. Our in vitro study showed the colocalization of CHOP and caspase-11 in renal tubule epithelial cells after HRI. The altered CHOP expression was consistent with mRNA levels of caspase-11 and with protein levels of pro-caspase-11 and caspase-11. Silence of CHOP significantly suppressed mRNA and protein expression of caspase-11 as well as pyroptosis in renal tubule epithelial cells upon HRI. These observations indicate that the CHOP-caspase-11 signaling pathway plays a critical role in the regulation of pyroptosis of renal tubule epithelial cells after renal IRI.
In conclusion, for the first time, we have shown that pyrop-
tosis might be a key biological event in IRI-induced renal
tubule epithelial cell death. Overactivated ER stress followed
by activation of the CHOP-caspase-11 signaling pathway may
be a novel molecular mechanism involved in IRI-induced
pyroptosis of renal tubule epithelial cells.

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DISCLOSURES
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REFERENCES
1. Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, Zak DE, 
   Tan MH, Cotter PA, Vance RE, Adrem A, Miao EA. Caspase-11
   protects against bacteria that escape the vacuole. Science 339: 975–978, 
   2013.
2. Aachoui Y, Sagulenko V, Miao EA, Stacey KJ. Inflammation-sensi-
   tive pyroptotic and apoptotic cell death, and defense against infection. 
3. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen GS, Thornberry 
   NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. 
5. Bergsbaken T, Cookson BT. Pyroptosis: host cell death and defense 
7. Buttelmann D, Kuch-Oliver C, Wendt D, Krammer HP, Krammer 
   PH. The inflammasomes in kidney disease. Nat Rev Nephrol 6: 408–417, 
   2010.
   Endothelial cell death by pyroptosis: caspase-11 and caspase-11 
   dependent NLRP3 inflammasome activation by gram-negative bacteria. 
9. Lauterbach D, Thiemann DR, Ferrario CM, de Groot GS, Cohn JN 
   et al. Angiotensin II and endothelial cell death: for the first time, 
   pyroptosis is an important component of acute heart failure. J 


