Pyroptosis as a Regulated Form of Necrosis
PI+/Annexin V−/High Caspase 1/Low Caspase 9 Activity in Cells = Pyroptosis?

Jun-ichi Abe, Craig Morrell

Pyroptosis is characterized by pore formation in the plasma membrane, cell swelling, and membrane disruption. These cellular events are also noted in necrosis, but not apoptosis, which is defined as a mode of regulated or programmed necrosis. Jorgensen et al have defined pyroptosis by the following 4 criteria: (1) programmed by an inflammatory caspase activation, (2) pore formation in the plasma membrane, (3) DNA damage with terminal deoxynucleotidyl transferase dUTP nick-end labeling positivity at a lower intensity than apoptosis, and (4) ADP-ribose polymerase activation after the pyroptosis-mediated DNA damage. The inflammasome is a large complex containing NOD (nucleotide oligomerization domain)-like receptors (NLRs), procaspase-1, and apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD), which regulates both pyroptosis and the maturation and secretion of proinflammatory interleukin (IL)-1β and IL-18. A study by Xi et al from Wang’s laboratory in this issue of Circulation Research has now indicated a critical role for the caspase-1–inflammasome in regulating hyperhomocysteinemia-induced pyroptosis and apoptosis. In particular, they show that hyperhomocysteinemia-mediated aortic endothelial dysfunction induced by the depletion of cystathionine β-synthase was rescued by the depletion of caspase-1 and NLRP3. This suggests that the inflammasome plays a crucial role in regulating hyperhomocysteinemia-induced endothelial dysfunction. In this editorial, we will briefly review the regulatory mechanism of caspase-1– and 4/5/11–mediated pyroptosis in modulating endothelial cell pyroptosis/apoptosis and consequent endothelial dysfunction.

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The caspase protease family has central roles in cell death and inflammation. Mammalian caspases can be divided into 3 groups based on their functions. Caspases 3, 6, and 7 are executioner caspases with a prodomain in the NH2-terminus that cleaves substrates essential for cellular homeostasis. Caspases 2, 8, 9, and 10 are initiator caspases with 2 DED (death-effector domain; caspases 8 and 10) or 1 CARD domain (caspases 2 and 9) in its NH2-terminus region and act as proteolytic signal transducers. Both executioner and initiator caspases contribute to apoptotic cell death. Caspases 1, 4, 5, 11, and 12 are so-called inflammatory caspases with the CARD domain in its NH2-terminus region. Caspase-11 is present only in rodents, and its human counterparts are caspases 4 and 5 (caspase-4/5/11). They are directly activated by cytosolic bacterial lipopolysaccharide and play a critical role in endotoxic shock. Caspase-12 is a dominant negative regulator of caspase-1. It has recently been shown that caspases 2, 3, 8, and 10 can regulate not only programmed cell death but also inflammatory and immune reactions to dying cells and microbial stimuli. In contrast, caspase-9 has not yet been directly associated with inflammatory responses.

The critical role of the caspase-1–inflammasome in regulating pyroptosis has been reported (canonical inflammasome pathway; Figure). However, this role was deduced from the use of caspase-1–deficient mice, which have been shown to also carry an inactivating passenger mutation in the caspase-11 gene, raising the question whether there is a role for caspase-11–related effects. In addition, a caspase-1/inflammasome-independent pyroptosis (noncannonical inflammasome pathway) has also been proposed. Both studies by Kayagaki et al and Shi et al from Dixit’s and Shao’s laboratories, respectively, reported the crucial role of gasdermin D (GSDMD) cleaved by caspase-1 and 4/5/11 in eliciting pyroptosis (Figure). An unbiased forward genetic screen with ethyl-N-mutagenized mice from the study by Kayagaki et al and Shi et al from Shao’s laboratory link GSDMD to lipopolysaccharide-mediated pyroptosis. The study by He et al from Han’s laboratory also detected GSDMD protein in nigericin-induced NLRP3 inflammasomes by a quantitative mass spectrometry–based analysis. GSDMD shows cytoplasmic and membranous expression in many cell types and lacks any obvious signal peptide or transmembrane segments.

Caspase-1 can be activated by various stimuli, including ATP, cholesterol crystals, double-strand DNA, and flagellin via several different types of inflammasomes. In contrast, as stated above, caspase-11 in the noncanonical inflammasome has been reported to directly sense cytosolic lipopolysaccharide. The caspase-11 CARD binds lipopolysaccharide, presumably via the acidic phosphate moieties of lipid A, and causes caspase-11 CARD oligomerization and activation. The depletion of GSDMD and caspase-11 inhibited cytosolic lipopolysaccharide-induced pyroptosis and the processing and

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From the Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston (J.i.A.); and Aab Cardiovascular Research Institute, University of Rochester, NY (C.M.).

Correspondence to Jun-ichi Abe, MD, PhD, Department of Cardiology, 2121 W, Holcombe Blvd, Unit 1101, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org

*Correspondence* to Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org

*Correspondence* to Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org

*Correspondence* to Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org

*Correspondence* to Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org

*Correspondence* to Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, Houston, TX, 77030. E-mail jabe@mdanderson.org
secretion of IL-1β. Interestingly, both caspases 1 and 4/5/11 directly cleave the 53-kDa inactive precursor form of GSDMD to generate GSDMD p30 of the propyroptotic NH2-terminus fragment. It was thought that the COOH-terminus of GSDMD (p23) inhibits the propyroptotic function of GSDMD p30, and cleavage at D276↓G277 releases GSDMD p23–mediated inhibition (Figure). Shi et al.1,11 have reported that aa residues 1–243 of GSDMD is the minimal fragment capable of triggering pyroptosis.

In the canonical pathway, caspase-1 activation induced by various types of inflammasomes triggers pyroptosis by releasing the cleaved GSDMD p30 fragment, which bears intrinsic pyroptosis-inducing activity described above.1,11 Because caspase-1–mediated pyroptosis is only delayed and not completely inhibited by the depletion of GSDMD, the existence of another caspase-1–dependent pyroptosis pathway was suggested (Figure).11 Caspase-1 can also cleave caspases-3 and 7 (3/7) independently on GSDMD as described in the current study by Xi et al.5 However, because GSDMD cleavage occurred much earlier than caspase-3/7 cleavage, Shi et al.11 have suggested that caspase-3/7 cleavage plays no role in caspase-1–mediated pyroptosis but may have some role in apoptosis.

In the current study by Xi et al.,5 caspase-3 inhibitor inhibited the R1 fraction (so-called pyroptosis fraction) after 24 hours of L-Hcy or lipopolysaccharide stimulation, suggesting that the R1 fraction in Figure 3B may not be a pure pyroptosis fraction and may include a large number of late apoptotic and necroptotic cells. In addition, the authors showed that a caspase-9 inhibitor significantly inhibited pyroptosis in PI+/annexin V− cells (Figure 3A and 3B) raising the question of how low caspase-9 activity was detected in the pyroptosis fraction of PI+/annexin V− cells. In the canonical pathway, the depletion of GSDMD has no effect on caspase-1–dependent IL-1β processing but shows some effect on IL-1β release.11

In the noncanonical pathway, direct activation of caspase-4/5/11 induced by cytosolic lipopolysaccharide cleaves 53-kDa inactive precursor form of GSDMD and converts it to the mature GSDMD p30 form. This is sufficient for inducing pyroptosis (Figure).1 The difference between the canonical (nonlipopolysaccharide) and noncanonical (cytosolic lipopolysaccharide dependent) pathways is that cleavage of GSDMD is required for caspase-1/IL-1β processing in the noncanonical pathway but not in the canonical pathway. Loss of inflammasome components, such as NLRP3,
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apoptosis-associated speck-like protein containing a CARD, or caspase-1, showed no effect on caspase-11–mediated GSDMD cleavage, suggesting that the cleavage of GSDMD is an upstream event of caspase-4/5/11–mediated inflammasome activation. In contrast, the cleavage of GSDMD is a downstream event in the canonical pathway and GSDMD p30 is not required for initial inflammasome activation (Figure). Of note, it remains unclear how GSDMD p30 can induce pyroptosis. Pore formation is a feature of pyroptosis, and it is suggested that GSDMD p30 may directly induce plasma membrane pore formation, but this needs further investigation.

In addition to GSDMD, the crucial role of pannexin-1 and purinergic receptor P2X7 ligand–gated ion channel (P2X7) in caspase-11–mediated pyroptosis has been reported. Pannexin-1 is a nonselective, large-pore channel, which releases nucleotide, including ATP. Exogenous ATP activates P2X7, leads to pore formation, depletes cytosolic K+, and induces pyroptosis. Yang et al reported that lipopolysaccharide-induced caspase-11 activation cleaves cytosolic domains of pannexin-1 and releases ATP in the extracellular medium, which activates P2X7 and causes pyroptosis. Because there were contradictory data to suggest that the depletion of pannexin-1 and P2X7 showed no effect on caspase-11–mediated events, further investigation is necessary to resolve these discrepancies.

Because inflammasome and pyroptosis regulation are so complex, it is difficult to definitively demonstrate the signaling mechanisms involved. In this current issue, Xi et al have focused on caspase-1 activity in hyperhomocysteinemia–induced pyroptosis. The potential contributions of caspase-4/5/11 were not explored. Lupfer et al have reported that reactive oxygen species increases caspase-11 expression and activation via an increase in JNK activation and subsequently activation of the noncanonical inflammasome (Figure). Xi et al studied pyroptosis after 24 hours of Hcy treatment and showed the important role of reactive oxygen species induction in regulating hyperhomocysteinemia-mediated pyroptosis. Therefore, the involvement of the noncanonical pathway in hyperhomocysteinemia-mediated pyroptosis cannot be excluded yet. Miao et al have described that pyroptotic cell death pathway–activated cells should be annexin V positive because pores open in the cell membrane, permitting annexin V to enter and stain the inner membrane leaflet. Dr Shao’s laboratory has also found that pyroptotic cells are annexin V using anthrax lethal toxin to trigger pure caspase-1–dependent pyroptosis in mouse macrophages (personal communication from Dr Feng Shao, National Institute of Biological Sciences, Beijing, 102206, China). Pyroptosis can occur within 6 hours of stimulation, but Xi et al detected pyroptosis after 24 hours of stimulation. Therefore, it is possible that the late apoptosis and necroptotic cells are also detected in the pyroptosis fraction. This study relied on the fractionation of PI/annexin V cells, but distinguishing between pyroptosis and other forms of necrosis can be difficult by simply using this narrow definition. The development of additional reliable markers is needed to better differentiate caspase-mediated cell death process, such as pyroptosis from apoptosis and necrosis.

Necrosis was once recognized as an accidental or physical cell death induced by physiochemical stress. This view has been greatly changed, and it is now clear that necrosis can take place in a genetically and well-controlled manner defined as regulated necrosis. Vanden Berghe et al have defined regulated necrosis as a genetically controlled cell death process, which leads to cellular leakage. Regulated necrosis is morphologically characterized by plasma membrane rupture, cytoplasmic granulation/vacuolization, and organelle or cellular swelling. These morphological hallmarks are shared by other forms of regulated necrosis, including necroptosis, parthanatos, oxytosis, ferroptosis, ETosis (extracellular trap), NETosis (neutrophil extracellular trap), pyronecrosis, and pyroptosis. All of these processes can not only be characterized by unique and distinct molecular mechanisms but also have numerous overlapping features of cell death. For example, necroptosis has been characterized by the signaling of receptor-interacting protein kinase 1 and mixed lineage kinase domain like. Therefore, to study the pathological role of each mode of regulated necrosis, it is important to define and clarify the unique signaling pathway for each form of regulated necrosis, the interplay between these different signaling pathways at the molecular level, and whether each path really has a distinct role in disease processes.

Xi et al have nicely shown the crucial role of caspase-1 and NLRP3 in hyperhomocysteinemia-induced endothelial dysfunction. In addition, the possible contribution of pyroptosis in regulating hyperhomocysteinemia-induced endothelial damage has been suggested. However, the definition of pyroptosis as PI+/annexin V+/high caspase-1/low caspase-9 activity in cells may be an oversimplification. Determining the role of GSDMD in the process of hyperhomocysteinemia-mediated endothelial dysfunction may also be important in clearly defining and identifying pyroptosis during this and other disease processes. This would involve exploring the distinct and unique molecular mechanisms that control each form of regulated necrosis. It is clear that we do not know the exact contribution of various modes of regulated necrosis in endothelial dysfunction. This study has opened the door to the future study of regulated necrosis in controlling endothelial dysfunction.

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Disclosures

None.

References


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