Dying as a Pathway to Death in Sepsis

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MORTALITY from sepsis remains high, with at least 270,000 deaths annually in the United States and more than 5 million deaths worldwide.[1,2] Despite increasing understanding of the pathophysiology of sepsis, outside of targeted antibiotic therapy and source control (when appropriate), treatment is generally supportive.[3] As such, improved understanding of mechanisms that lead to mortality as well as development of new therapeutics are of paramount importance to treat sepsis and septic shock, which have mortality rates of 20 to 40%. In this light, a study by Song *et al.* in this issue of Anesthesiology examining macrophage pyroptosis in sepsis from intraperitoneal infection of *Escherichia coli*, identifying a new pathway of mortality from sepsis, has potential therapeutic implications.[4]

"…[I]ncreasing macrophage pyroptosis is maladaptive in sepsis, and targeting this process of cell death could potentially have therapeutic promise..."

It has long been known that cell death plays a critical role in mortality from sepsis. However, there are multiple forms of cell death including apoptosis, necroptosis, necrosis, and pyroptosis (fig. 1). An extensive literature exists on the importance of apoptosis—mostly occurring in rapidly dividing cells such as lymphocytes and the gut epithelium—in sepsis.[5,6] However, much less is known about the role of other forms of cell death. Pyroptosis is a lytic form of programmed cell death mediated by inflammatory caspases and characterized by the formation of large pores on the plasma membrane. Pyroptosis is induced *via* the inflammasome, a cytosolic multiprotein oligomer responsible for activation of inflammation. The canonical (or classical) inflammasome induces pyroptosis by activation of caspase-1 while the noncanonical inflammasome involves murine caspase-11 or its human orthologues caspase-4/5.[7] Pyroptosis functions as a method to remove intracellular bacteria and release damage-associated molecular pattern molecules and inflammatory mediators such as interleukin-1β, thereby protecting the host from invading pathogens. However, excessive activation of pyroptosis can lead to widespread cell death, causing extensive inflammation and tissue damage and organ failure, in turn inducing lethal septic shock.[8] While it is known that activated caspases can cleave downstream gasdermin D, which mediates membrane damage,[9] the upstream signals of pyroptosis remain a subject of intense investigation.

Within this context, Song *et al.* examined septic sphingosine-1-phosphate receptor 2 (S1PR2) genetically deficient mice. Sphingosine-1-phosphate is a biologically active lipid, generated from metabolites of membrane sphingolipids, which exerts its effects by binding to five different receptors, of which S1PR2 is the dominant one in macrophages. *S1pr2*-deficient (*S1pr2*$^{-/-}$) mice had decreased peritoneal macrophage pyroptosis and proinflammatory cytokine production, and this was associated with improved survival. Furthermore, caspase-11 activation was decreased in macrophages from *S1pr2*$^{-/-}$ mice and higher in S1PR2-overexpressing macrophages, whereas it was unaffected in nucleotide-binding oligomerization domain-like receptor 3 knockout (*Nlrp3*$^{-/-}$) mice, demonstrating that pyroptosis occurred through the noncanonical pathway. This was mediated by Ras homolog gene family, member A signaling, as a Ras homolog gene family, member A inhibitor prevented the amplified caspase-11 activation in both wild-type and S1PR2-overexpressing cells *in vitro.* To increase the clinical relevance of their results, the authors also examined expression of caspase-4 and S1PR2 in human monocytes taken from patients with Gram-negative sepsis and from critically ill nonseptic controls. Levels of both caspase-4 and S1PR2 were higher in septic subjects, and increased caspase-4 expression positively correlated with S1PR2 levels. This suggests that increasing macrophage pyroptosis is maladaptive in sepsis, and targeting this process of cell death could potentially have therapeutic promise in patients.

While the mechanisms identified are novel, the potential for translation of the findings must be understood within the context of this preclinical study. Due to rapid lysis of bacteria by complement, a model of live bacterial injection is more similar to endotoxemia than actual infection.[10] This

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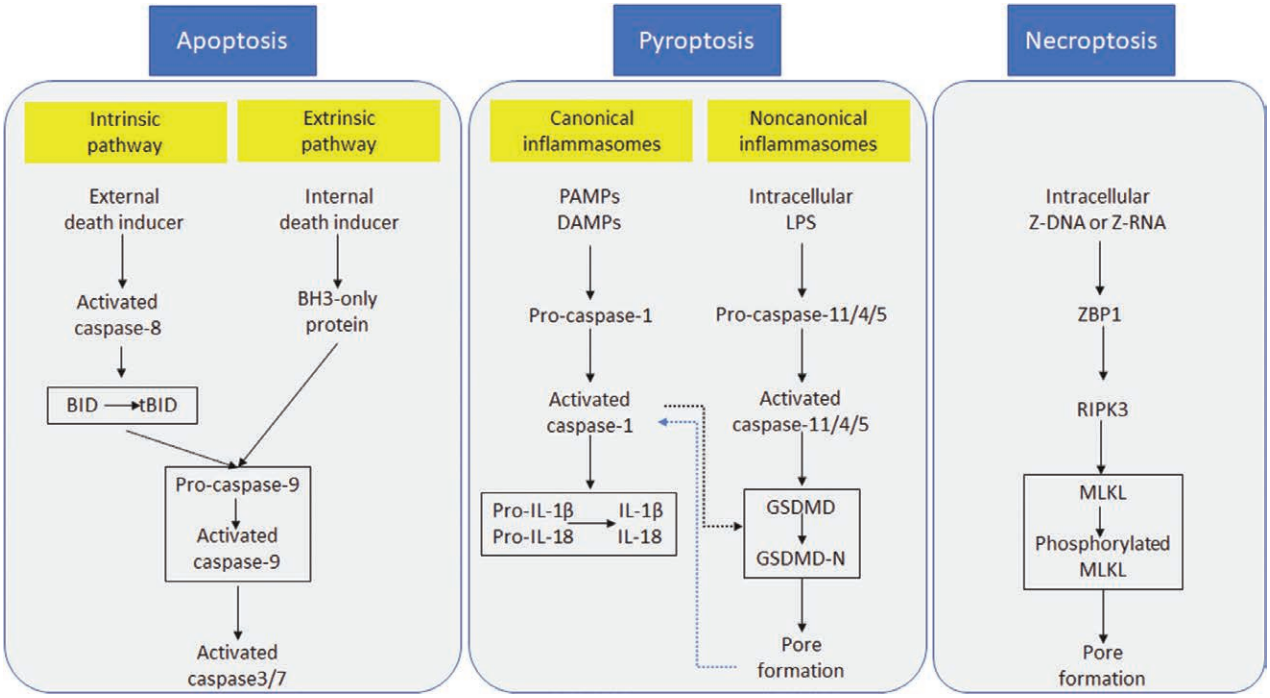

Fig. 1. Pathways of programmed cell death. Apoptosis can be triggered through intrinsic or extrinsic pathways, which can induce caspase-9 activation. As a result, caspase-3 and caspase-7 are cleaved into their active forms and lead to apoptosis. In pyroptosis, canonical inflammasome complexes activate caspase-1, which in turn converts pro–interleukin (IL)-1β and pro–IL-18 into their active forms. Sensing of intracellular lipopolysaccharide (LPS) can activate caspase-11, triggering noncanonical inflammasome. Activated caspase-11 then cleaves gasdermin D (GSDMD) and releases the N-terminal p30 domain, which induces membrane pores. External death inducers can activate receptor-interacting protein kinase 3 (RIPK3), which phosphorylates mixed lineage kinase domain-like (MLKL). Phosphorylated MLKL binds to the plasma membrane and forms the necroptotic pore. Both necroptosis and pyroptosis induce cell membrane rupture, releasing cellular components which may trigger inflammation. BID = BH3-interacting domain death agonist; DAMP = damage associated molecular pattern; PAMP = pathogen associated molecular pattern; tBID = truncated BID; ZBP1 = Z-DNA binding protein 1.

is highlighted by the 80% mortality within 24 h of bacterial injection in this study, a mortality and time course far more severe than human sepsis. Previous data from the same group demonstrated that mortality is improved in *S1pr2*$^{-/-}$ mice after cecal ligation and puncture (a more commonly used preclinical model of sepsis) by inhibiting bacterial phagocytosis, although this was also in the setting of 100% lethality.[11] This is directly relevant because numerous interventions that are effective in highly lethal preclinical models of sepsis lose their efficacy or even become harmful when given in models of sepsis with decreased mortality, more representative of the human syndrome. In addition, while inhibiting S1PR2 led to similar findings as knockout animals, the inhibitor was given either as a pretreatment before the onset of sepsis or immediately after. Given the difficult in identifying "time zero" in septic patients (who almost assuredly have sepsis before the onset of clinical diagnosis), the benefit of S1PR2 inhibition in a patient population that is likely to have a mixed inflammatory picture with immunosuppression is unclear.

It is also unclear what the specific downstream effects of decreasing macrophage pyroptosis are that improve survival. While inflammation is decreased in *S1pr2*$^{-/-}$ mice, it is unclear if that is the mechanism through which these animals have higher survival. This, of course, is a critical question in light of the failure of multiple studies of antiinflammatory agents in human sepsis. Further, as a G protein–associated receptor, S1PR2 has diverse functions in multiple organ systems,[12] and its inhibition would thus be expected to have numerous effects beyond blocking macrophage pyroptosis, and off-target effects on other cell types could lead to unexpected side effects. Additionally, functional differences between human caspase-4 and murine caspase-11 have recently been described,[13] and thus the significance of caspase-11 changes in mice need to be replicated in patients above and beyond demonstration of increased caspase-4 concentrations.

When these and related questions are answered, the potential for targeting S1PR2 or caspase-4 in patients will be better framed. Until then, the elegant mechanistic studies demonstrate a new role for pyroptosis in mortality in a highly lethal model of sepsis. While the majority of host cells are viable after an infection, increased death (apoptosis, necroptosis, pyroptosis) of multiple cell types increasingly appears to be important in the dysregulated host response[14] that causes so many patients with sepsis to die.

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Competing Interests

The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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