Prevention of Lymphocyte Apoptosis—A Potential Treatment of Sepsis?

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Sepsis is the leading cause of death in surgical intensive care units and is a major cause of morbidity and mortality in neonatal and medical intensive care units. The Centers for Disease Control and Prevention estimates that, in the United States alone, ∼500,000 people develop sepsis and 175,000 people die each year. Sepsis is a growing problem; its incidence has tripled from 1972 to 1992. Recently, apoptosis has been identified as an important mechanism of cell death in animal models of sepsis and endotoxemia. During sepsis, there is extensive apoptotic death of lymphocytes and gastrointestinal epithelial cells. The extensive apoptotic death of lymphocytes is likely an important cause of the profound immunosuppression that is a hallmark of patients with sepsis. The apoptosis of gastrointestinal epithelial cells may compromise the integrity of the bowel wall, resulting in translocation of bacteria or endotoxins into the systemic circulation. The potential importance of apoptosis in the pathophysiology of sepsis is illustrated by results from animal models that demonstrate that blocking lymphocyte apoptosis improves survival in sepsis. A variety of strategies to inhibit apoptosis may ultimately provide an effective therapy for this highly lethal disorder.

Although both apoptotic and necrotic cell death occur during sepsis, the predominant form of cell death in patients with sepsis who do not have severe ischemia-reperfusion injury is apoptosis [1]. There is extensive apoptosis of lymphocytes and gastrointestinal epithelial cells during sepsis [1–7]. It is thought that these cell types are particularly prone to undergo apoptosis because they are rapidly dividing cells. A key question with regard to the death of lymphocytes and gastrointestinal epithelial cells during sepsis is whether the net outcome is beneficial or detrimental to the host. There is convincing laboratory evidence from our group that prevention of gastrointestinal epithelial cell death during sepsis improves survival [2]. However, this review focuses on sepsis-induced apoptosis of lymphocytes. One possibility is that death of cells of the adaptive immune system is beneficial to the host by down-regulating the inflammatory response to sepsis.

Alternatively, the extensive loss of immune cells may compromise the ability of the host to eliminate the invading pathogens. Recent studies using a variety of strategies to inhibit apoptosis in lymphocytes suggest that blocking programmed cell death is beneficial to survival in sepsis. Transgenic mice that overexpress the antiapoptotic protein Bcl-2 in lymphocytes have improved survival in sepsis [5, 7, 8]. Administration of compounds that block the Fas death pathway has shown promise in animal models of sepsis [4]. The use of drugs that block the cell proteases involved in performing the death program (e.g., caspase inhibitors) also seems to be effective in sepsis [9, 10]. Thus, if the research findings are validated in more clinically relevant models, new compounds may be available to prevent the morbidity and mortality associated with sepsis [11]. Here, we discuss the potential therapeutic efficacy and problems of antiapoptotic strategies in sepsis.

**THE DEATH PATHWAYS—EXTRINSIC AND INTRINSIC ROADS TO DEATH**

Two major pathways are involved in the initiation of apoptosis (figure 1) [13–15]. The first apoptotic pathway involves transmission of a death signal via receptors...
Figure 1. Major pathways involved in the initiation of apoptosis. The extrinsic, death receptor–mediated apoptotic pathway involves the binding of a death ligand (e.g., TNF or Fas ligand) to its cell surface receptor, resulting in activation of caspase 8 and, subsequently, caspase 3. The intrinsic, mitochondria-mediated apoptotic pathway results in the release of cytochrome C (Cyto C), which binds to apoptotic protease–activating factor 1 (APAF-1) and caspase 9, thus forming the apoptosome. This complex activates caspase 3, resulting in additional caspase activation. The final result is activation of a protease cascade that dismantles the cell. In certain types of cells, there is an interconnection between the extrinsic and intrinsic pathways. Caspase 8 cleaves Bid to form tBid, which subsequently activates the mitochondria-mediated pathway. Figure is modified from Roy and Nicholson [15]. cFLIP, cellular Flice-like inhibitory protein; FADD, Fas-associated death domain; IAPs, inhibitor of apoptosis proteins.

that are located on the cell surface [13–15]. This pathway is termed the extrinsic pathway and is mediated by caspase 8. Caspase 8 can be activated by a number of death ligands that belong to the TNF superfamily: TNF-α, Fas, and Dr3. The second apoptotic pathway (the intrinsic pathway) is activated by mitochondrial injury and is mediated by caspase 9 [13–15]. The mitochondrial caspase 9–mediated pathway can be activated by a diverse number of stimuli, including reactive oxygen species, radiation, and chemotherapeutic agents, that are believed to disrupt the mitochondrial membrane potential. Both caspase 8 and caspase 9 ultimately activate caspase 3, which is involved in the final common pathway of the cell death program. Understanding the particular pathway of sepsis-induced apoptosis is important, because it provides insight into potential factors responsible for initiating cell suicide and may allow for the development of more targeted therapy.

Studies involving animal models of sepsis support the concept that lymphocyte apoptosis in sepsis results from both death receptor–mediated and mitochondria-mediated pathways. Work from the laboratory of Ayala et al. [16] has shown that survival in sepsis was improved by administration of compounds that block the Fas death receptor–mediated pathway. Studies from 3 independent laboratories have shown that lymphocytes from mice that overexpress the antiapoptotic protein Bcl-2 are resistant to sepsis-induced apoptosis and have improved survival [5, 7, 8]. Bcl-2 localizes to the mitochondrial membrane and is thought to inhibit loss of mitochondrial membrane potential. Therefore, the fact that overexpression of Bcl-2 was able to block sepsis-induced lymphocyte apoptosis is highly consistent with a mitochondria-mediated apoptotic pathway in sepsis. Recently, a live baboon model of sepsis due to Escherichia coli infection showed that both receptor- and mitochondria-mediated apoptotic pathways are responsible for cell death in splenocytes [17]. Serial sections of the septic baboon spleen showed specific regions containing apoptotic splenocytes positive for Fas ligand (which activates the death receptor pathway), whereas other apoptotic cells in different locations were positive for active caspase 9 and apoptosis-inducing factor (evidence of mitochondria-mediated pathway). These results suggest that both the mitochondria- and receptor-mediated pathways may be involved in mediating lymphocyte apoptosis in different lymphocyte subsets.

Recently, we examined the role of the extrinsic and intrinsic death pathways in circulating lymphocytes from patients with sepsis by determining the percentage of apoptotic lymphocytes positive for active caspase 8 and active caspase 9, respectively
Figure 2. Functions of caspases. Although first identified as proteases that are essential for mediating apoptotic cell death, caspases are now recognized to have numerous functions, including cell proliferation, cell differentiation, and cytokine activation.

In both healthy volunteers and critically ill patients without sepsis, ~5% of circulating lymphocytes were apoptotic. In contrast, ~10% of lymphocytes from patients with sepsis were apoptotic. Both active caspase 8 and active caspase 9 were detected in apoptotic lymphocytes by 3 independent methods. These results suggest that lymphocyte apoptosis in sepsis may be occurring by both death receptor–mediated injury (the extrinsic pathway) and mitochondrial stress–mediated injury (intrinsic pathway). Other experimental findings also indicated that lymphocyte apoptosis was due to activation of both death pathways in sepsis. A percentage of apoptotic lymphocytes had decreased concentrations of Bcl-2 (suggesting a mitochondrial death pathway), whereas other apoptotic lymphocytes had normal concentrations of Bcl-2 (authors’ unpublished data). Sepsis is a highly complex disorder involving activation of numerous overlapping cascades, including proinflammatory, anti-inflammatory, coagulation, and complement systems [18–21]. It is, therefore, not surprising that multiple pathways of cell death may be involved in sepsis. Furthermore, different bacteria possess different toxins that may activate unique cell death programs.

One possible complicating factor in trying to decipher specific death pathways involves “cross-talk” between the death receptor pathway and the mitochondria-mediated pathway (figure 1) [14, 15]. In certain types of cells, activation of the death receptor pathway results in cleavage of the proapoptotic Bcl-2 family member Bid, which subsequently activates the mitochondria-mediated death pathway [14, 15]. However, this cross-talk between the 2 pathways appears to be cell type specific, and, although it has been described for hepatocytes, it may not be applicable for circulating lymphocytes [22].

Currently, there is an intense debate within the apoptosis community about the preeminence of the mitochondrial versus caspase activation as the key to the cell death process [23–25]. Proponents of the mitochondrial theory hold that numerous cell death stimuli act via the mitochondria to cause opening of mitochondrial membrane channels, with subsequent release of cytochrome C and other proteins (e.g., apoptosis-inducing factor and Smac/Diablo) that then activate caspases and result in cell death. Pro-apoptotic Bcl-2 family members, such as Bax and Bak, assist in channel opening, whereas Bcl-2 itself blocks channel opening [23–25]. Other investigators believe that the mitochondria are only secondary players (serving to amplify the death signal) and that the real actors are the family of cell caspases [22, 23]. Support for their contention is based on the fact that, in such animals as Caenorhabditis elegans, the whole apoptosis machinery involves just 4 main proteins and does not appear to involve mitochondria. Other evidence for the primary role of caspases comes from recent work showing that, in certain cells under stress, the endoplasmic reticulum can activate caspase 12 and trigger apoptosis without involvement of mitochondria [23]. Finally, some studies show that pharmacological compounds that inhibit caspases can prevent apoptotic cell death [9, 10, 26, 27].

**POTENTIAL ANTIAPOPTOTIC THERAPY IN SEPSIS**

A number of pharmaceutical companies are working to develop compounds that inhibit activation of caspases [28]. It has been speculated that these caspase inhibitors could be effective in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis and Parkinson disease [28]. Apoptosis is also an important mechanism of cell death in the liver [29]. Hepatocytes express a great deal of Fas receptor and undergo apoptosis in response to certain types of injury. A preliminary study involving treatment of patients with liver disease with a caspase inhibitor showed beneficial effects [30]. The compound was well tolerated, and the elevated levels of liver enzymes on liver function testing of the patients decreased during treatment with the caspase inhibitor. Possible future therapies may include the use of small interfering RNA to block caspase activation or inhibit expression of Fas [31]. In this regard, work by Wesche-Soldato et al. [32] has shown that administration of small interfering RNA against caspase 8 improves survival in a model of sepsis.

**CASPASE INHIBITORS IN SEPSIS**

Apoptosis is regulated by a family of cysteine-aspartyl proteases (caspases) that are activated in response to proapoptotic stimuli and result in disassembly of the cell through cleavage of a variety of cellular proteins. Caspases are critical effector molecules in cell death, as shown by the inhibition of cell death and improved organ function and/or survival in various models of ischemia-reperfusion injury, burns, endotoxemia, and sepsis after administration of drugs that block caspases [33–36]. Our laboratory reported that treatment with broad-spectrum caspase inhibitors decreased lymphocyte apoptosis, decreased blood bacterial counts, and improved survival in sepsis [37]. The protection was dose related, and, at higher concen-
trations, the survival benefit was lost. In a related study, Fauvel et al. [35] noted that caspase inhibitors prevented cardiac apoptosis and improved contractility in an endotoxin model.

At present, however, there have not been numerous studies showing efficacy of caspase inhibitor therapy in sepsis. A potential rationale for the difficulty in use of caspase inhibitors in sepsis was recently presented by Méthot et al. [11]. These investigators conducted a detailed study of the effect of caspase inhibitors on various markers of apoptosis, such as membrane injury and nuclear fragmentation, during sepsis. They reported that caspase inhibitors were able to block apoptotic manifestations both in vitro and in vivo but with different efficacy for different cell death markers. Prevention of annexin V cell surface labeling of phosphatidylcholine was readily accomplished by caspase inhibitors, but inhibition of DNA fragmentation was difficult to achieve. The investigators concluded that small quantities of uninhibited caspase 3 (that were not blocked by caspase inhibitor therapy) were sufficient to initiate breakdown of genomic DNA. This finding suggests that successful antiapoptotic therapy in sepsis with caspase inhibitors may be challenging, because it would be difficult to get nearly complete inhibition of active caspase 3 [11]. In addition, when examining antiapoptotic therapy, it is not advisable to use prevention of annexin V labeling as a sole means to determine efficacy of caspase inhibitors.

It is also becoming apparent that caspases are multifunctional enzymes that have many effects, in addition to mediating cell death (figure 2). Although it has been known for a long time that caspase 1 (also termed IL-1–converting enzyme) was essential for processing pro–IL-1 into the active cytokine, new studies show that caspases are required for lymphocyte activation and proliferation [38–41]. Mack and Hacker [12] demonstrated that caspases are also activated during primary T cell activation in the absence of apoptosis. Olson et al. [42] showed that caspase activity is required for stimulated B lymphocytes to enter the cell cycle. Caspase 6–like activity was necessary for induction of genes required for entry of the B cell into G1. [42]. Beisner et al. [43] reported that Fas-associated death domain and likely caspases transduce a signal for either survival or apoptosis. Other studies show that inhibition of apoptosis can actually worsen cell death in TNF-mediated shock, thereby indicating that caspase activation is not synonymous with apoptotic cell death [44]. Studies in mice that had knockout of caspase 8 selectively in T cells showed that the cells failed to proliferate in response to antigenic challenge [45]. These mutant mice were unable to mount an immune response to viral infection, indicating that caspase 8 deficiency leads to an immune-deficient state. Similarly, 2 patients with deficiency in caspase 8 were identified [46]. These 2 patients had recurrent viral infections, and their T cells had decreased proliferative responses to pokeweed mitogens. In short, it is a remarkable paradox of life that caspases, which are key components of the cell death machinery, are also required for cell proliferation and differentiation.

Given the findings showing that caspases are so essential for certain cell functions, it is likely that antiapoptotic therapy in sepsis may best be accomplished by targeted selection of specific caspases. In particular, caspase 3 appears to be an optimal target because of its location in the final common pathway of both the extrinsic and intrinsic death programs. Survival studies of caspase 3–knockout mice have shown improved survival, compared with wild-type mice (authors’ unpublished data). Interestingly, lymphocyte apoptosis was not totally prevented in the caspase 3–knockout mice, and this finding may be the result of up-regulation of activity of other executioner caspases.

CONCLUSION

Apoptosis is a major mechanism of cell death in sepsis and occurs in both animal models and patients with sepsis. Sepsis-induced lymphocyte apoptosis is mediated by both the extrinsic death receptor pathway and the intrinsic mitochondria-mediated pathway. The potential importance of apoptosis in the pathophysiology of sepsis is illustrated by results from animal models that demonstrate that blocking lymphocyte apoptosis improves survival in sepsis. A variety of strategies to inhibit apoptosis may ultimately provide an effective therapy for this highly lethal disorder.

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