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Desperate Times Call for Desperate Measures: Self-Cannibalism Is Protective During Sepsis*

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Advances in the care of sepsis, including improved compliance with sepsis bundles conforming to best practice, have resulted in improved sepsis mortality. However, the prevalence of sepsis continues to rise, and sepsis remains a leading cause of in-hospital mortality. Recent trends in sepsis mortality indicate that more patients are surviving the early phase of sepsis during which acute inflammation promotes acute organ failures, only to succumb to secondary infections within a few weeks or months due to postsepsis “immune paralysis” (1). In this regard, immune cell depletion due to programmed cell death (apoptosis) is shown to be the primary mechanism of sepsis-induced immune suppression. T cells are particularly depleted in human blood and tissues following sepsis, and it is shown that inhibition of T-cell apoptosis improves long-term sepsis survival in animal models (2). However, the mechanisms influencing T-cell apoptosis during sepsis are complex and elusive.

Recent evidence indicates that autophagy, a physiological process by which cytoplasmic organelles are recycled, plays a critical role in regulating the functions of T cells during host-pathogen interactions. Autophagy is essential for the normal functioning of long-lived cells, particularly under stressful situations such as sepsis, during which damaged organelles are removed and are eventually replaced to sustain normal cell and organ functions (3). Autophagy is also known to play a key role as a tumor suppressor by regulating inflammation and stimulating antitumor immunity (4). Given the many parallels that exist in immune dysfunction in cancer and sepsis (5), it is probable that autophagy plays a similar role in modulating the immune response in sepsis. ATG5 is an essential proximal component of the autophagy-signaling complex during which a dual-membrane “phagophore” selectively engulfs cytoplasmic organelles to form an “autophagosome,” which subsequently merges with lysosomes producing an “autophagolysosome.” Cytoplasmic components are

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enzymatically degraded or “self-cannibalized” within autophagosomes. The functional implications of autophagy in T cells are complex, wherein ATG5 is shown to be essential for T-cell activation, differentiation, and proliferation and may also mitigate T-cell apoptosis and depletion during severe infections (6, 7).

In this issue of *Critical Care* Medicine, the study by Oami et al (8) is the first to consider the role played by autophagy in the depletion and altered function of T cells during severe experimental sepsis. Strengths of the study design include the use of conditional CD4 T-cell–selective ATG5 knockout mice and green fluorescent protein (GFP)-LC3 transgenic mice, allowing for the effective inhibition of and accurate detection of autophagy, respectively. Genetically unaltered B cells served as internal controls in the conditional T-cell ATG5 knockout mice, and septic wild-type mice with intact autophagy pathways (normal ATG5 expression) served as controls for these experiments. Severe sepsis was induced by cecal-ligation and puncture (CLP), which resulted in 100% mortality within 5 days in the wild-type mice. The CLP protocol provided fluid resuscitation, but unlike the human condition, no antibiotics or sedatives were administered. In CD4-Cre/ATG5 knockout mice, the allele conforming to the deleted ATG5 gene was shown to be selectively deleted in CD4 and CD8 T-cell catheters, but not in B cells.

The experimental results were intriguing in that autophagy was shown to be strongly induced in T cells during sepsis. The rate of autophagosome formation, reflected by mean fluorescence index in GFP-LC3 transgenic mice, increased significantly over time in the CD4 T cells of septic mice compared with sham-operated controls. In contrast, autophagy increased transiently in B cells and then returned to baseline within 24 hours. Despite the increased rate of autophagy in septic T cells, the expression of p62, which identifies aggregated proteins and dysfunctional organelles targeted for autophagy, was markedly increased. This finding indicates that the rate of autophagy was unable to keep up with the pace at which damaged proteins and organelles were forming. This is an important finding because p62 accumulation promotes caspase-induced apoptosis (9), and this mechanism could contribute to T-cell depletion during sepsis. Although not reported in this study, it is likely that suppression of the rate of autophagy in ATG5 knockout mice would lead to even greater levels of p62 accumulation in T cells during sepsis.

As hypothesized, suppression of autophagy in T-cell selective ATG5 knockouts resulted in accelerated rates of apoptosis and greater depletion of T cells in the spleen during sepsis. The connection between autophagy and apoptosis is unclear in this setting but could relate to the accumulation of p62 (i.e., insufficient autophagy), as discussed previously, or the accumulation of damaged mitochondria. Damaged mitochondria are capable of promoting cell apoptosis through induction of the intrinsic apoptosis pathway, wherein depolarization (dissipation of the electrochemical gradient) of the mitochondria and/or the actions of proapoptotic Bcl-2 family proteins (e.g., Bcl-2-like protein 11 [BIM], Bcl-2-like protein 4 [BAX]) leads to the release of cytochrome c from the mitochondria to promote the formation of a protein complex including adaptor protein apoptotic protease factor 1 (Apaf 1) and the protease caspase-9, referred to as the “apoptosome.” The apoptosome triggers a proteolytic cascade that leads to the enzymatic self-destruction of the cell (10). The observed increase in BIM, which promotes the proapoptotic actions of BAX (10), and the accumulation of

deenergized mitochondria in the T cells of septic ATG5 knockout mice supports the likely role of the intrinsic apoptotic pathway in T-cell apoptosis in this sepsis model.

Results of experiments conducted on T cells in vitro provide insight into the immunological implications of autophagy. T cells derived from sham-operated and CLP-treated ATG5 knockout mice exhibited increased interferon γ production, and T cells from CLP-treated ATG5 knockouts also had significantly higher interleukin (IL)-10 production. IL-10 is a potent suppressor of adaptive immune responses and is capable of promoting T-cell apoptosis (11). Although T-cell proliferation was not considered in this study, previous studies have shown that ATG5 promotes T-cell proliferation (12). It follows that ATG5 depletion could further influence T-cell populations through this mechanism. Other mechanisms of T-cell dysfunction have been reported during sepsis, including impaired proliferation and effector function due to histone methylation events (13) and increased expression of “gene related to anergy in lymphocytes” (14) or changes in the expression of proapoptotic receptors (e.g., programmed cell death receptor-1) (15). Thus, there are likely multiple mechanisms contributing to CD4 T-cell depletion and dysfunction during sepsis, and it remains unclear to what extent these mechanisms are individually influenced by autophagy.

In keeping with previous reports, there appeared to be a correlation between T-cell apoptosis and sepsis survival. Short-term survival (at 72 hr) was significantly reduced in the septic CD4 ATG5 T cell knockouts compared with sepsis controls; however, sustained survival was not observed in either group. The limitation of this study being that the sepsis model used for this study (100%, 5-d mortality) does not match the condition of sepsis or septic shock in humans (10%–50%; 28-d mortality) (16), wherein most human deaths occur in a later phase of sepsis (17). The putative mechanisms linking T-cell dysfunction to delayed sepsis mortality in humans relates to increased susceptibility to secondary infections (18). Nonetheless, the short-term survival advantage demonstrated in this extreme sepsis model in animals with intact T-cell autophagy suggests that autophagy is clinically relevant and should be further investigated.

In summary, these exciting findings from Oami et al (8) add to the increasing evidence of the essential role that autophagy plays in regulating inflammation and immunity. The impact of autophagy on immune regulation was already known in cancer and, with the present work, has now been established in sepsis. Their work is the first to demonstrate the linkage between T-cell autophagy and apoptosis in sepsis, that is, inhibiting autophagy accelerates T-cell apoptotic death. Inhibiting T-cell autophagy also increased the potent immunosuppressive cytokine IL-10, thereby underscoring the role of autophagy in modulating sepsis-induced inflammation. Collectively, these findings lead us to speculate that manipulation of autophagy could be a new therapeutic approach in sepsis. Drugs that regulate autophagy are in clinical trials in cancer and neurodegenerative diseases and could play a therapeutic role in sepsis as well.

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