Lymphocyte Apoptosis in Sepsis

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Introduction

Sepsis and multiple organ failure are reported to be responsible for upwards of 60% of the deaths which occur in the surgical intensive care unit [1,2]. However, the precise etiology contributing to the development of this state remains unclear. A number of studies have suggested that the link between cell and organ dysfunction associated with multiple organ failure lies in the initial presentation of sepsis (gram-negative, gram-positive, and/or fungal in nature) [2–7]. Clinically, sepsis commonly manifests itself as a two phase process in which the patient initially demonstrates a hyperdynamic-hypermetabolic phase (increased cardiac output, fever, enhanced metabolic rate) and subsequently a hypodynamic-hypometabolic state [8,9]. The latter stage of this process is typically referred to as “septic shock” in which the circulatory system is compromised, organ function becomes more depressed, and the animal or patient eventually dies.

With respect to the host’s defense against microbial challenge, studies from a number of laboratories indicate that the immune system appears to exhibit a bi-phasic response, comparable to the metabolic and circulatory responses seen in sepsis [4,8,10]. Initially there is evidence of an exaggerated systemic inflammatory response thought to be due to immune cell (monocyte/macrophage) stimulation by microbes and/or their products released following the onset of sepsis [11–17]. A number of investigators have suggested that mediators released during this early phase, in response to trauma, shock and/or sepsis, may indeed be the nidus for the hyperdynamic-hypermetabolic response seen [18–20]. Over time, however, this stage gives way to what appears to be a state of generalized immune cell hyporesponsiveness [21–28]. This latter state may contribute directly and/or indirectly to the inability of the animal to ward off the septic challenge. However, our understanding of the mechanisms contributing to this latter state of immune cell hyporesponsiveness, as well as its potential significance in aiding the septic patient in warding off the infection, remain poorly understood. This is in part because of the heterogenous nature of the patient population that has been studied. Components such as nutritional state, age, sex, type of prior injury, nidus of infection, time since onset of sepsis, etc., cannot be controlled in studies carried out in patients. Experimental animals, therefore, provide an important means of investigating the effects of trauma, shock and sepsis in a controlled setting. In this respect, the mouse provides one of the best experimental models in which to assess the effect of polymicrobial sepsis on immune responsiveness. The use of inbred strains and controlled experimental conditions provide an appropriate model in which to examine the mechanism of the pathobiology of sepsis. In this article, we will review our findings and those of other laboratories with respect to lymphocyte apoptosis/programmed cell death and its possible involvement in the induction of lymphocyte dysfunction during polymicrobial sepsis in the mouse.

Polymicrobial Sepsis and Lymphocyte Immune Dysfunction

With respect to the early stage of sepsis, this is characterized by the detection in circulation of pro-inflammatory cytokines, such as TNF, IL-1, IL-6, and chemokines, typically of macrophage derivation [11–17]. In this regard, elevated circulating levels of both TNF and IL-6 have been detected in both the mouse and rat model of polymicrobial sepsis, i.e., cecal ligation and puncture (CLP) (Figure 1) [21,29,30]. Hadjiminas et al. [31], using a chronic model of CLP, further documented that enhanced TNF-α release observed following sepsis was not only a reflection of increasing secretion but also elevated transcriptional activity of the gene early following CLP. Interestingly, we have also found that both endotoxin-tolerant C3H/HeJ mice and endotoxin-sensitive C3H/HeN mice exhibit a similar systemic pro-inflammatory cytokine response (increased TNF and IL-6), as well as show comparable rates of mortality after CLP [22]. These findings not only demonstrate the ability of this murine polymicrobial sepsis model to

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mimic/produce this early systemic inflammatory response, which is associated with the early hyperdynamic-hypermetabolic response seen in the septic patient, but also suggest that sensitivity and/or responsiveness to endotoxin does not markedly alter this response.

Alternatively, there is little evidence of lymphocyte-mediated systemic lymphokine production/release or of up regulation of the expression of genes for these products early following the onset of sepsis [22,25,27]. Doherty et al. [32] have, however, observed that if the T-lymphocyte product IFN-\(\gamma\) is administered immediately after mice have been subjected to endotoxemia, it significantly augments the severity/mortality observed in that model. These investigators, nonetheless, did not report elevated levels of this macrophage activator in circulation following endotoxemia. In this regard, we [22], as well as others [25], have not found evidence of early augmented T\(_{h1}\)-lymphocyte IL-2/IFN-\(\gamma\) release capacity. Similarly, early following the onset of experimental sepsis, there is little evidence of a depression in nonspecific T-cell mitogenic responses [22,25].

With the progression of sepsis, i.e., >12 h after CLP, there is a marked decline in the capacity of macrophages and lymphocytes to respond to stimulation. These cells therefore appear to become dysfunctional or immune compromised with the progression of sepsis. This has also been referred to by some investigators as a form of “anergy” [33]. Recent findings [25,28,34] indicate that, at least with respect to splenic lymphocytes, the decrease in immune responsiveness appears to be related to a concomitant decrease in cellular ATP stores and an increase in cellular calcium levels. Meldrum et al. [28] observed that the depression in splenocyte immune response appears to correlate with a marked decrease in intracellular ATP levels, as detected by \(^{31}\text{P}\)-NMR [28]. Analysis of total splenocyte calcium levels at 24 h after the onset of sepsis also indicated that there was a marked increase in this cation’s concentration [34,35]. In this respect, recent studies by Choudhry et al. [25] indicated not only that splenocyte functions, such as IL-2 release and mitogen responsiveness, were depressed late following gram-negative septic insult in the rat but that both of these events were associated with an increase in the basal levels of intracellular calcium as well as changes in calcium mediated signal transduction events. With respect to splenocyte IL-2 and IFN-\(\gamma\) release capacity, we have also reported that by 24 h post-CLP there is a marked reduction in the release of these lymphokines [27]. However, as much of this work has been restricted to splenic or circulating lymphocytes, it remains to be determined whether a comparable induction of lymphocytic immune depression is evident in other lymphocytic populations, such as the thymus, bone marrow, lymph nodes, etc.

Before discussing the data associating apoptosis with lymphocyte alterations in immune function, it is important to first describe what are some of the potential mediators which have been identified up to this point as possible contributors to lymphocyte immune depression following the onset of sepsis (Figure 2).