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T-cell mediated immunity and the role of TRAIL in sepsis-induced immunosuppression

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Abstract

Sepsis is the leading cause of death in most intensive care units, and the death of septic patients usually does not result from the initial septic event but rather from subsequent nosocomial infections. Patients who survive severe sepsis often display severely compromised immune function. Not only is there significant apoptosis of lymphoid and myeloid cells that depletes critical components of the immune system during sepsis, there is also decreased function of the remaining immune cells. Studies in animals and humans suggest the immune defects that occur during sepsis may be critical to the pathogenesis and subsequent mortality. This review is focused on sepsis-induced alterations with the CD8 T-cell compartment that can affect the control of secondary heterologous infections. Understanding how a septic event directly influences CD8 T-cell populations through apoptotic death and homeostatic proliferation and indirectly by immune-mediated suppression will provide valuable starting points for developing new treatment options.

Keywords

Sepsis; apoptosis; CD8 T-cells; TRAIL; homeostatic proliferation; tolerance

I. INTRODUCTION

Sepsis, defined as a “generalized inflammatory state caused by an infectious agent”,¹ is a term covering a spectrum of clinical states. The severity of a septic event ranges from patients with a defined set of clinical symptoms, such as the suspicion of infection or confirmed infection (clinical sepsis), to multiple organ failure (severe sepsis) and even circulatory collapse (septic shock).² Septic patients have been described throughout recorded medical history – dating back to pathology accounts by Hippocrates (depicting it as *sepidon*, “the decay of [tissue] webs”)³ and the works of Ignaz Semmelweis proposing puerperal sepsis to be the result of “cadaveric animal matter” entering systemic circulation.⁴

Collectively, the clinical states of sepsis cause ~200,000 deaths per year in the United States, making it the 10th leading cause of death. Additionally, retrospective analysis demonstrates that the incidence of sepsis cases per 100,000 people has increased from 82.7 in 1979 to 240.4 in 2000.⁵ Several factors have been proposed to contribute to the increase in sepsis incidence, such as increased diagnostic and therapeutic procedures, frequent use of immunosuppressive drugs, and the growing number of multiresistant bacterial infections – especially within the nosocomial environment.⁶ Consequently, mortality due to a septic event remains high, with conservative mortality estimates ranging between 25–35%, despite enormous efforts to standardize and improve the supportive care measures.⁷ The elderly are a high-risk group with an incidence rate of nearly 60% of all septic cases.⁸ This patient population is particularly vulnerable to the consequences of sepsis, showing a 100-fold increase in mortality above the general population,⁹ and the survival of such events is not without sequelae.¹⁰ The social and economic burden associated with sepsis is also concerning, with ~\$22,000 spent per patient,^{11, 12} and the long-term health-related quality of life of survivors of sepsis is significantly lower than that of the general population.¹³

With the advent of molecular immunology, a new scientific view of the pathophysiology of sepsis has emerged arguing that an aggressive invasion of pathogens into tissues triggers massive immune retaliation to regain homeostasis.¹⁴ The systemic inflammatory response syndrome (SIRS) is the spillover of elevated inflammatory mediators [such as tumor necrosis factor (TNF), platelet activating factor (PAF), interleukin(IL)-1, or IL-6]^{15–17} into the circulation that are released during the course of an infection. Locally, these mediators normally promote leukocyte recruitment, cell death, and coagulation events – an uninviting environment for the offending pathogen – that limit systemic spread of infection. However, when amplified systemically the same inflammatory mediators that promote vascular leakage and neutrophil infiltration can cause circulatory dysfunction.¹⁸ Similarly, molecular mediators that induce local thrombosis cause disseminated intravascular coagulation.¹⁹ What emerges is a situation where pro-inflammatory mediators work locally for the host, but systemically against it.

Perhaps a more important contribution to new ideas into the pathophysiology of sepsis was the re-examination of the effects of sepsis on the immune system. Bone noted several observations that supported the idea of re-evaluating the SIRS model.²⁰ First, in addition to proinflammatory cytokines seen in SIRS, anti-inflammatory molecules such as IL-4, IL-10, transforming growth factor (TGF)- β , and colony stimulating factors are present in sepsis.²¹ Second, a massive amount of apoptotic death occurs in a variety of immune cell types during sepsis. Finally, studies from the 1970s and 1980s conducted in critically ill patients (due to sepsis or traumatic injury) showed impairment in delayed-type hypersensitivity (DTH) skin reactions.^{22–25} Collectively, these three underappreciated pieces of the sepsis puzzle led to the notion that a large population of patients surviving the early events would enter into an immunological unresponsive state characterized by decreased T-cell function, as well as defects in antigen presentation that was termed a compensatory anti-inflammatory response syndrome (CARS). It was then proposed that with adequate supportive care most patients would survive the “cytokine storm” during the SIRS phase, but there would remain the high risk for mortality long after survival of the septic episode. The plausibility that other factors present during the CARS phase could be accounting for this mortality became more evident, since septic patients are severely immunocompromised and highly susceptible to secondary nosocomial infections that are easily eliminated by T-cells when a normally functioning immune system is in place. Of particular interest, data from a number of studies in animals and humans suggest the apoptotic cell death of lymphoid and nonlymphoid tissue,^{26, 27} the subsequent restoration of the components of the immune system, and the suppression of lymphocyte responses seen after the acute phase events²⁸ play significant roles in the increased mortality after sepsis.²⁹ With this information in mind, this review will

focus on the sepsis-induced alterations within the T-cell compartment that result in its reduced ability to respond to secondary infection after sepsis and possible mechanisms by which the T-cells that remain after a septic event are suppressed from responding to pathogenic challenge.

II. SEPSIS-INDUCED CHANGES IN IMMUNE SYSTEM CONTRIBUTE TO OVERALL MORTALITY

The diagnostic capacity of inflammatory acute events in sepsis, along with the high mortality rates that the diagnosis carries, resulted in labeling sepsis as a deadly disease with an acute etiology. Thus, it is not surprising that the early events seen in clinical sepsis have dictated the focus of basic and translational research for the last 30 years.³⁰ Unfortunately, the majority of clinical trials aiming to block the activity of inflammatory cytokines, such as TNF,^{31–33} PAF,³⁴ or IL-1,^{35, 36} failed to substantially improve overall mortality. One exception to this was Drotrecogin-alfa,³⁷ a recombinant form of human activated protein C that completed successful nationwide (U.S.) phase III clinical trials (PROWESS)³⁸ and was available to patients for several years. The PROWESS trials and the FDA licensing of this drug were, however, controversial.³⁹ The compound was withdrawn from clinical usage in 2011 due to failure to show improvement in a multinational extension (PROWESS-SHOCK) of earlier results.^{40, 41} A recent trial focused on the inhibition of toll-like receptor (TLR) 4 signaling yielded similarly disappointing results.⁴²

The results from the Surviving Sepsis campaign have clearly shown that standardization of care in sepsis has significantly reduced the number of early deaths.⁴³ However, overall mortality has remained the same or modestly decreased. The failure of more than 25 clinical trials, along with several observations regarding alterations in the immune system, resulted in the development a variety of novel immunologically-based therapeutic concepts for sepsis. The study of long-term mortality in septic patient populations contributed to steering investigators toward some of these new immunological ideas. The long-term immunological effects were a part of the sepsis pathophysiology that had been poorly studied, primarily due to sepsis being defined as an acute disease.^{44, 45} In agreement with the reduction in acute deaths, but not the overall mortality of sepsis, several studies tracking septic patients showed the number of deaths in sepsis that occur after the acute onset of disease is indeed a significant cause of mortality.^{46, 47} Yet, longer-term follow-up studies have shown a similar trend in mortality after a septic event has subsided. Quartin and colleagues used a retrospective model to examine a cohort of hospitalized patients with sepsis from a randomized trial and concluded that when compared to other causes of hospitalization, sepsis increased the risk for death for a period of 5 years.⁴⁸ Moreover, Perl and colleagues tracked long-term survival in a cohort of sepsis patients and noted the sepsis survivors had a significantly higher risk of non-septic causes of death after the initial hospitalization.⁴⁹ Perl's group also noted an interesting correlation between the severity of the initial, acute inflammatory response during sepsis and the ability of the host to deal with a longer-term, chronic challenge.

Building on these observations, a significant amount of data has since intricately linked the immune cell apoptosis that occurs during the acute phase of sepsis and the subsequent prolonged immune suppression that can render septic individuals highly susceptible to secondary infection to pathogens that a normal immune system readily eliminates. Focusing first on septic patients, Hotchkiss and colleagues showed that post-mortem tissue samples had some degree of apoptosis in 56% of spleens, 47% of colons, and 28% of ileums sampled.⁵⁰ Furthermore, tissue immunohistochemistry revealed increased active caspase-3 expression in septic vs. nonseptic patients ($p < 0.01$), with 25–50% of cells staining positive for active caspase-3 in the splenic white pulp of six septic but in no nonseptic patients,

providing evidence that lymphocyte apoptosis was significantly increased in septic patients. When examining immunophenotypic and apoptosis markers in spleens from 27 patients with sepsis and 25 patients with trauma, they showed a profound loss in B cells with increased duration of sepsis with ~25% decrease in B cell area at earlier time points (<7 days post-onset) in septic patients and a 52% decrease at later time points (>7 days post-onset) compared to trauma patients. Additionally, they observed a decrease in CD4 T-cells that was specific and profound for sepsis.⁵¹ A number of other studies have added credibility to the theory that lymphocyte apoptosis during the early stages of a septic event plays a role in the immune suppressive characteristics of the late events in sepsis. Lymphocyte apoptosis has been observed in the circulating blood of septic patients in intensive care units.^{28, 52, 53} Le Tulzo *et al.* reported an increase in apoptosis in circulating lymphocytes from septic patients compared to healthy volunteers. Lymphocyte apoptosis leads to persistent lymphopenia that is associated with poor prognosis for septic patients.⁵² Hotchkiss *et al.* also demonstrated an increase in lymphocyte apoptosis in septic patients compared to non-septic patients.⁵³ Interestingly, the onset of sepsis correlated with an increase in lymphocyte apoptosis and resolved when lymphocyte apoptosis decreased. These results demonstrate that the degree of lymphocyte apoptosis correlated with septic severity and subsequent patient outcome. Despite the well-characterized immune cell apoptosis during sepsis, the impact of sepsis on protective T-cell responses (especially CD8 T-cells) against secondary pathogen challenge remains poorly understood.

III. SEPSIS-INDUCED ALTERATIONS OF CD8 T-CELL RESPONSES

The use of clinically relevant mouse models of sepsis, especially the cecal-ligation and puncture (CLP) model,⁵⁴ has provided valuable insight into the relationship between lymphocyte apoptosis during sepsis and the sepsis-induced immune suppression. Sepsis results in the apoptotic death of multiple lymphoid and myeloid immune cell populations in a variety of locations in the body, including thymus, spleen, gut, and peripheral blood.^{26, 50, 51} As seen in a number of other reports,^{53, 55–57} we observe a significant reduction in the number of CD8 T-cells throughout the body of CLP-mice compared to sham controls early after sepsis induction (2 days post-CLP surgery).⁵⁸ Based on this observation, the prevailing question posed was, “What are the consequences of the rapid reduction in CD8 T-cell numbers on subsequent CD8 T-cell responses for the host?” CD8 T-cells play an essential role in the control and elimination of invading intracellular pathogens,⁵⁹ and alterations in the CD8 T-cell compartment can seriously compromise T-cell mediated immunity. Here we will discuss the factors that influence CD8 T-cell responses to infection and how sepsis may impact them.

A. CD8 T-cell repertoire diversity and generation of a primary response

Pre-immune hosts cannot predict which pathogen-derived antigen will be encountered, thus the immune system relies on the generation of a diverse CD8 T-cell T-cell receptor (TCR) repertoire. The naïve CD8 T-cell repertoire is composed of relatively small numbers of single antigen-specific naïve CD8 T-cell precursors that are able to respond to virtually any pathogen-derived antigen (epitope). Diversity arises from re-arrangement of TCR- $\alpha\beta$ gene segments composed of 2 polypeptide chains with variable and constant domains. The composition of the naïve CD8 T-cell repertoire is important in shaping the overall immune response to any given antigen.

Primary CD8 T-cell responses to infection can be divided into four distinct phases: activation, expansion, contraction and memory generation.⁶⁰ Activation (phase I) is dependent on interactions between antigen-specific naïve CD8 T-cells bearing the appropriate TCR and an APC (i.e., dendritic cell (DC)) presenting cognate antigen on MHC I (signal 1).⁶¹ Complete activation requires co-stimulation (signal 2) provided by CD80/86-

CD28 interactions between a mature DC and antigen-specific CD8 T-cell, respectively. Finally, the cytokine milieu (signal 3) at the time of activation also provides signals that allow optimal accumulation of the responding CD8 T-cell.^{60, 62–66}

To respond to vast diversity of pathogens, antigen-specific naïve CD8 T-cells that recognize specific pathogen-derived peptides are infrequent in the total CD8 T-cell repertoire (ranging from 10–1,000 cells in an inbred laboratory mouse).^{67–72} As such, once activated these rare antigen-specific naïve CD8 T-cells must undergo massive clonal expansion (phase II) (proliferating more than 10,000-fold) and differentiate into effector cells, enabling them to defend against the invading pathogen.^{73–75} CD8 T-cells acquire effector functions, such as cytotoxicity (expressing cytotoxic perforin and Granzyme B molecules) and cytokine production [interferon (IFN)- γ and TNF]⁷⁵, that enable CD8 T-cells to provide sterilizing immunity to infection.

Following the peak of expansion, CD8 T-cells undergo a contraction phase (phase III), which results in the death of 90–95% of responding effector CD8 T-cells.⁷⁶ Subsequently, the antigen-experienced effector CD8 T-cells that survive the contraction phase (5–10% of the effector CD8 T-cells) constitute the primary antigen-specific memory CD8 T-cell pool (phase IV; Figure 1).⁷⁶ Memory CD8 T-cells provide life-long protective immunity and mount rapid recall responses following pathogen re-encounter. Both antigen-specific naïve and memory CD8 T-cells play an important role in T-cell mediated immunity. Several factors can influence the initial CD8 T-cell response to infection and subsequent memory generation, thus compromising the capacity of the host to mount an effective immune response to infection.

Studies examining sepsis-induced lymphocyte apoptosis typically grossly separate lymphocytes into CD4 and CD8 T-cells. Although informative on a population level, the impact of sepsis-induced lymphocyte apoptosis on the generation of a primary antigen-specific CD8 T-cell response to a bacterial infection is currently poorly understood. Utilizing the CLP mouse model of sepsis work by Gurung *et al.* demonstrated impaired antigen-specific CD8 T-cell responses to experimental *Listeria monocytogenes* infection early after sepsis induction.⁵⁵ CLP and sham-treated mice were infected with an attenuated recombinant strain of *L. monocytogenes* expressing ovalbumin (LM-OVA) on day 2 following the induction of sepsis and antigen-specific CD8 T-cell responses were examined 7 days post-infection. OVA-specific CD8 T-cell responses (specifically, the number of IFN- γ -producing OVA-specific CD8 T-cells in the spleens after *ex vivo* OVA_{257–264} re-stimulation) were significantly reduced in CLP-treated mice compared to sham controls. Perhaps more importantly, CLP-treated mice had a reduced ability to control the infection compared to sham controls. We have detected a similar impairment in virus-specific CD8 T-cell responses in CLP-treated mice compared to sham controls.⁵⁸ These results together suggest that sepsis-induced apoptosis can impact antigen-specific CD8 T-cell responses to secondary infections early after the start of a septic event. These data also raise the question to what extent sepsis impacts long-term CD8 T-cell responses by altering the abundance of antigen-specific naïve CD8 T-cell precursors and CD8 T-cell repertoire diversity.

B. Alterations in T-cell repertoire impact subsequent immune responses

Advances in flow cytometric techniques and reagents in recent years has made it possible to quantitate the small number of naïve antigen-specific CD8 T-cell precursors mice using peptide:MHC I tetramers.^{70, 71, 77} Obar *et al.* determined the number of antigen-specific naïve CD8 T-cell precursors ranged from 80–1,200 cells per mouse [average precursors enumerated: OVA_{257–264} 130 cells; LCMV glycoprotein (GP)_{33–41} 287 cells; LCMV nucleoprotein (NP)_{396–404} 151 cells; MCMV M45_{985–993} 603 cells; influenza A virus (IAV) polymerase acidic protein (PA)_{224–233} 120 cells, and vesicular stomatitis virus (VSV)

nucleocapsid (N)₅₂₋₅₉ 166 cells].⁷⁰ Kotturi *et al.* also showed that the number of LCMV-specific naïve CD8 T-cell precursors ranged from 15 polymerase L segment (L)₃₃₈₋₃₄₆-specific to 449 GP₃₃₋₄₁-specific CD8 T-cells in C57BL/6 mice.⁷⁷ Given the low number of naïve precursors, the impact of sepsis-induced apoptosis on antigen-specific CD8 T-cell precursors likely occurs stochastically. Consequently, the composition of the CD8 T-cell repertoire diversity may be altered after sepsis creating ‘holes’ in the repertoire that ultimately impact CD8 T-cell responses to infection. Utilizing the peptide:MHC I tetramer-based enrichment technique, we observed a significant reduction in the number of antigen-specific naïve CD8 T-cell precursors to LCMV early after sepsis induction (2 days post-CLP surgery) compared to sham controls. Moreover, this reduction was followed by an incomplete and variable recovery of precursors at late time points after sepsis induction (month post-CLP surgery). Furthermore, we observed a sepsis-associated loss in the CD8 T cell response to some LCMV epitopes suggesting that ‘holes’ in the CD8 T-cell repertoire can be generated via sepsis-induced apoptosis⁵⁸ (Figure 2). These data suggest that one of the factors that may contribute to long-term immune suppression in individuals that have survived sepsis is a numerical change in the composition of the CD8 T-cell compartment that renders them unable to elicit an effective immune response upon encountering a new pathogen later in life.

The concept that changes in the breadth and depth of the T-cell repertoire affect the ability to deal with infection is not new, as there are several examples within the aging literature that demonstrate a narrowing of the CD8 T-cell repertoire with age is directly associated with impaired immune responses.⁷⁸ A study by Yager *et al.* showed an age-associated loss of specific CD8 T-cell responses to IAV infection in mice resulting from ‘holes’ in the IAV-specific CD8 T-cell repertoire, and in some cases IAV NP₃₆₆₋₃₇₄-specific CD8 T-cell responses were completely abrogated.⁷⁹ Furthermore, reduced NP-reactivity correlated with an impaired ability to control heterosubtypic IAV secondary challenge. Age-associated changes in the T-cell repertoire are not limited to experimental animals, as work by Lee *et al.* showed CD8 T-cells obtained from geriatric donors (age 65 over) lacked the broad CD8 T-cell IAV-specific memory response observed in younger donors (aged 21–42), resulting in a narrowed CD8 T-cell response to IAV.⁸⁰ Young donors were able to respond to several IAV epitopes [including matrix protein 1 (M1)₅₈₋₆₆, polymerase basic protein 1 (PB1)₄₁₃₋₄₂₁, non-structural protein 1 (NS1)₁₂₃₋₁₃₂, neuraminidase (NA)₂₃₁₋₂₃₉, NA₇₅₋₈₄, PA₄₆₋₅₄ and PA₂₂₅₋₂₃₃], whereas geriatric donors could only respond to M1₅₈₋₆₆. Thus, the observed sepsis-associated impairment in CD8 T-cell immune responses may be akin to the age-associated decline in CD8 T-cell responses where alterations in antigen-specific CD8 T-cell repertoire diversity (repertoire ‘holes’) can profoundly impact CD8 T-cell responses to infection.

In contrast to the preferential loss of particular CD8 T-cell populations, CD8 T-cell clones bearing a single TCR can clonally expand with age.⁸¹ CD8 T-cell clonal expansions (TCE) have been correlated with reduced CD8 T-cell repertoire diversity resulting in impaired responses to infection.⁷⁸ Greater than 90% of CD8 T-cells in C57BL/6 mice responding to herpes simplex virus (HSV-1) infection elicit a response directed against the glycoprotein B (gB)₄₉₈₋₅₀₅ epitope and predominantly use Vβ10 (~ 50–70%) and Vβ8 (~ 20–25%) TCRs.⁸² The presence of TCE in aged mice result in reduced CD8 T-cell diversity and impaired CD8 T-cells responses to *de novo* HSV-1 infection. Aged mice bearing Vβ10 and Vβ8 CD8 TCE failed to mount an effective antigen-specific CD8 T-cell response against HSV-1 (based on CD8⁺ gB₄₉₈₋₅₀₅ tetramer⁺ staining), demonstrate that the presence of TCE impaired antiviral CD8 T-cell immunity in an antigen-specific manner. In humans TCE have been observed with respect to CD8 T cells specific for cytomegalovirus (CMV). CMV latently persists in the majority of adults and viral reactivation often occurs. CMV reactivation results in chronic stimulation of CD8 T-cells, which can lead to immune senescence and

ultimately impairment in immune responses.⁷⁸ Using a mouse model of CMV (MCMV) Cicin-Sain *et al.* reported that latent MCMV infection resulted in an accumulation of MCMV-specific CD8 T-cells, which led to distorted CD8 T-cell diversity and impaired CD8 T-cell responses to *de novo* viral challenge.⁸³ Interesting, mice also exhibited significantly lower antigen-specific CD8 T-cell responses when challenged with IAV, West Nile virus or HSV-1 months after the initial MCMV infection. Together, these data demonstrate that the loss of a diverse CD8 T-cell repertoire – an event that can occur in a number of physiological setting, including sepsis – can impair a host's capacity to elicit effective immune responses.

C. Sepsis-induced homeostatic proliferation

Antigen-experienced (effector/memory) CD8 T-cells can be distinguished phenotypically from naïve (cognate antigen-inexperienced) CD8 T-cells by increased expression of a number of cell surface molecules such as CD44, CD11a, CD122 and Ly6c.^{61, 84–88} When lymphocyte numbers reach levels below a certain threshold a lymphopenic environment is created.^{89, 90} Once lymphopenia has occurred, it is important that homeostasis is restored to the immune system for proper function. To restore homeostasis CD8 T-cells present in the periphery will undergo lymphopenia-induced expansion known as homeostatic proliferation.^{89, 91} Naïve CD8 T-cells that undergo lymphopenia-induced homeostatic proliferation exhibit a 'memory-like' phenotype comparable to 'true' effector/memory CD8 T-cells, as evidenced by increased expression of cell surface molecules (i.e. CD44, CD11a, CD122, Ly6c).^{88, 92–96} Naïve CD8 T cells also demonstrate functional qualities much like true antigen-experienced effector/memory CD8 T-cells, based on increased IFN- γ production after cognate antigen-stimulation and the ability to control bacterial infection as effectively as 'true' memory CD8 T-cells.^{93, 95, 96} Together these results suggest that 'memory-like' naïve CD8 T-cells are 'better' than normal naïve CD8 T-cells since they develop effector functions much like true effector/memory T cells allowing them to respond to antigenic challenge. Thus, it is reasonable to believe that following a septic episode 'memory-like' naïve CD8 T-cells generated from lymphopenia-induced homeostatic proliferation may provide a survival advantage for the host.

Using the CLP mouse model of sepsis, Unsinger *et al.* demonstrated lymphopenia-induced homeostatic proliferation of CD8 T-cells, but not CD4 T-cells, following septic injury.⁵⁶ Splenic CD8 T-cells obtained from septic mice (21 days post-CLP surgery) exhibited a decrease in CFSE staining and an increase in CD44 expression compared to sham controls, leading to the conclusion that after sepsis-induced apoptosis the remaining naïve CD8 T-cells undergo homeostatic proliferation to replenish CD8 T-cell numbers. We have observed a similar significant increase in naïve CD8 T-cells exhibiting a 'memory-like' phenotype in the peripheral blood 30 days post-CLP surgery, but we further characterized the HP phenotype to include CD44, CD11a, CD122 and Ly6c⁵⁸ (Figure 3). Thus, homeostatic proliferation of naïve CD8 T-cells following sepsis-induced lymphocyte apoptosis may be considered ideal situation for improving post-sepsis survival, by increasing T-cell numbers to 'pre-sepsis' levels and providing the cells with enhanced function.

Interestingly, the study by Unsinger *et al.* that showed CD8 T-cells underwent homeostatic proliferation after sepsis also found that CD4 T-cells did not under the same circumstance.⁵⁶ In recent years, the importance of CD4 T-cells in the induction of CD8 T-cell responses has been highlighted,^{97–100} where most CD8 T-cell-mediated responses require concomitant CD4 T-cell priming to be effective. This concept holds true for homeostatic proliferation-induced CD8 T-cells also. The enhanced protective function of CD8 T-cells that experienced homeostatic proliferation is dependent on CD4 T-cell help.⁹⁶ The priming of CD8 T-cells in the absence of CD4 T-cell help alters CD8 T-cell programming such that the CD8 T-cells express TNF-related apoptosis-inducing ligand (TRAIL) and undergo activation-induced

cell death (AICD) upon secondary antigenic stimulation.¹⁰⁰ TRAIL expression in this setting is regulated by Nab2,¹⁰¹ and the full proliferative and functional capacity of the 'helpless' CD8 T-cells can be regained with IL-2 (but not IL-7 or IL-15) are added.¹⁰² Thus, it is reasonable to hypothesize one possible mechanism for the lack of protective CD8 T-cell-mediated immunity to secondary infection after sepsis is that there is an increased frequency of CD8 T-cells that undergo homeostatic proliferation but do not receive the necessary CD4 T-cell help at the time of antigen-mediated stimulation to inhibit TRAIL-mediated AICD. Consequently, it could then be predicted that protective CD8 T-cell responses to secondary infection after septic insult would be restored with the disruption of the TRAIL-DR5 pathway. Data from the study by Gurung et al. suggest this to be the case, as septic *Trail*^{-/-} or *Dr5*^{-/-} mice were able to control a secondary *Listeria* infection to the same degree as sham-treated mice.⁵⁵ Similar protection from secondary *Listeria* infection after sepsis was obtained in wild-type mice given a blocking anti-TRAIL mAb therapeutically. Thus, the identification of a role for TRAIL in this capacity provides the possibility for another therapeutic target for reducing the complications associated with secondary infection in septic patients.

These hypotheses and experimental data should be approached with caution, though, as the altered T-cell repertoire that is produced after homeostatic proliferation may impact the generation of a protective CD8 T-cell response against infection in a combination of ways. There is evidence that some T-cells may not undergo homeostatic proliferation,¹⁰³ and TCR repertoire diversity may be altered with homeostatic expansion of polyclonal T-cell populations.¹⁰⁴ As a result, only certain T-cells will expand following lymphopenia-induced homeostatic proliferation, which once again leads to the generations of 'holes' in the CD8 T-cell repertoire. Additionally, lymphopenia-induced homeostatic proliferation could skew the TCR repertoire towards greater self-reactivity leading to potential autoimmune consequences.^{105–107} As much as the sepsis-induced decrease in T-cell numbers (for the sake of this discussion) and potential change in T-cell repertoire as a result of homeostatic proliferation are key factors in the alterations in the immune system's ability to respond to secondary infection after a septic event, the sepsis-induced lymphopenia is more than just a passive event that decreases cell numbers. In the next section, we will discuss how the large number of dead immune cells generated during sepsis can actively lead to the generation of immune suppression.

IV. THE INFLUENCE OF CELL DEATH ON THE IMMUNE RESPONSE

We have concentrated our discussion so far on the changes within the CD8 T-cell repertoire that develops after a septic event and how these changes themselves can impact the type of response generated. It is important to note that the lymphopenia and (potentially) altered T-cell repertoire are not the only events responsible for the long-term immune suppression after sepsis – the remaining cells are actively suppressed. Thus, it is also important to understand the relationship between the immune cell death and the induction of active immune suppression. As is the case for normal cellular turnover and the contraction of the number of effector T-cells after they have cleared the activating antigen, the large amount of death seen in immune system early after a septic event is via an apoptotic mechanism.⁵⁰ Apoptosis is a well-characterized cell death process that can occur via extrinsic (i.e. death ligand/death receptor engagement) and intrinsic (i.e. mitochondrial driven) pathways.¹⁰⁸ Interestingly, there is data to suggest that both the extrinsic and intrinsic apoptosis pathways are involved in the sepsis-induced lymphocyte death.⁵³ There is considerable interest in the therapeutic modulation of the apoptotic death of immune cells during sepsis, and a recent review by Hotchkiss and Nicholson excellently covered this topic.¹⁰⁹ Instead, we will focus our discussion on the link between apoptotic cells and sepsis-induced immune suppression, and one possible mechanism by which suppression is maintained.

The original descriptions of apoptosis characterized it as a “silent death” whereby the immune system was not alerted to the various antigens and damage-associated molecular patterns (DAMPs) present within a cell.¹¹⁰ The reticuloendothelial system is capable of dealing with the “normal” load of apoptotic cells that are induced during tissue turnover, such that the apoptotic cells are quickly eliminated before cell membrane integrity can be compromised and the pro-inflammatory cellular components (i.e., DAMPs) are released. It is conceivable that the magnitude of the wide-spread apoptotic death within the immune system during sepsis may be too large to allow for efficient clearance, so that the body (and immune system) is being exposed to increased numbers of apoptotic cells that have progressed farther down the path of cellular destruction and are now more immunogenic and help drive the hyper-inflammatory state seen early after sepsis induction and its associated mortality. Inhibition of apoptosis, using caspase inhibitors or overexpression of anti-apoptotic proteins, improves survival in septic animals.^{111, 112} Similarly, administration of recombinant IL-7 or IL-15 can prevent immune cell apoptosis and increase survival after a severe septic event.^{113, 114} However, there is now substantial data describing the induction of immune tolerance using apoptotic cells, and the immune suppression that develops during sepsis is now considered to be a major factor leading to the increased mortality of septic patients long after the septic event has occurred and resolved.

The induction of immunological tolerance by apoptotic cells has been attributed to a number of mechanisms, including the production of immunosuppressive cytokines from phagocytic cells,¹¹⁵ production of inhibitors from the dead cell itself,^{116, 117} and effects on the maturation of DCs.^{99, 118} Clearance of apoptotic cells by DCs can impair CD8 T-cell responses. When DCs uptake apoptotic cells co-stimulatory molecules are typically not up-regulated.¹¹⁹ As a result CD8 T-cells are not efficiently activated and become anergic, and/or undergo apoptosis.^{119, 120} T-cell anergy is a tolerance mechanism that occurs when a T-cell is functionally inactivated following antigen-encounter.¹²¹ Additionally, uptake of apoptotic cells can induce the release of anti-inflammatory cytokines thus suppressing the production of pro-inflammatory cytokines.^{53, 120} Using the CLP mouse model of sepsis work by Hotchkiss *et al.* demonstrated that transfer of apoptotic cells 5 days prior to sepsis induction decreased the survival rate of septic mice.¹²⁰ Furthermore, the pro-inflammatory cytokine, IFN- γ , was decreased significantly in CLP-apoptotic treated mice compared to CLP-treated mice. These results demonstrate that uptake of apoptotic cells exacerbate the sepsis-induced decrease of IFN- γ and is associated with increased mortality. These data also illustrate that due to a decrease in pro-inflammatory cytokine production and reduction of co-stimulatory molecule up-regulation the post-septic environment is not optimal for CD8 T-cell activation, thus impairing CD8 T-cell responses.

We have had a long-standing interest in the tolerogenic nature of apoptotic cells, and have assessed the tolerogenicity (or immunogenicity) of apoptotic cells on the induction of T cell-mediated immunity in several experimental systems.^{117, 122–125} One method, described initially by Battisto and Bloom,¹²⁶ involves i.v. injection of antigen (hapten)-modified self cell that results in significant inhibition of cell-mediated immune responses (e.g., DTH). A reexamination of the immune tolerance generated by the i.v. injection of haptenated apoptotic cells revealed TRAIL-expressing CD8 T-cells were required for the observed tolerance.¹²⁴ Similarly, TRAIL-expressing CD8 T-cells were found to be responsible for the tolerance observed in soluble peptide antigen is administered systemically to induce the peripheral deletion of an antigen-specific T-cell population,¹²³ as well as in a model of tolerance where immunization occurs via injection of antigen into the anterior chamber of the eye.¹²⁵ The identification of TRAIL-expressing CD8 T-cells in multiple tolerance models was quite surprising since TRAIL was initially characterized (and most heavily studied) as a potent inducer of tumor cell apoptosis, but had little-to-no cytotoxic activity against normal cells and tissues.¹²⁷ It was not until the incorporation of *Trait*^{-/-} mice¹²⁸ into

a variety of immunological models did additional physiological roles for TRAIL outside of inducing apoptosis in tumor cells come to light. One key feature of the above mentioned tolerance models is the association between apoptotic cells and the induction of tolerance – much like that for sepsis-induced immune suppression. With this idea in mind, Unsinger et al. examined the hypothesis that sepsis-induced apoptosis may be related to the established natural suppressive mechanisms that deal with apoptotic cells.⁵⁷ Sepsis-induced immune cell apoptosis lead to the generation of a TRAIL-expressing CD8-T cell population that was essential for the loss of DTH in sepsis. TRAIL-mediated immune suppression appears to be transient in these conditions, which may relate to the suicide of the TRAIL-expressing CD8 T-cells (see the discussion of ‘helpless’ CD8 T cells above).

CONCLUDING REMARKS

It is clear from the numerous studies examining sepsis in preclinical animal models and the assessment of humans with sepsis that this is a condition that exerts a variety of effects on the immune system. The emphasis of this review article was examining the impact of sepsis on CD8 T-cell immunity to newly encountered infection. There is the potential for multiple mechanisms to explain sepsis-induced immune suppression of CD8 T-cell responses. Having seen the interesting relationship between sepsis-induced immune cell death and the induction of TRAIL-mediated immune suppression, we propose the following model of TRAIL-dependent suppression of T cell immunity during sepsis. Sepsis induces massive apoptosis of multiple immune cell populations, which is required for the induction of the TRAIL-dependent suppressive mechanism. If a new infection is then acquired, the TRAIL-dependent suppressive mechanism prevents pathogen clearance and the animal succumbs to the infection. Data suggests that blocking TRAIL, using the clinically-relevant approach of systemic anti-TRAIL mAb administration, will prevent the sepsis-induced immune suppression, keeping normal T cell-mediated immune function to clear the infection, and increase the chance for survival. We understand and acknowledge that this is one of many changes that occur within the immune system after a septic event. It remains to be seen how this potential therapeutic target, by itself or combined with any of the approached designed to protect against apoptosis and/or facilitate more robust immune cell recovery, will eventually improve the outcome of septic patients that are stricken with an additional infection.

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Abbreviations

AICD	activation-induced cell death
APC	antigen presenting cell
CARS	compensatory anti-inflammatory response syndrome
CD	cluster differentiation
CLP	cecaligation and puncture
CMV	cytomegalovirus
DAMP	damage-associated molecular pattern
DR5	death receptor five

DC	dendritic cell
DTH	delayed-type hypersensitivity
gB	glycoprotein B
GP	glycoprotein
HSV	herpes simplex virus
IAV	influenza A virus
IFN-γ	interferon gamma
IL	interleukin
L	polymerase L segment
LCMV	lymphocytic choriomeningitis virus
LM-OVA	attenuated recombinant strain of <i>L. monocytogenes</i> expressing ovalbumin
M1	matrix protein 1
MCMV	mouse cytomegalovirus
MHC	major histocompatibility complex
N	nucleocapsid protein
NA	neuraminidase
NP	nucleoprotein
NS1	non-structural protein 1
OVA	ovalbumin
PA	polymerase acidic protein
PAF	platelet activating factor
PB1	polymerase basic protein 1
SIRS	systemic inflammatory response syndrome
TCE	T-cell clonal expansions
TCR	T-cell receptor
TGF-β	transforming growth factor beta
TLR	toll-like receptor
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
VSV	vesicular stomatitis virus

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Figure 1. Primary CD8 T-cell responses to infection

An antigen-specific naïve CD8 T-cell becomes fully activated (Phase I) after interacting with an antigen-presenting cell (i.e., dendritic cell (DC)) presenting cognate antigen on MHC I to the T-cell receptor of a CD8 T-cell. Once activated, CD8 T-cells undergo rapid proliferative expansion (Phase II) resulting in the generation of effector CD8 T-cells that are able to secrete cytokines to combat the invading pathogen. Following the peak of expansion, CD8 T-cells undergo a contraction phase (Phase III) whereby 90–95% of responding effector cells will die. The remaining 5–10% cells transit into long-lived memory CD8 T-cells (Phase IV).

Figure 2. Sepsis-induced lymphocyte apoptosis changes the composition of the naïve CD8 T-cell repertoire

The naïve CD8 T-cell repertoire is composed of relatively small numbers of single antigen-specific naïve CD8 T-cell precursors that enable the host to respond to virtually any pathogen encounter. The impact of sepsis-induced apoptosis on particular antigen-specific naïve CD8 T-cell precursors likely occurs stochastically. Depicted in A, B and C are several scenarios that may occur following sepsis-induced lymphocyte apoptosis. **A.** Incomplete recovery of naïve CD8 T-cell precursors specific for antigen-(A) after sepsis. **B.** Complete recovery of naïve CD8 T-cell precursors specific for antigen-(B) after sepsis. **C.** Outgrowth of naïve CD8 T-cell precursors specific for antigen-(C) after sepsis. Taken together these scenarios illustrate that sepsis could alter the composition of the naïve CD8 T-cell repertoire which may have consequences on CD8 T-cell responses to infection.

Figure 3. Sepsis-induced homeostatic proliferation changes the phenotype of naïve CD8 T-cells

A lymphopenic environment is created after sepsis-induced lymphocyte apoptosis. The remaining naïve CD8 T-cells present replenish the environment via lymphopenia-induced homeostatic proliferation. As a consequence, CD8 T-cell numerical recovery is accompanied by a phenotypic change in naïve CD8 T-cells through increased expression of cell surface molecules (such as CD44, CD11a, CD122, Ly6c), resulting a 'memory-like' phenotype.