The Role of TCR Engagement and Activation-Induced Cell Death in Sepsis-Induced T Cell Apoptosis

Jacqueline Unsinger, John M. Herndon, Christopher G. Davis, Jared T. Muenzer, Richard S. Hotchkiss and Thomas A. Ferguson

*J Immunol* 2006; 177:7968-7973; doi: 10.4049/jimmunol.177.11.7968
http://www.jimmunol.org/content/177/11/7968

**References**
This article cites 37 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/177/11/7968.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Role of TCR Engagement and Activation-Induced Cell Death in Sepsis-Induced T Cell Apoptosis

Jacqueline Unsinger,* John M. Herndon,† Christopher G. Davis,* Jared T. Muenzer,* Richard S. Hotchkiss,* and Thomas A. Ferguson†

Sepsis induces extensive apoptosis in T and B cells suggesting that the loss of immune effector cells could be one explanation for the profound immunosuppression observed in this disorder. Unfortunately, the mechanisms responsible for lymphocyte apoptosis in sepsis remain unknown. In T cells, apoptosis can occur through activation-induced cell death (AICD) in which engagement of the Ag receptors by cognate Ag or polyclonal activators such as bacteria-derived superantigens induces activation, proliferation, and apoptosis. We examined whether proliferation and AICD are necessary for apoptotic cell death in sepsis using normal and TCR transgenic mice. Results show that although sepsis resulted in activation of a small percentage of T cells, no proliferation was detected during the first 48 h following onset, a time when extensive apoptosis is observed. We also observed that T cells do not enter the cell cycle, and stimulation via the TCR in TCR transgenic animals does not enhance or decrease cell death in sepsis. Interestingly, T cells recovered from septic mice retained their ability to proliferate and synthesize cytokines albeit at reduced levels. With the exception of IL-10, which was increased in lymphocytes from mice with sepsis, sepsis caused a decrease in the production of both proinflammatory and anti-inflammatory cytokines. We conclude that lymphocyte apoptosis in sepsis does not require proliferation, TCR engagement, or AICD. Thus the immunosuppression observed in sepsis cannot be the result of T cell deletion via the TCR.

The Journal of Immunology, 2006, 177: 7968–7973.

Sepsis and the resultant multiorgan failure are the leading causes of death in intensive care units (1, 2). Patients with sepsis have massive apoptosis in lymphoid organs such as thymus, spleen, lymph nodes, and gastrointestinal associated lymphoid tissue (3–5). Apoptosis is notable in most subpopulations of lymphoid cells including B cells and CD4+ and CD8+ T cells. The death of these cells may result in depletion of key immune effector cells and this effect may account for the immunosuppression that is a central pathogenic event (6, 7). Down-regulation of the immune response by regulatory T cells as well as the induction of a Th2 type immune deviation have also been proposed as contributory to immune suppression in sepsis (8, 9).

During normal immune responses, T cell fate is determined to a large degree by signals received through their Ag receptors (10–12). The developmental and maturational stage, the availability of co-stimulation, and the quality of the ligand stimulation all determine the outcome of the response (11). Apoptosis of T cells in the thymus and periphery also requires TCR engagement and is critical for thymic development and maintaining balance in the immune system (13, 14). In the thymus, negative selection eliminates unwanted T cell specificities, whereas T cell death in the periphery controls the extent of T cell expansion and helps eliminate cells following the induction of a productive immune response (14). One mechanism for T cell death is activation-induced cell death (AICD)3 in which activation through the TCR with Ag or polyclonal activators (e.g., superantigens) results in apoptosis (11). AICD can occur through a cell autonomous manner that is determined by a number of factors such as the nature and intensity of TCR signaling (11, 15, 16) and the availability of cytokine growth factors. It has been suggested that a common feature linking many of the causes of AICD is an aberration in the cell cycle program (17, 18).

Although lymphocyte apoptosis is a common feature in sepsis (19), the precise death-inducing stimuli and other critical elements remain unknown. During sepsis the immune system is exposed to bacterial products as well as Ags from damaged tissues. Thus, one possible mechanism for the induction of T cell apoptosis and the loss of immune function in sepsis is AICD in which engagement of the TCR by Ags or polyclonal activators leads to cellular proliferation and death (11). The purpose of this study was to determine whether activation and proliferation were responsible for sepsis-induced apoptosis and immune suppression. Our results show that although sepsis induces early T cell activation it does not induce proliferation in these cells. In addition, T cells removed from septic mice retained their ability to proliferate, but are compromised in their ability to make key cytokines. Collectively, these results suggest that the mechanism of killing of lymphocytes in sepsis does not require cognate Ag receptor interaction and is probably distinct from classical AICD. Thus the loss of immune functions is not directly related to the death of Ag-specific T cells but may be the result of a more generalized, nonspecific loss of immune function.

3 Abbreviations used in this paper: AICD, activation-induced cell death; CLP, cecal ligature and puncture.
Materials and Methods

Mice

BALB/c, DO11.10, C57BL/6, and OT-1 mice were purchased from The Jackson Laboratory. Mice weighing 18–28 g were housed for at least 1 wk before manipulations. Male or female mice were age- and sex-matched for all experiments, and at least five mice were used per group. All animal procedures were performed according to National Institutes of Health guidelines and approved by the Washington University Institutional Animal Care and Use Committee.

Abs and reagents

Unless otherwise indicated, all Abs were purchased at BD Pharmingen. The DO11.10 Ab KJ-1–26 was purchased from Caltag Laboratories (catalogue no. MM7501). CFSE was purchased from Molecular Probes. The OVA\textsubscript{223–239} peptide was synthesized by Sigma-Genosys. For fixation and permeabilization for the Ki-67 staining the BD Cytofix/Cytoperm fixation/permeabilization solution kit was used (catalogue no. 554714; BD Biosciences). The staining for apoptotic cells via TUNEL was performed using the APO-BrdU kit (Phoenix Flow Systems). For CD4\textsuperscript{+} T cell isolation, we used the magnetic bead-based CD4\textsuperscript{+} T cell isolation kit from Miltenyi Biotec (catalogue no. 130-090-860). Cytokine production was determined using the BD Cytometric Bead Array (CBA) Mouse Th1/Th2 cytokine kit and the BD CBA Mouse Inflammation kit according to the manufacturer’s instruction (BD Pharmingen). Data were obtained and analyzed using the BD FACSArray system and software (BD Pharmingen).

Quantification of cell activation

Approximately 18–22 h after CLP or sham surgery, mice were killed and single-cell suspensions were prepared. Cells were stained for CD4 and T cell activation markers CD25 and CD69. Cells were then analyzed by flow cytometry as described.

Sepsis model of cecal ligation and puncture (CLP)

The CLP model was used to induce intraabdominal peritonitis as previously described (20). Earlier studies from our laboratory include positive blood cultures for polymicrobial organisms (aerobic and anaerobic bacteria) from CLP, but not sham-operated mice (19–21). Mice were anesthetized with halothane and an abdominal incision was performed. The cecum was identified, ligated, and punctured once with a 30-gauge needle. The abdomen was closed in two layers and 1 ml of 0.9% saline was s.c. administered. Sham-operated mice were treated identically except the cecum was not ligated or punctured.

Results

Sepsis does not induce T cell proliferation

One of the main characteristics of AICD is T cell activation via the TCR followed by proliferation and programmed cell death. Thus, it is possible that during sepsis bacterial products that are released into the circulation could act as superantigens by binding to TCRs and resulting in cellular proliferation. To examine this in vivo, we used normal BALB/c spleen cells and CD4\textsuperscript{+} TCR transgenic DO11.10 T cells labeled with CFSE (22, 23). Because most of the T cell apoptosis occurs within the first 24–48 h following sepsis (20), we assessed cellular proliferation during this period. Spleen cells from BALB/c mice or TCR transgenic DO11.10 T cells (CD4\textsuperscript{+} KJ-1–26\textsuperscript{+}) isolated from the spleen were labeled with CFSE, and infused into recipient wild-type BALB/c mice. Twenty-four hours later, mice were subjected to a sham operation or CLP. Data in Fig. 1A show that no proliferation could be detected at either 24 or 48 h after CLP surgery in either CD4\textsuperscript{+} T cells. Similarly, CD4\textsuperscript{+} KJ-1–26\textsuperscript{+} T cells obtained from sham-operated or CLP mice demonstrated no proliferation at 24 or 48 h postsurgery (Fig. 1B). Sham-operated animals in all experiments showed no cellular proliferation (data not shown). (Note, we also tested CFSE content in normal CD8\textsuperscript{+} T cells and transgenic OT-I CD8\textsuperscript{+} T cells. These cells showed no significant proliferation following sham or CLP operations (data not shown). Because these results were also negative, we did not include these data in this study.) Thus, sepsis does not induce proliferation of T cells early in the response.

Sepsis does not induce T cells to enter the cell cycle

It has been suggested that cells that have entered the cell cycle are more vulnerable to apoptosis from external stimuli (17, 18, 24). Because cells were not proliferating following sepsis, it was possible that T cells become activated during sepsis, enter the cell cycle, but die before they can complete a round of proliferation. As a result changes in CFSE content would not be detected. We tested this possibility by using the well-established proliferation marker Ki-67. Ki-67 is a nuclear Ag that is not expressed in resting cells but increases in late G\textsubscript{1} phase of the cell cycle and remains elevated throughout mitosis (25). When we compared the percentage...
of splenic CD4+ T cells from BALB/c and DO11.10 mice that express Ki-67 following sham or CLP surgery, we found that the numbers were similar, i.e., 7% at 18 h postsurgery (Fig. 2). Thus, sepsis does not appear to induce T cells to enter the cell cycle.

Sepsis induces CD69 but not CD25 expression in T cells
Because sepsis does not induce T cells to proliferate or enter the cell cycle, we tested for expression of activation markers. Activated T cells demonstrate increased expression of a number of cell surface markers including CD69 (a very early T cell activation marker) (26) and CD25 (the IL-2R). C57BL/6 mice underwent CLP or sham surgery and were sacrificed 18–22 h later. Splenocytes were prepared and stained for CD4 and for the activation markers CD25 and CD69. Although there was a small increase in the expression of CD69 (***, p ≤ 0.001), but minimal change in the expression of CD25.

Engagement of the TCR is not necessary for sepsis-induced lymphocyte apoptosis
Next, we examined the role of TCR engagement in the induction of apoptosis in T cells using TCR transgenic T cells from the DO11.10 mice. Sham surgery or CLP was performed on DO11.10 and BALB/c mice and apoptosis was evaluated in the spleen 18–22 h postsurgery by TUNEL staining. Both CD4+ T cells in BALB/c mice and KJ1-26+ CD4+ T cells in DO11.10 mice underwent apoptosis (Fig. 4). Thus, populations of CD4+ T cells containing multiple receptor specificities as well as T cells with a single receptor specific for OVA323–339 peptide undergo equal apoptosis following sepsis.

Sepsis does not alter the ability of surviving T cells to proliferate to antigenic challenge
Our results thus far demonstrate that T cells do not get fully activated or proliferate in response to a septic challenge. The question then arose as to whether sepsis could impair the ability of cells to proliferate in response to Ag. CFSE-labeled DO11.10 CD4+ T cells were injected into BALB/c mice and 24 h later the animals were injected with OVA323–339 peptide. After an additional 24 h, sham or CLP operation was performed and proliferation was assessed 1 day later by examining CFSE content. As shown in Fig. 5, KJ1-26+ CD4+ T cells from both sham- and CLP-treated mice underwent equivalent cellular proliferation, i.e., three to four cell divisions occurred in both T cell groups. Thus, sepsis does not
interrupt the capacity of proliferating T cells to further respond to their cognate Ag.

**Proliferation neither enhances nor protects against sepsis-induced apoptosis**

We then examined whether the induction of proliferation increased the susceptibility of splenic T cells to apoptosis. Apoptosis during sepsis occurs to a large degree in T cells in spleen and thymus as well as in the intestinal epithelial cells in the crypt (27). These cells are known to be rapidly proliferating and it has been reported that these types of cells, i.e., rapidly dividing cells, are more vulnerable to apoptosis (17, 18, 24). We tested this hypothesis in DO11.10 TCR transgenic CD4+ T cells by inducing proliferation and then determining whether the level of apoptosis in dividing KJ1-26+, CD4+ T cells was increased over the level in resting TCR transgenic cells. DO11.10 mice were injected with OVA323-339 peptide or left untreated. Twenty-four hours later, mice were subjected to sham or CLP. After a second 24 h period, the percentage of proliferating cells was determined by flow cytometry comparing completed cell cycles of sham- or CLP-treated T cells by CFSE content. T cells underwent uninterrupted cellular proliferation with sepsis challenge. Data represent four individual experiments.

We then examined whether the induction of proliferation increased the susceptibility of splenic T cells to apoptosis. Apoptosis during sepsis occurs to a large degree in T cells in spleen and thymus as well as in the intestinal epithelial cells in the crypt (27). These cells are known to be rapidly proliferating and it has been reported that these types of cells, i.e., rapidly dividing cells, are more vulnerable to apoptosis (17, 18, 24). We tested this hypothesis in DO11.10 TCR transgenic CD4+ T cells by inducing proliferation and then determining whether the level of apoptosis in dividing KJ1-26+, CD4+ T cells was increased over the level in resting TCR transgenic cells. DO11.10 mice were injected with OVA323-339 peptide or left untreated. Twenty-four hours later, mice were subjected to sham or CLP. After a second 24 h period, the percentage of proliferating cells was determined by flow cytometry comparing completed cell cycles of sham- or CLP-treated T cells by CFSE content. T cells underwent uninterrupted cellular proliferation with sepsis challenge. Data represent four individual experiments.

**Cytokine production in T cells recovered from septic mice**

Because sepsis does not alter the ability of T cells to proliferate in response to cognate Ag, it was possible that it could alter the cytokine production of these cells as has been observed in other models of chronic injury (7, 9, 28). We tested whether cells recovered from septic animals maintained their ability to proliferate and produce cytokines after a protracted period of sepsis. DO11.10 mice were subjected to sham or CLP and 4 days later the CD4+ KJ1-26+ T cells were harvested from spleen, labeled with CFSE, and placed in culture with freshly irradiated APCs and peptide Ag. Forty-eight hours later, cells were assessed for proliferation by examining CFSE content and cytokine production was examined in the supernatants. Although there were slight differences in the percentages of cells found in each cell division, proliferation of DO11.10 cells in response to OVA323-339 peptide was essentially the same whether the cells were obtained from sham or CLP mice (Fig. 7A).

The cytokine response of these cells is shown in Fig. 7B. The proinflammatory cytokines TNF-α and IL-6, as well as IL-2, IL-4, and IL-5 levels were significantly reduced (Fig. 7B). Conversely, the anti-inflammatory cytokine IL-10 was significantly enhanced. IFN-γ values (942 ± 72.2 in CLP mice vs 1141 ± 129 pg/ml in sham) were unaffected. These results show that there is no definitive shift in the cytokine profile toward a Th2 type in fact most of the Th2 cytokines are reduced. The fact that IFN-γ levels are unchanged also argues against a definitive Th1/Th2 shift. However, the increase in IL-10 production suggests that the immunosuppression following sepsis could be mediated by this cytokine.

**Discussion**

Over 220,000 patients die of sepsis each year in the United States making it a major health care problem (1, 2). A central pathologic process in sepsis is apoptotic death that occurs in lymphoid cells and intestinal epithelial cells (3, 7, 29). Data obtained from autopsies of patients who died of sepsis as well as data from animal models suggest that there is extensive apoptosis and depletion of CD4 T cells as well as B cells (3–5, 30). That this is an important pathologic feature of sepsis is emphasized by data showing that prevention of apoptosis improves survival (7, 19, 20, 27, 29). A key goal of sepsis research has been to identify the mechanisms responsible for sepsis-induced apoptosis thereby allowing the design of effective therapies (6, 27).

We examined several aspects of T cell death in a clinically relevant mouse model of polymicrobial sepsis. We tested whether...
AICD, an important mechanism for T cell death that requires engagement of the TCR by cognate Ag or polyclonal activators, might be involved. We observed that although there is limited activation of CD4⁺ T cells (as measured by increased CD69 expression) the cells do not increase IL-2R (CD25 receptor) expression during the critical period of apoptosis induction. Thus, it is unlikely AICD as induced by engagement of the TCR has a role in the T cell apoptosis observed. A recent study using the burn injury model demonstrated that 12 h after injury certain VB TCR subpopulations increased Ki-67 expression in the lymph nodes (31). They did not observe differences at 24 h in the lymph nodes, or at any time postinjury in the spleen. We have reported our results on splenic T cells, but we have examined proliferation in the mesenteric lymph node of septic mice. We did not observe proliferation or increases in Ki-67 expression (data not shown). We also examined Ki-67 expression at 12 h in the spleen and did not find any differences in expression (data not shown). Perhaps difference in the burn model and the CLP model could account for these results.

We also explored whether sepsis could influence the response of T cells that were already induced to proliferate by cognate Ag. Our studies reveal several novel findings. First, induction of sepsis in proliferating T cells does not appear to influence this outcome, i.e., it does not alter the course of cellular division. Second, sepsis does not influence the ability of T cells to enter the cell cycle following Ag stimulation. Third, proliferating T cells are not more vulnerable to apoptosis during sepsis. Because sepsis does not interrupt active cycling of Ag-stimulated T cells, apoptosis in this model is not related to stages of the cell cycle. Fourth, T cells recovered from septic mice retain their ability to proliferate to Ag if given fresh APC, ruling out a form of clonal anergy mediating immunosuppression (32).

In contrast to the findings regarding the ability of lymphocytes to proliferate, we did observe a significant impact of sepsis on lymphocyte cytokine production. With the exception of IL-10, T cells from septic mice had reduced production of both proinflammatory and anti-inflammatory cytokines. The fact that IL-10 production was increased in lymphocytes from septic mice indicates that sepsis does not induce a global effect on cytokine production but rather it must be selectively induce this specific pathway that leads to the production of this potent immunosuppressive cytokine. Increased circulating IL-10 has been correlated with worsened mortality in both animal models of sepsis and in human clinical studies (33) and targeting IL-10 therapeutically has been shown to be beneficial (9). However, why sepsis specifically induces IL-10 production while suppressing other cytokines is unclear. That IFN-γ levels remain unchanged and that both Th1 and Th2 cytokines are inhibited argues against a polarization of T cells responses toward one specific T cell type. This observation is at odds with observations in burn models (28) suggesting different mechanisms of immunoregulation between these two types of injury. One possibility for the impairment in T cell function in septic mice is the loss of key APCs such as dendritic cells early in sepsis that compromises T cell function. We would suggest that surviving T cells in septic animals retain their ability to proliferate, but that the loss of key APC or APC functions impairs T cell function and alters their cytokine profile. The loss of critical APCs (i.e., dendritic cells) has been observed in several models (34–37).

Because TCR engagement is not involved, it is unlikely that adaptive immunity plays a role this early in the response. Adaptive immunity requires stimulation through the TCR along with clonal expansion, clonal selection, and progression to effector status. Because the TCR and proliferation are not involved there is no mechanism to drive T cells toward effector function. This result suggests that examining the functions of other cells involved in T cell responses (e.g., APCs), as well as determining how specific cytokine pathways might be targets during sepsis, will shed light on the immunosuppression and provide information for the rational design of treatments.

**Disclosures**

The authors have no financial conflict of interest.
References