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Adoptive Transfer of In Vitro-Stimulated CD4⁺CD25⁺ Regulatory T Cells Increases Bacterial Clearance and Improves Survival in Polymicrobial Sepsis

Josef G. Heuer,¹* Tonghai Zhang,* Jingyong Zhao, † Chunjin Ding,* Martin Cramer,* Kathy L. Justen, ‡ Steven L. Vonderfecht,* and Songqing Na¹†

Regulatory CD4⁺CD25⁺ T cells (Tregs) suppress autoimmune and inflammatory diseases through mechanisms that are only partly understood. Previous studies suggest that Tregs can suppress bacterially triggered intestinal inflammation and respond to LPS through TLRs with enhanced suppressive activity. In this study, we have used murine cecal ligation and puncture as a model of polymicrobial sepsis to explore the effects of adoptive transfer of Tregs on septic outcome. Adoptive transfer of in vitro-stimulated Tregs in both prevention and therapeutic modes significantly improved survival of cecal ligation and puncture mice. Furthermore, the effect was dependent on both the number of Tregs adoptively transferred and the presence of host T cells. Animals that received stimulated Tregs had significantly increased peritoneal mast cells and peritoneal TNF-α production. More importantly, adoptive transfer of in vitro-stimulated Tregs significantly improved bacterial clearance, which resulted in improved survival. Our results suggest a novel role for Tregs in sepsis. The Journal of Immunology, 2005, 174: 7141–7146.

Naturally arising CD4⁺CD25⁺ regulatory T cells (Tregs)² are essential for maintenance of peripheral tolerance through their suppression of autoreactive T cells (1, 2) and have also been found to inhibit several inflammatory and autoimmune diseases in animal models such as experimental autoimmune encephalomyelitis (3), inflammatory bowel disease (4), bacterial-induced colitis (5), collagen-induced arthritis (6), type I diabetes (7), graft-vs-host disease (8), and organ transplantation (9). The in vitro suppressive activity of Tregs on T cell activation is thought to be through a cell contact-dependent and cytokine-independent mechanism (10, 11), although the mechanisms of Treg activity in vivo are less understood. A recent study suggested that the suppressive activity of Tregs may be enhanced by microbial products through the TLRs, implicating potential suppression of innate immune responses (12). However, another recent study demonstrated Toll pathway-dependent blockade of Treg-mediated suppression by dendritic cells that was dependent in part on IL-6 (13); thus the exact role that Tregs play in the response to microbial products is presently not clear.

Sepsis results from a microbial infection and is associated with severe inflammatory and procoagulant activities concomitant with multiple organ failure and substantial mortality (14, 15). Upon infection, innate immune cells recognize microbial products and initiate a host immune response by releasing chemokines and cytokines to recruit leukocytes, enhance microbial clearance, and activate the adaptive immune system to generate long-lasting immunity (16). Cecal ligation and puncture (CLP) is a clinically relevant animal model of sepsis (17) that creates bowel perforation with devitalized tissue and subsequent polymicrobial infection resulting in peritonitis. Therefore, control of the host’s response to overwhelming microbial infection through increased clearance is beneficial by a reduction in inflammatory organ damage and improved survival. Tregs are believed to inhibit adaptive immune responses through inhibition of T cell activation in autoimmune diseases (18). Furthermore, they are also responsive to microbial products through TLRs, which potentiate their in vitro suppressive activity (12). The role of Tregs in innate immune responses such as leukocyte recruitment and bacterial clearance is unknown. In the present study, we sought to address this issue by investigating the effects of adoptive transfer of naïve or in vitro anti-CD3/anti-CD28-stimulated Tregs to CLP mice on septic outcome. Our data show that transfer of stimulated Tregs significantly improves CLP survival through increased bacterial clearance.

Materials and Methods

Mice, CLP, and adoptive transfer

Female BALB/c and BALB/c athymic nude mice were purchased from Harlan, acclimated for 1 wk, and used at 8–10 wk of age. Mice were housed in a room that maintained constant temperature and humidity, and were subjected to one 12-h light/dark cycle per day. Mice received normal rodent chow and water ad libitum. The experiments were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

Mice were anesthetized with an i.p. injection of ketamine HCl (87 mg/kg body weight; Ketaset; Fort Dodge Animal Health) and xylazine (13 mg/kg body weight; The Butler Company). The cecum was exposed aseptically through a 1-cm incision of the lower abdomen, tightly ligated with a 4-0 silk suture below the ileocecal valve without causing bowel obstruction, and punctured through-and-through once with a 27-gauge-diameter needle. The ligated and perforated cecum was replaced in the peritoneal cavity, and surgical incision was closed with 4-0 silk sutures and stainless-steel wound clips (BD Biosciences). All of the mice received 1 ml of prewarmed (37°C) normal saline s.c. for fluid resuscitation and were placed on a heating pad until they recovered from anesthesia. Mice were injected with 3 × 10⁴ CD4⁺ or CD4⁺CD25⁺ T cells in 0.5 ml of sterile HBSS by tail vein just before CLP surgery or 6-h post-CLP surgery. The mice were monitored four times per day for 14 days.
In vitro-stimulated Tregs improve survival dose dependently in a mouse CLP model. A, In vitro Treg suppression of CD4⁺ T cell proliferation is greater with Tregs previously stimulated with IL-2, anti-CD3/anti-CD28 for 3 days. The data represent mean ± SEM of triplicate wells. B, Survival curves of mice receiving either 3 × 10⁵ naive CD4⁺, naive CD4⁺ CD25⁺, or in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP. The data are a composite of two independent experiments, each with similar results. C, Survival curves of mice receiving either 3 × 10⁵ naive CD4⁺ cells or 3 × 10⁶, 3 × 10⁵, or 3 × 10⁴ in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP. D, Survival curves of mice receiving either vehicle, 3 × 10⁵ in vitro-stimulated CD4⁺ CD25⁺ cells, or 3 × 10⁴ in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP.

**Cell culture reagents and condition**

Supplemented RPMI 1640 consisted of RPMI 1640 with 10% FBS, 55 mM 2-ME, and 1% antibiotic-antimycotic. All tissue culture reagents were purchased from Invitrogen Life Technologies. All cells were cultured at 37°C with 5% CO₂.

**Cell isolation and in vitro stimulation of CD4⁺ T cells and CD4⁺ CD25⁺ Tregs**

All cell populations were isolated with Ab-tagged magnetic beads and AutoMACS (Miltenyi Biotec). Mouse CD4⁺ T cells and dendritic cells were isolated from mouse splenocytes by positive selection with anti-CD4 and anti-CD11c microbeads, respectively. CD4⁺ cells isolated in this manner or CD4⁺ CD25⁺ cells stimulated in vitro for 3 days (as described below) were used as control cells in CLP experiments. CD4⁺ CD25⁺ Tregs were isolated from mouse splenocytes with a CD4⁺ CD25⁺ Regulatory T Cell Isolation kit (Miltenyi Biotec). CD4⁺ CD25⁺ T cells or Tregs (5 × 10⁵ cells per well) were stimulated by culture with 4 ng/ml recombinant mouse IL-2 (R&D Systems) for 72 h in 24-well plates, which were precoated with 1 μg/ml anti-mouse CD3 and anti-mouse CD28 Abs (BD Pharmingen) before adoptive transfer into CLP mice. The purity of isolated Tregs was >90% by flow cytometric analysis with anti-CD4 and anti-CD25 staining (data not shown).

**In vitro Treg suppression assay**

CD4⁺ T cells (2 × 10⁴ per well) were cultured with splenic dendritic cells (2 × 10⁴ per well), the indicated number of CD4⁺ CD25⁺ Tregs, and 0.1 mg/ml anti-mouse CD3 Ab in 96-well round-bottom plates for 66 h. Cells were pulsed with 1 μCi/well [³H]thymidine during the last 16 h of incubation. T cell proliferation was determined by the incorporation of [³H]thymidine. To calculate the suppression percentage, the cpm value from each well was divided by the average cpm value from six wells without Tregs.

**Peritoneal wash collection**

Mice were sacrificed at 24 h post-CLP by CO₂ asphyxiation. Two milliliters of sterile PBS was injected into the peritoneal cavity and mixed by gentle squeezing of the abdomen. The peritoneal wash fluid was collected with a syringe and an 18-gauge needle and stored on ice. Samples were removed immediately for bacterial CFU analysis, and then cells were pelleted by centrifugation for differential analysis, and supernatants were aliquoted and stored at −80°C for cytokine/chemokine analysis.

**Measurement of peritoneal and plasma cytokines/chemokines**

Peritoneal lavage fluid was subjected to ELISA with the use of Mouse Quantikine kits (R&D Systems) according to the manufacturer’s instructions. Absorbance readings were obtained on a Versamax microplate reader (Molecular Devices), and data were analyzed with Microsoft Excel 2000 software. Cytokines/chemokines in EDTA plasma were analyzed at Rules-based Medicine by a proprietary technology similar to luminex for cytokines and chemokines.

**Measurement of peritoneal bacterial CFU**

Peritoneal fluid was analyzed for bacterial CFU/milliliter by dilution plating onto trypticase soy agar with 5% sheep blood (BBL; BD Biosciences) and colony counting following overnight incubation.

**Coulter count, cytopsin, and differential determination**

The peritoneal cell suspension was centrifuged at 350 × g for 10 min at 4°C in a Beckman GPR centrifuge. The supernatant was discarded, and the cell pellet resuspended in 1 ml of Dulbecco’s PBS (DPBS; Invitrogen Life Technologies). From this cellular suspension, the number of infiltrating leukocytes was enumerated with a Z2 Coulter Particle Counter and Size Analyzer (Beckman Coulter). The average of two counts was recorded. Instrument settings were as follows: aperture size, 100 μm; lower threshold (TL), 3 μm; count mode above TL. Differentiation of cell populations was

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**FIGURE 1.** In vitro-stimulated Tregs improve survival dose dependently in a mouse CLP model. A, In vitro Treg suppression of CD4⁺ T cell proliferation is greater with Tregs previously stimulated with IL-2, anti-CD3/anti-CD28 for 3 days. The data represent mean ± SEM of triplicate wells. B, Survival curves of mice receiving either 3 × 10⁵ naive CD4⁺, naive CD4⁺ CD25⁺, or in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP. The data are a composite of two independent experiments, each with similar results. C, Survival curves of mice receiving either 3 × 10⁵ naive CD4⁺ cells or 3 × 10⁶, 3 × 10⁵, or 3 × 10⁴ in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP. D, Survival curves of mice receiving either vehicle, 3 × 10⁵ in vitro-stimulated CD4⁺ CD25⁺ cells, or 3 × 10⁴ in vitro-stimulated CD4⁺ CD25⁺ cells just before CLP.
determined by microscopic morphology of the cells. Cell concentration was adjusted in a cytofunnel (Thermo Electron Corporation) to $1 \times 10^5$ cells/funnel with the addition of DPBS. Cells were centrifuged at 700 rpm for 10 min (Cytospin 2; Shandon/Thermo Electron Corporation). Slides were stained in HEMA 3 staining set (Fisher Scientific), rinsed in Millipore water, and dried at room temperature. Leukocytes were classified as neutrophils, eosinophil, lymphocytes, macrophage, or mast cells. A percentage of each population was estimated by counting 100 cells, twice, in various random fields by two blinded independent observers. The mean for each cell type was then calculated.

**Statistical analysis**

Data are expressed as the mean ± SEM. Survival analysis was done by the Kaplan-Meier method, and statistical comparisons between groups were performed by a log rank test with JMP 4.0.4 software (SAS Institute). Statistical comparisons for all other data were conducted by ANOVA analysis with JMP 4.0.4 software. Data that were skewed were transformed by Box Cox transformation and analyzed by ANOVA with JMP 4.0.4 software. A value of $p < 0.05$ was considered significant.

**Results**

**Adoptive transfer of in vitro-stimulated Treg just before CLP improves survival in a dose-dependent fashion**

To examine the effect of Tregs on septic outcome, we adoptively transferred $3 \times 10^5$ naive syngeneic Tregs into BALB/c mice just before CLP and followed the mice for survival up to 2 wk. Mice that received unstimulated Tregs exhibited only 53% mortality at 2 wk, whereas mice that received either vehicle or naive CD4$^+$ cells exhibited 67% and 70% mortality, respectively (data not shown; $n = 30$, $p = 0.0507$ for naive CD4$^+$ vs naive CD4$^+$). It has been shown that both polyclonal and Ag-specific stimulation of Tregs results in increased suppressive activity in vitro and in vivo (19–21). To determine whether in vitro-stimulated Tregs might have a greater effect on CLP survival, we adoptively transferred $3 \times 10^5$ Tregs stimulated in vitro with IL-2, anti-CD3, and anti-CD28 for 3 days just before CLP. An in vitro Treg suppression assay was used to measure their suppressive activity (11). As expected, the in vitro-stimulated Tregs exhibited significant enhanced suppression of CD4$^+$ T cell proliferation in vitro (Fig. 1A, $p < 0.05$). Adoptive transfer of in vitro-stimulated Tregs resulted in a significant improvement in 2-wk survival of CLP mice compared with groups receiving either naive CD4$^+$ or nonstimulated Tregs (Fig. 1B; $p < 0.005$ and 0.02, respectively). Furthermore, the improvement in survival with stimulated Tregs resulted in a significant improvement in 2-wk survival of CLP mice compared with groups receiving either naive CD4$^+$ or nonstimulated Tregs (Fig. 1B; $p < 0.005$ and 0.02, respectively). Because in vitro-stimulated Tregs exhibited a significant improvement in survival, all subsequent experiments were done with these cells in our studies.

**The effect of Treg transfer on CLP survival is host T cell dependent and occurs with therapeutic administration**

Treg activity on suppression of immune responses has been demonstrated to be mediated through suppression of T cell activation both in vitro and in vivo (3, 7, 10, 11). To determine whether the improved survival of CLP mice by Treg transfer is also mediated...
through host T cells in vivo, we adoptively transferred syngeneic Tregs into BALB/c athymic nu/nu mice just before CLP. Interestingly, unlike our observations in wild-type mice, we did not observe any effect of Tregs on improved survival in CLP nude mice, suggesting that host T cells are required for the protective effect (Fig. 2A). To test whether therapeutic treatment with Tregs could also improve survival, we adoptively transferred syngeneic Tregs to BALB/c mice at 6 h post-CLP and monitored survival for 2 wk. The delayed administration of $3 \times 10^5$ Tregs also resulted in a significant improvement in survival compared with transferred naive CD4$^+$ or in vitro-stimulated CD4$^+$CD25$^+$ cells just before CLP. The data are represented as mean ± SEM. There were no detectable mast cells in mice that received naive CD4$^+$ cells. C, Peritoneal TNF-α levels at 24 h post-CLP in mice that received either $3 \times 10^5$ naive CD4$^+$ or in vitro-stimulated CD4$^+$CD25$^+$ cells just before CLP. The data are represented as mean ± SEM.

### Table I. Comparison of plasma cytokines, peritoneal cytokines/chemokines, and peritoneal leukocytes in mice at 24 h post-CLP with adoptive transfer of naive CD4$^+$ versus in vitro-stimulated CD4$^+$CD25$^+$ cells

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Control</th>
<th>Naive CD4$^+$</th>
<th>Stimulated CD4$^+$CD25$^+$</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma IL-2 (pg/ml)</td>
<td>10</td>
<td>23 ± 5</td>
<td>90 ± 17</td>
<td>94 ± 27</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/ml)</td>
<td></td>
<td>BLD</td>
<td>715 ± 96</td>
<td>869 ± 278</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma IL-10 (pg/ml)</td>
<td>208</td>
<td>± 15</td>
<td>724 ± 111</td>
<td>855 ± 145</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma TNF-α (pg/ml)</td>
<td></td>
<td>BLD</td>
<td>461 ± 104</td>
<td>330 ± 92</td>
<td>NS</td>
</tr>
<tr>
<td>Peritoneal IL-6 (pg/ml)</td>
<td>5</td>
<td>BLD</td>
<td>2,757 ± 302</td>
<td>3,090 ± 530</td>
<td>NS</td>
</tr>
<tr>
<td>Peritoneal TNF-α (pg/ml)</td>
<td></td>
<td>BLD</td>
<td>32 ± 16</td>
<td>96 ± 30</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Peritoneal MCP-1 (pg/ml)</td>
<td></td>
<td>BLD</td>
<td>2,141 ± 351</td>
<td>2,650 ± 330</td>
<td>NS</td>
</tr>
<tr>
<td>Peritoneal eotaxin (pg/ml)</td>
<td></td>
<td>BLD</td>
<td>254 ± 44</td>
<td>284 ± 57</td>
<td>NS</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>ND</td>
<td>8</td>
<td>584,257 ± 194,289</td>
<td>443,782 ± 133,709</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>ND</td>
<td>37,439 ± 11,291</td>
<td>31,298 ± 16,855</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>ND</td>
<td>1,316,562 ± 211,787</td>
<td>1,313,689 ± 304,736</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>ND</td>
<td>0</td>
<td>4,115 ± 1,776</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>ND</td>
<td>20,147 ± 7,717</td>
<td>31,584 ± 9,594</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Control mice were age-matched naive female BALB/c mice. Peritoneal cell counts are in cells/milliliter. BLD, Below limit of detection.
Adoptive transfer of stimulated Tregs leads to improved bacterial clearance and increased recruitment of peritoneal mast cells and TNF-α production

To understand the mechanism of action of adoptively transferred Tregs on CLP survival, we examined peritoneal bacterial load in CLP mice at 24 h post-CLP. Mice that received Tregs just before CLP exhibited significantly lower peritoneal bacterial load compared with mice that received naive CD4+ T cells (205 ± 108 vs 1891 ± 520 × 10^5 CFU/ml); Fig. 3A; p < 0.002). To determine whether the increased bacterial clearance was associated with increased leukocyte infiltration into the peritoneum, peritoneal lavages from mice at 24 h post-CLP were examined by cytoospin and differential cell counts. Significant cell infiltration of mononuclear cells, neutrophils, eosinophils, and lymphocytes was clearly evident in both the Treg and naive CD4+ treated groups (Table I). The majority of infiltrated cells were neutrophils in both groups, which constituted ~70% of total cells. Interestingly, animals that received Tregs were found to have significantly greater numbers of mast cells in peritoneal lavages (Table I; Fig. 3B; p < 0.02), with no other notable differences.

To determine whether adoptive transfer of Tregs suppressed systemic or peritoneal cytokine/chemokine production, plasma and peritoneal lavage fluids were analyzed for either TNF-α, IL-6, IL-2, IL-10, eotaxin-1, or MCP-1 at 24 h post-CLP. There were no significant differences between the groups in plasma levels of IL-6, IL-2, IL-10, or TNF-α, although both groups exhibited elevated plasma levels relative to control mice as expected (Table I). Analysis of TNF-α, IL-6, MCP-1, and eotaxin in peritoneal lavage fluids indicated that these markers were significantly elevated above control naïve mice in both groups as expected (Table I). However, only TNF-α was significantly higher in the group that received Tregs (Fig. 3C; p < 0.03).

Discussion

This is the first study that demonstrates that administration of Tregs in a clinically relevant model of sepsis significantly improves survival. Furthermore, we have demonstrated that in vitro stimulation of Tregs before adoptive transfer results in increased potency for improving survival, which is host T cell dependent. In vitro stimulation of Tregs has been shown to enhance suppressor function (19), and other studies have shown that adoptive transfer of in vitro-stimulated Tregs can improve outcome in several disease models including graft-vs-host disease, murine colitis, and autoimmune diabetes (19–21). Although we did not examine the classical suppressor function in vivo of adoptively transferred Tregs in CLP mice, we suggest that this classical suppressor function is not a plausible explanation for the improved survival we observed. The idea that suppression of endogenous T cell function could lead to improved CLP survival is not supported by our observation that nude mice, which lack T cells, exhibit greater mortality with CLP. In addition, previous studies have demonstrated that RAG−/− mice that lack an adaptive immune system also exhibit greater mortality in CLP than wild-type mice, which could be ameliorated by adoptive transfer of syngeneic T cells (22, 23). Furthermore, promotion of T cell survival through inhibition of lymphocyte apoptosis improved outcome in CLP mice (22, 24, 25), and host immunosuppression accompanying sepsis (decreased T cell proliferation and cytokine production) is believed to contribute to poor outcome and is thought to be due in part to apoptosis of host lymphocytes (26, 27). Taken together, the evidence indicates that proper T cell function plays an important role in survival following CLP. However, these studies did not address whether the protective role of T cells is due to Tregs or effector T cells. Our study is the first one to demonstrate that Tregs improve sepsis survival through host T cells. It is also possible that the observed improvement in survival was due to potential contamination with effector memory CD4+ T cells directed against host bacterium, which were isolated along with Tregs. Although we tried to detect any memory CD4+ T cells in our Treg preparation by staining for CD45RB expression (data not shown), this marker was unable to distinguish these two cell populations because both expressed low levels of CD45RB. Unfortunately, there are currently no well-defined cell surface markers that allow for clear discrimination between these two cell populations. However, we believe that the improved survival by the administered Tregs was not simply due to potential contamination with memory CD4+ T cells based on the following: 1) unlike memory CD4+ T cells, the isolated Treg population used in these studies exhibited potent suppressor function in vitro (Fig. 1A); 2) these cells failed to proliferate in the presence of anti-CD3 stimulation in vitro (data not shown); 3) these cells expressed FoxP3 mRNA by TaqMan analysis, a Treg-specific transcription factor (data not shown); and 4) transfer of these cells into nude mice led to no improvement in survival (Fig. 2A), which one might expect if the effect were due to effector memory T cells to host bacteria.

The improvement in survival accompanying adoptive transfer of stimulated Tregs could be attributed to the increased peritoneal bacterial clearance we observed in these animals. We have recently demonstrated the importance of bacterial clearance with outcome in a rat CLP model (28). Consistent with our previous findings, improved bacterial clearance was observed with adoptive transfer of stimulated Tregs, suggesting a more effective innate immune response with a concomitant improvement in survival. We did observe greater peritoneal TNF-α levels in animals that received Tregs at 24 h post-CLP, suggesting that sepsis-associated immunosuppression may have been less with Treg transfer, although we did not investigate this in the present study. We also observed greater mast cell recruitment into the peritoneum with Treg transfer. Mast cell-deficient mice are less efficient in clearing enterobacteria than wild type (29), and purified mast cells have been shown to release TNF-α upon stimulation with bacteria (30). Current thought suggests that mast cells modulate neutrophil influx into the peritoneum accompanying local bacterial infection through TNF-α (31). We did not see an increase in peritoneal neutrophil numbers at 24 h post-CLP with adoptive transfer of Tregs, although we did not look at earlier time points in this study.

Previous in vivo studies with adoptively transferred Tregs (3, 8, 20, 21) have demonstrated immunosuppressive effects, yet surprisingly in our study we did not observe an immunosuppressive effect on local or systemic inflammation accompanying adoptive transfer of Tregs in mouse CLP. In fact, we actually observed greater peritoneal TNF-α production with Treg transfer. This could indicate that, under septic conditions, Tregs may have unique and undefined functional roles in the innate immune response.

In summary, we have shown that the adoptive transfer of in vitro-stimulated Tregs, but not CD4+CD25− cells, significantly improves survival in a mouse CLP model. Furthermore, we have demonstrated that the effect on survival is accompanied by improved peritoneal bacterial clearance, enhanced peritoneal mast cell recruitment, and TNF-α production. Further studies are warranted to examine the role of Tregs in the innate immune response accompanying sepsis.

Disclosures

The authors have no financial conflict of interest.
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