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Overexpression of Suppressor of Cytokine Signaling-5 in T Cells Augments Innate Immunity during Septic Peritonitis

Hiroyuki Watanabe,† Masato Kubo,‡ Kosuke Numata,† Katsumasa Takagi,† Hiroshi Mizuta,† Seiji Okada,§ Takaaki Ito,* and Akihiro Matsukawa2*¶

Suppressors of cytokine signaling (SOCS) proteins are negative regulators of cytokine signaling by inhibiting the JAK-STAT signal transduction pathway, but their role in innate immunity remains to be investigated. In the present study, we demonstrate that overexpression of SOCS5 in T cells augments innate immunity during septic peritonitis induced by cecal ligation and puncture (CLP). Mice with a cell-specific overexpression of SOCS5 in T cells (SOCS5 transgenic (Tg)) were resistant to the lethality relative to the wild-type (WT) mice. This was most likely due to the enhanced innate immunity in SOCS5Tg mice, as bacterial burden in SOCS5Tg mice was significantly lower than WT mice. Accumulation of neutrophils and macrophages was augmented in SOCS5Tg mice, an event that was accompanied by increased peritoneal levels of IL-12, IFN-γ, and TNF-α. In vitro bactericidal activities of macrophages and neutrophils were enhanced in SOCS5Tg mice. Both neutrophils and macrophages from WT mice adopted enhanced bacterial killing activity when cocultured with CD4+ T cells from SOCS5Tg mice, relative to CD4+ T cells from WT mice. Adoptive transfer of SOCS5Tg-CD4+ T cells into T- and B cell-deficient RAG-2-/- mice resulted in augmented leukocyte infiltration and increased peritoneal levels of IL-12, IFN-γ, and TNF-α after CLP, as compared with the controls. Furthermore, CLP-induced bacterial burden in RAG-2-/- mice harboring SOCS5Tg-CD4+ T cells was significantly reduced relative to the controls. These findings provide evidence that intervention of SOCS5 expression in T cells affects innate immunity, which highlight a novel role of T cells during sepsis. The Journal of Immunology, 2006, 177: 8650–8657.

Sepsis is a severe illness caused by overwhelming infection of the bloodstream by toxin-producing bacteria that can originate anywhere in the body, the frequent sites of which are lung, urinary tract, and abdomen (1, 2). Sepsis may be initialized by intense local inflammation to inactivate and clear the invading pathogens (3, 4). Evidence indicates that cytokines that include IL-10, IL-12, and IL-13 control a wide variety of inflammatory responses during sepsis (5–8).

Many of these cytokines exert their biological functions through the JAK/STAT pathway (9, 10). STAT3/4/6 are transcription factors that mediate IL-10/IL-12/IL-13 cytokine signaling, respectively. We have heretofore shown in a murine model of septic peritonitis that mice with targeted disruption of the Stat3 gene in macrophages and neutrophils exhibit increased lethality by exaggerated local and systemic inflammation through overzealous production of cytokines (11). STAT4- and STAT6-deficient mice are resistant to the lethality by balancing local type 1 and systemic type 2 cytokine responses (12). Thus, Stat proteins are crucial in innate immunity during septic peritonitis.

Balanced cytokine responses are important in immune system. Several mechanisms for negative regulation of cytokine signaling have been recently identified. Suppressor of cytokine signaling (SOCS)1 proteins are a family of Src homology 2 domain-containing cytoplasmic proteins that complete a negative feedback loop to attenuate signal transduction from cytokines that act through the JAK/STAT pathway (13, 14). SOCS5, a member of SOCS proteins, is predominantly expressed in Th1 cells and inhibits the Th2 immune responses, indicating a regulatory role of SOCS5 in Th1 and Th2 balance (15). However, little is known about the role of SOCS5 in innate immunity. Because T cells are present in the peritoneal cavity even on a steady basis (Thy1.2-positive cells, 11–16%, our own data by flow cytometry), SOCS5 in T cells may affect innate immunity during septic peritonitis, in which a type 1 response is important.

In this study, we have extended our previous work and attempted to explore the role of SOCS5 in innate immunity during sepsis. For this purpose, we here used mice with a cell-specific overexpression of SOCS5 in T cells (SOCS5 transgenic (Tg)) (15). The mice underwent a well-established murine septic peritonitis model, cecal ligation and puncture (CLP), a clinically relevant model of intra-abdominal sepsis (16). We here provide evidence that SOCS5 in T cells serves a beneficial role in host defense during septic peritonitis by augmenting the innate immune responses. Thus, innate immunity can be controlled under T cells, particularly by SOCS5.

Materials and Methods

Mice

The Flag-tagged SOCS5 was expressed under the control of the lck proximal promoter Egr enhancer and backcrossed with C57BL/6J mice over 10 generations, as described (15). In the mice, SOCS5 was overexpressed in...
were positively purified with anti-mouse CD4 microbeads using Midi-Teria. After a 4-h culture in a 5% CO2 incubator, plates were placed at recovered from mice undergone CLP. Control wells contained only bac-
cells were washed three times with RPMI 1640, after which CD4

described above.

In vitro bactericidal activities of leukocytes

In vitro bactericidal activities of the cells were determined by a classical CFU assay with minor modifications (11, 19). In brief, cells (3 × 10^6/well) were infected with 1 × 10^6 CFU of live bacteria recovered from mice undergone CLP. Control wells contained only bac-
teria. After a 4-h culture in a 5% CO2 incubator, plates were placed at 37°C for 3 h and the colonies were counted. Bacterial activity was expressed as the percentage of bacteria death = (CFU from control wells (without cells) − CFU from experimental wells)/CFU from control wells (without cells) × 100. In other experiments, bacterial activities of cells were assessed in the presence of CD4+ T cells (1 × 10^6/well) from nontreated WT mice or SOCS5Tg mice. CD4+ T cells were purified from spleens as described above.

Cell culture

Macrophages (1 × 10^6/ml) and CD4+ T cells (0.5 × 10^6/ml) were cocul-
tured in RPMI 1640 supplemented with 5% FCS, glutamine, and antibiotics in a 5% CO2 incubator for 24 h with a TLR4 ligand, Escherichia coli LPS (100 ng/ml, 0111:B4; Difco Laboratories) and in the presence of rabbit anti-murine IFN-γ IgG or control IgG (10 μg/ml). The culture supernatants were used for measurements of cytokines. In some experiments, cells were cultured in a dual chamber transwell culture apparatus (Corning Scientific Products), in which macrophages were cultured in a lower chamber and CD4+ T cells were done in an upper chamber.

Superoxide production

The production of superoxide was measured using a highly reactive oxygen species detection reagent, aminophenyl fluorescein (APF; Daichi Pure Chemicals), as described (20). In brief, macrophages and neutrophils (2 × 10^6 cells/ml), prepared as described above, were stimulated with LPS (100 ng/ml) for 24 h in phenol red-free RPMI 1640 in the presence of APF (1 mM), after which the culture supernatants were harvested. Superoxide production was measured spectrophotometrically using FLX800 Fluores-
cence Microplate Reader (Bio-Tek Instruments). The excitation wave-
length was 490 nm and the emission was 515 nm.

Measurements of cytokines and myeloperoxidase (MPO)

Murine cytokines were quantitated using a standard method of sandwich ELISA as previously described (6, 18). The captured Abs, detection Abs, and the recombinant cytokines were purchased from R&D Systems. The ELISAs used in this study did not cross-react with other murine cytokines available. MPO levels in the kidney were measured by ELISA kit (Cal-
biotech). The kidneys were homogenized in PBS containing 0.1% Triton X-100 and complete protease inhibitor (Roche), centrifuged, and the cleared supernatants were obtained. Protein concentrations in the extracts were measured by protein dye-binding assay (Bio-Rad).

Clinical chemistry

Serum levels of blood urea nitrogen (BUN) and creatinine were measured using standardized techniques.

Western blotting

Leukocytes were dissolved in Laemmli buffer (1 × 10^6/50 μl), sonicated, boiled, fractionated on SDS-polyacrylamide gel (10 μl), and transferred to a nitrocellulose membrane. After blocking with TBST containing 5% skim milk at room temperature, the membrane was incubated with Abs to STAT6 or control rabbit IgG at 5 μg/ml. The membrane was washed, the reaction was developed with diaminobenzidine (Sigma-Aldrich). Counterstaining was done with hematoxylin.

Immunocytochemistry

Cytospin preparations of peritoneal exudates at 24 h after CLP were immediately fixed in 100% methanol. After blocking endogenous peroxidase using 0.3% H2O2 in methanol, the slides were rehydrated in TBS and blocked with 10% normal goat serum for 1 h at room temperature. The slides were incubated with anti-tyrosine-phosphorylated STAT6 (Cell Signaling) overnight at 4°C. After washing with TBST, the membrane was incubated with anti-
HRP-linked Ab for 1 h at room temperature and visualized with an ECL system (Cell Signaling), photographed, digitized, and the band densities were measured with NIH image.

Flow cytometry analysis

Peritoneal cells were harvested from nontreated WT and SOCS5Tg mice and the cells (1 × 10^6 cells/ml) were suspended in PBS supplemented with 2% FCS and 0.1% sodium azide. Cells were stained with mAb specific for mouse CD4, CD8, CD44, CD62L, and CD69 (BD Pharmingen). Stained cells were analyzed using a FACS Calibur (BD Biosciences).

Statistics

Statistical significance was determined by ANOVA. In case of survival curve and CFU count, the data were analyzed by the log-rank test and Mann-Whitney U test, respectively. A p value <0.05 was regarded as statistically significant. All data were expressed as mean ± SEM.
Results
Improved survival to CLP in SOCS5Tg mice
To understand whether overexpression of SOCS5 in T cells would affect host defense during CLP, initial studies were conducted to determine mice survival in SOCS5Tg and WT mice after CLP. As shown in Fig. 1, survival rate in SOCS5Tg mice was significantly higher than that in WT mice. Of the 29 SOCS5Tg mice, 15 survived for 7 days (51.7%); whereas only 7 of 30 WT mice survived for 7 days (23.3%) (p < 0.001). Thus, SOCS5Tg mice were resistant to the lethality induced by CLP, thereby indicating that overexpression of SOCS5 in T cells was beneficial in host defense during septic peritonitis.

Augmented bacterial clearance in SOCS5Tg mice
CLP-induced lethality is firmly linked with bacterial burden (17, 18). In an attempt to identify the basis whereby SOCS5Tg mice were resistant to CLP, we first examined the bacterial load in the peritoneum after CLP. Although bacterial burden at 6 h post-CLP was unchanged between SOCS5Tg and WT mice (data not shown), SOCS5Tg mice exhibited an enhanced bacterial clearance at 24 h post-CLP, as indicated by smaller numbers of recovered CFU counts in the peritoneum (Fig. 2). At this time point, the bacterial load recovered from peripheral blood in SOCS5Tg mice was 23-fold lower than that in WT mice, although it was not statistically significant (p = 0.107, Fig. 2).

Phagocytes, such as neutrophils and macrophages, are cells responsible for clearing bacteria (21–23). Experiments were next conducted to examine bactericidal activities of neutrophils and macrophages. For this purpose, neutrophils and macrophages from WT and SOCS5Tg mice were infected with live bacteria recovered from the peritoneum of WT mice that had undergone CLP. As shown in Fig. 3A, bactericidal activities of macrophages from SOCS5Tg mice were higher than those from WT mice. There was a trend toward increase in SOCS5Tg neutrophils. Supernoxide generation, an effector molecule for bacterial killing (21), was subsequently examined in the culture supernatants of LPS-stimulated neutrophils and macrophages, which demonstrated that cells from SOCS5Tg mice produced higher levels of superoxide generation than those from WT mice (Fig. 3B). Thus, SOCS5Tg mice cleared bacteria more effectively than WT mice, possibly due to enhanced bactericidal activities of phagocytes.

Enhanced local inflammation in SOCS5Tg mice
The leukocyte infiltration in the peritoneum after CLP was next investigated, which demonstrated that the numbers of infiltrating neutrophils and macrophages were significantly augmented in SOCS5Tg mice, resulting in a 1.5- (at 24 h) and 2.1-fold (at 6 h) increase, respectively, relative to the WT mice (Fig. 4A). Consequently, cytokine levels in the peritoneum after CLP were measured. The data in Fig. 4B demonstrated that levels of IL-12 and IFN-γ were elevated relative to the WT mice. In addition, TNF-α level in SOCS5Tg mice was significantly higher than that in WT mice (Fig. 4B). These cytokines are known to enhance bacterial clearance in a variety of infectious models including septic peritonitis (8, 24, 25). Anti-inflammatory cytokines were also measured, which showed that no appreciable levels of IL-4 and IL-13 were detected in the peritoneum from WT and SOCS5Tg mice after CLP. There were no statistical differences in the peritoneal IL-10 level between WT and SOCS5Tg mice (6 h: 1.0 ± 0.3 vs 1.3 ± 0.3 ng/cavity, 24 h: 2.1 ± 0.5 vs 1.1 ± 0.3 ng/cavity, respectively, eight mice each, not significant). Upon stimulation with LPS, peritoneal macrophages from SOCS5Tg mice produced higher levels of TNF-α and IL-12, but not IFN-γ, relative to the controls (Table I). No substantial IL-4 and IL-13 were detected (data not shown). IL-10 level was not significant between WT and
SOCS5Tg macrophages (Table I). STAT6 activation in peritoneal exudates at 24 h post-CLP were subsequently examined, which showed that the expression of phosphorylated STAT6 (pSTAT6) was reduced in SOCS5Tg mice compared with that showed that the expression of phosphorylated STAT6 (pSTAT6) was reduced in SOCS5Tg mice. At 6 and 24 h after CLP, mice were killed, bled, and the peritoneal fluids were harvested. A. The numbers of infiltrating leukocytes in the peritoneum were counted. ○, WT mice; ●, SOCS5Tg mice. B. Peritoneal levels of IL-12, IFN-γ, and TNF-α were measured by ELISA. □, WT mice; ■, SOCS5Tg mice. The data represent the mean ± SEM of 8–11 estimations from separate mice. †, p < 0.05 vs WT mice.

Adoptive transfer of resident CD4+ T cells augments innate immune response

These results suggest that overexpression of SOCS5 in T cells affects innate immune response during septic peritonitis. We then asked whether CD4+ T cells could be responsible for the enhanced innate immune response in SOCS5Tg mice. For this, phagocytes (neutrophils and macrophages) from WT mice were cocultured with CD4+ T cells from WT or SOCS5Tg mice and the cells were infected with live bacteria recovered from CLP mice, after which the bacterial killing activities of phagocytes were examined. The data in Fig. 6A showed that both neutrophils and macrophages exhibited enhanced bacterial killing activities in the presence of CD4+ T cells from SOCS5Tg mice, as compared with the controls.

Table I. Cytokine production from peritoneal macrophages

<table>
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<th>IL-12</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>IL-10</th>
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<tr>
<td>Medium</td>
<td>0.20 ± 0.02</td>
<td>0.09 ± 0.00</td>
<td>0.38 ± 0.04</td>
<td>2.28 ± 0.09</td>
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<tr>
<td>LPS</td>
<td>0.21 ± 0.01</td>
<td>0.10 ± 0.00</td>
<td>0.46 ± 0.02</td>
<td>2.29 ± 0.08</td>
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To further investigate the role of CD4+ T cells during CLP, CD4+ T cells from WT or SOCS5Tg mice were transferred into RAG-2−/− mice, T- and B cell-deficient mice, and the mice underwent CLP. As a result, mice harboring SOCS5Tg-CD4+ T cells cleared bacteria more effectively than those with WT-CD4+ T cells (Fig. 6B). The numbers of infiltrating leukocytes were increased in mice with SOCS5Tg-CD4+ T cells (Fig. 6C). RAG-2−/− mice received SOCS5Tg-CD4+ T cells produced significantly higher levels of IL-12 and IFN-γ in the peritoneum relative to the mice with control CD4+ T cells. TNF-α level was also increased by ~150%, albeit they were not statistically significant (p = 0.12) (Fig. 6D). No apparent IL-4 and IL-13 was detected in the peritoneum (data not shown). There was no statistical differences in the peritoneal IL-10 level between mice with WT- and SOCS5Tg-CD4+ T cells (0.62 ± 0.14 vs 0.87 ± 0.16 ng/cavity, respectively, 10 mice each, not significant). Accordingly, these data clearly indicate that SOCS5 in T cells is responsible for the skewed innate immune response in this particular model.

To understand the mechanism by which SOCS5Tg-CD4+ T cells influence the innate immune response, WT macrophages were stimulated with LPS in the presence of WT- or SOCS5Tg-CD4+ T cells, after which a proinflammatory cytokine TNF-α level in the culture supernatants was measured. As shown in Fig. 6E, TNF-α level was augmented when WT macrophages were cocultured with SOCS5Tg-CD4+ T cells as compared with WT-CD4+ T cells, suggesting that SOCS5Tg-CD4+ T cells might augment TNF-α production. The augmented TNF-α level was not found when cells were cultured using transwell apparatus (WT-CD4+ T cells vs SOCS5Tg-CD4+ T cells = 1.06 ± 0.03 vs 1.09 ± 0.03 ng/ml, respectively, n = 5, not significant). In addition, there was no difference in TNF-α level when macrophages were cultured with LPS supernatants of WT- or SOCS5Tg-CD4+ T cells (0.99 ± 0.01 vs 0.95 ± 0.04 ng/ml, n = 5, not significant).

To examine the molecular mechanism behind the augmented TNF-α, cocultured cells were stimulated with LPS in the presence of neutralizing anti-IFN-γ IgG. As a result, the augmentation in TNF-α level by SOCS5Tg-CD4+ T cells was completely abrogated. Thus, SOCS5Tg-CD4+ T cells appear to influence TNF-α production through IFN-γ production.

FIGURE 4. Leukocyte infiltration and production of cytokines in SOCS5Tg mice. At 6 and 24 h after CLP, mice were killed, bled, and the peritoneal fluids were harvested. A. The numbers of infiltrating leukocytes in the peritoneum were counted. ○, WT mice; ●, SOCS5Tg mice. B. Peritoneal levels of IL-12, IFN-γ, and TNF-α were measured by ELISA. □, WT mice; ■, SOCS5Tg mice. The data represent the mean ± SEM of 8–11 estimations from separate mice. †, p < 0.05 vs WT mice.

FIGURE 5. STAT6 activation in SOCS5Tg mice. A. Infiltrating leukocytes were harvested at 24 h after CLP and the lysates were immunoblotted with anti-STAT6 or anti-tyrosine-phosphorylated STAT6. Upper, Representative photos. Lower, Blots were photographed, digitized, and the densities were measured with NIH image. The pSTAT6/STAT6 ratio was calculated and the data were expressed as relative index from four independent experiments. †, p < 0.05 vs WT controls. B. Peritoneal exudate cells at 24 h post-CLP were harvested from WT mice and the cells were stained with anti-pSTAT6 IgG. A representative photograph. In addition to cytoplasm, nuclei were faintly stained. Original magnification, ×400.
The nature of peripheral T cells in SOCS5Tg mice

The different response of SOCS5Tg-CD4⁺ T cells may result from different nature of peritoneal T cells. We then examined the surface marker expressions of peritoneal T cells in these nontreated mice. Representative data are shown in Fig. 7, which demonstrated that CD4⁺ T cells were similar between WT- and SOCS5Tg-CD4⁺ T cells. An early activation marker CD69⁺ was not increased both in WT- and SOCS5Tg-CD4⁺ T cells. Interestingly, CD4⁺CD44⁺ double-positive T cells (memory T cells) were increased in SOCS5Tg mice whereas CD4⁺CD62L⁻ double-positive cells (naive T cells) were decreased (Fig. 7). Thus, SOCS5Tg mice showed distinct phenotype of peritoneal CD4⁺ T cells as compared with the controls.

Attenuated renal injury in SOCS5Tg mice

Sepsis frequently causes multiple organ failure. Kidney is a major target organ during sepsis, and the dysfunction can be fatal to the

![FIGURE 6. CD4⁺ T cells from SOCS5Tg augment innate immune responses. A, Phagocytes (neutrophils and macrophages, 3 × 10⁶/ml) cocultured with CD4⁺ T cells (1 × 10⁶/ml) from either WT mice or SOCS5Tg mice were infected with 1 × 10⁶ CFU of live bacteria recovered from CLP mice and the killing activities of phagocytes were examined. Shown are representative data from three independent experiments. B–D, CD4⁺ T cells from WT or SOCS5Tg mice were transferred into RAG-2⁻/⁻ mice (3 × 10⁶ cells/peritoneum, 10 mice each) and the mice underwent CLP. At 24 h post-CLP, mice were killed and the peritoneal fluids and peripheral blood were harvested. B, Ten microliters of peritoneal fluids and blood were serially diluted and plated on TSA-blood plates. Line represents mean CFU count. C, The numbers of neutrophils and macrophages were counted. D, Peritoneal cytokine levels were measured. E, WT macrophages (1 × 10⁶/ml) were cocultured with WT-CD4⁺ T cells (●) or SOCS5Tg-CD4⁺ T cells (■) (0.5 × 10⁶/ml), and the cells were stimulated with LPS (100 ng/ml) in the presence of control IgG or neutralizing anti-IFN-γ IgG (10 μg/ml). Twenty-four hours later, the culture supernatants were harvested and TNF-α level was measured. Shown are representative data from two independent experiments. ‡, p < 0.05; †, p < 0.01; §, p < 0.001; ¶, p < 0.0001 vs controls.

![FIGURE 7. Representative flow cytometry data of peritoneal T cells. Peritoneal cells were harvested from nontreated WT and SOCS5Tg mice, and the cells were stained with CD4, CD8, CD44, CD62L, and CD69. Percentages for individual cell types are shown. Data are from two to three independent experiments with similar results.

![FIGURE 8. Ameliorated renal injury in SOCS5Tg mice. At 24 h after CLP, mice were killed, bled, and the sera were harvested. A, BUN and creatinine level in sera was measured (WT, 11 mice; SOCS5Tg, 13 mice). ‡, p < 0.05; †, p < 0.01 vs WT mice. B, Shown are representative histological sections of the kidney (H&E) at 24 h after CLP. Original magnification, ×200.]

8654 SOCS5 IN T CELLS DURING SEPTIC PERITONITIS by guest on April 17, 2017 http://www.jimmunol.org/ Downloaded from
host (1, 6, 22). Effective bacteria killing in SOCS5Tg mice may attenuate systemic inflammatory response and tissue damage induced by CLP. As shown in Fig. 8A, renal injury was reduced in SOCS5Tg mice, as evidenced by decreased serum levels of BUN and creatinine at 24 h after CLP relative to the WT mice. RAG-2−/−mice transferred with SOCS5Tg-CD4+ T cells were also exhibited decreases in the BUN and creatinine level at 24 h after CLP as compared with the mice received WT-CD4+ T cells (BUN: 42.9 ± 4.2 vs 62.5 ± 6.6 mg/dl, creatinine: 0.21 ± 0.03 vs 0.38 ± 0.06 mg/dl, respectively, 10 mice each, p < 0.05). In WT mice underwent CLP, epithelial cells in proximal tubules demonstrated karyorrhexis and granary pink cytoplasm of epithelial cells in proximal tubules, indicative of acute tubular necrosis. In contrast, CLP caused little histological change in the SOCS5Tg mice (Fig. 8B). MPO level in the kidney, a marker of neutrophil infiltration, was decreased, and renal levels of TNF-α, MIP-2, and KC were significantly reduced in SOCS5Tg mice, compared with WT mice (Fig. 9). Thus, SOCS5Tg mice evaded renal injury induced by CLP, probably resulting in the improved mice survival post-CLP.

**Discussion**

SOCS5 is a Th1-specific protein that inhibits Th2 differentiation (15), and is highly expressed in lymphoid organs in human tissues (26). Allergic conjunctivitis, a Th2-polarized inflammation model, was attenuated in SOCS5Tg mice (27). Thus, SOCS5 appears to skew acquired immunity in favor of Th1 response. In the present study, we have attempted to explore the role of SOCS5 in T cells in host defense during sepsis. Given our recent data showing that a balanced Th1/Th2 response controlled by STAT proteins is critical in host defense during sepsis (6, 11, 12), we hypothesized that SOCS5 would play a beneficial role during sepsis. As expected, mice with a cell-specific overexpression of SOCS5 in T cells were resistant to CLP-induced lethality.

The innate immune response is the first line of host defense in infection, in which neutrophils and macrophages are cells in charge, enabling the host to achieve efficient removal of invading microbes (4, 28). In contrast, evidence indicates that T cells are also involved in host defense during infection. Mice genetically deficient in lymphocytes (nude and RAG−/− mice) were susceptible to various types of bacterium infection (29–33). Hotchkiss et al. (34) has demonstrated that RAG−/− mice were vulnerable to CLP; an event that was accompanied by increased blood bacteria counts. Immunocompromised hosts whose T cell function can be impaired are at high risk for opportunistic infections (35, 36). Thus, T cells appear to play a protective role in innate immunity during infection. Our present data highlight a new aspect that T cells, particularly CD4+ T cells, are capable of enhancing the innate immunity when SOCS5 is overexpressed in the cells, allowing the host to decrease bacteria burden, leading to subsequent alleviation of renal injury, a major cause of death during sepsis.

A question arises how SOCS5 in T cells affects innate immune response during septic peritonitis. Several mechanisms might contribute to the enhanced innate immunity in SOCS5Tg mice. Seki et al. (15) have demonstrated that overexpression of SOCS5 in T cells negatively regulates IL-4-dependent STAT6 activation without inhibiting IL-12-mediated STAT4 activation and produces higher IFN-γ. IFN-γ primes/stimulates macrophages to increase their responsiveness to microbial products such as bacterial LPS that include up-regulation of TLR4 expression, induction of anti-bacterial mediator NO, and production of IL-12 and TNF-α (37–39). In the present study, we have shown that phagocytes from SOCS5Tg mice exhibited augmented bactericidal activities and increased production of IL-12 and TNF-α by nature. WT phagocytes adopted enhanced bactericidal activities when cocultured with SOCS5Tg-CD4+ T cells. Considering that T cells are present in the peritoneum under physiological conditions (Thy1.2-positive cells, 11–16%, our own data), these findings suggest that peritoneal resident T cells in SOCS5Tg mice might influence innate immune responses. In the current study, we have shown that LPS-induced TNF-α production from macrophages was increased when macrophages were cultured with SOCS5Tg-CD4+ T cells relative to the WT-CD4+ T cells. This appeared to need cell-cell contacts, as no augmentation was found when macrophages and CD4+ T cells were separately cultured using transwell apparatus, and culture supernatants of CD4+ T cells failed to transfer the augmentation. More importantly, enhanced TNF-α production by SOCS5Tg-CD4+ T cells was diminished by neutralization of IFN-γ. Considering the current data showing that macrophages are capable of producing TNF-α but not IFN-γ, enhanced IFN-γ production by SOCS5Tg-CD4+ T cells appear to be responsible for the augmented production of TNF-α by macrophages. In addition, we have demonstrated in the current study that SOCS5Tg-CD4+ T cells bear an increased memory cell-like phenotype. Acquisition of the ability to produce IFN-γ is a fundamental property of memory T cells (40). These results suggest that peritoneal SOCS5Tg-CD4+ T cells may rapidly become activated and produce higher IFN-γ in an initial phase of infection and then influences the innate immune response of phagocytes. Interestingly, STAT6 activation in peritoneal neutrophils and macrophages was impaired in SOCS5Tg mice. We have demonstrated that STAT6−/−mice are resistant to septic peritonitis by exerting an improved bacterial clearance via an altered cytokine profile in the peritoneum in favor of bacterial clearance (12). Attenuated STAT6 activation in phagocytes possibly through type I-polarized peritoneal cytokine response also appears to contribute to the skewed immune response in SOCS5Tg mice.

Other mechanism(s) might be involved in the increased survival in SOCS5Tg mice. Studies in experimental animals and critically ill patients have demonstrated that increased apoptosis

**FIGURE 9.** MPO and cytokine level in the kidney in SOCS5Tg mice. At 24 h after CLP, mice were bled and euthanized. MPO (A) and cytokines/chemokines levels (B) in kidney extracts were quantitated by ELISA. The data represent the mean ± SEM (10 mice, each). ‡, p < 0.05; †, p < 0.01 vs WT mice.
of lymphoid organs may provoke a marked immune suppression and organ system dysfunction (41). Prevention of thymic apoptosis protects mice from CLP-induced lethality (42, 43). Given the observation that SOCS5 is expressed in thymus of non-treated SOCS5Tg mice (15), the thymic T cells may fulfill a protective role during CLP. Alternatively, overexpression of SOCS5 in T cells may affect the recruitment of T cells into the thymus through decreased IL-4 signaling. The contribution of SOCS5 in lymphoid organs during sepsis is under investigation.

An attractive question is whether endogenous SOCS5 could regulate host defense during sepsis. Could SOCS5 deletion interfere with innate immune responses? A recent study by Brender et al. (44) demonstrated that SOCS5-deficient CD4+ T cells differentiated in vitro into either Th1 or Th2 cells with the same efficiency as WT cells. Furthermore, the mice mounted a protective Th1 response when infected with the intracellular parasite Leishmania major. Thus, endogenous SOCS5 seems not to be essential for Th1 cell generation. Endogenous SOCS5 in T cells may not play a role in innate immunity, however, the findings here and elsewhere (15) provide evidence that overexpression of SOCS5 in T cells appears to bias innate immunity in favor of a type 1 response. Another interest is whether SOCS5Tg mice are sensitive to LPS-induced shock due to the skewed immune response. Our preliminary data suggest that this is not likely as there were no significant differences in mice survival between WT and SOCS5Tg mice after i.p. injection of 100mg/kg LPS (data not shown). This may result from discrepancies between infectious (CLP) and noninfectious (endoxin) models of sepsis, which differ in their complexity and in their pathogenesis (45).

Our present data challenge a current concept that lymphocytes do not play a principal role in an initial phase of infection (46). We have provided evidence in the present study that the T cell response appears rapidly during infection, activating phagocytes to enhance their bactericidal activities and cytokine production within hours. Sepsis is a fatal disease and its mortality rate has not improved over the past three decades (1, 2). The treatment of sepsis and septic shock remains a clinical conundrum and recent prospective trials with biological response modifiers aimed at the inflammatory response have shown only modest clinical benefit (47, 48). The present findings raise the new prospect that up-regulation of SOCS5 in T cells may provide a novel therapeutic strategy in the management of sepsis.

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Disclosures

The authors have no financial conflict of interest.

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3. Fearon, D. T., and R. M. Locksley. 1996. The instructive role of innate immunity to bias innate immunity in favor of a type 1 response. Another interest is whether SOCS5Tg mice are sensitive to LPS-induced shock due to the skewed immune response. Our preliminary data suggest that this is not likely as there were no significant differences in mice survival between WT and SOCS5Tg mice after i.p. injection of 100mg/kg LPS (data not shown). This may result from discrepancies between infectious (CLP) and noninfectious (endoxin) models of sepsis, which differ in their complexity and in their pathogenesis (45).

Our present data challenge a current concept that lymphocytes do not play a principal role in an initial phase of infection (46). We have provided evidence in the present study that the T cell response appears rapidly during infection, activating phagocytes to enhance their bactericidal activities and cytokine production within hours. Sepsis is a fatal disease and its mortality rate has not improved over the past three decades (1, 2). The treatment of sepsis and septic shock remains a clinical conundrum and recent prospective trials with biological response modifiers aimed at the inflammatory response have shown only modest clinical benefit (47, 48). The present findings raise the new prospect that up-regulation of SOCS5 in T cells may provide a novel therapeutic strategy in the management of sepsis.

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Disclosures

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