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Review

STAT Proteins in Innate Immunity during Sepsis: Lessons from Gene Knockout Mice

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The innate immune system provides immediate defense against infection and serves as the first line of host defense during infection. In innate immunity, leukocytes such as neutrophils and macrophages recognize and respond to pathogens in a non-specific manner. Therefore, the recruitment and activation of leukocytes are essential in innate immunity, and are governed by a variety of chemical mediators including cytokines. Cytokines are generally divided into 2 types, termed type-1 and type-2 cytokines. Type-1 cytokines are important in local host defense, while type-2 cytokines play a protective role when inflammatory response spreads to the body. These cytokines exert their biological functions through the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. STAT1/3/4/6 are transcription factors that mediate IFNγ/IL-10/IL-12/IL-13 cytokine signaling, respectively. Evidence indicates that STAT proteins have a significant impact on innate immunity during sepsis. This review focuses on recent understandings in the regulation of innate immunity by STAT proteins during sepsis and septic shock. The suppressor of cytokine signaling (SOCS) proteins are a family of SH2 domain-containing cytoplasmic proteins that complete a negative feedback loop to attenuate signal transduction from cytokines that act through the JAK/STAT pathway. The participation of SOCS proteins in sepsis is also discussed.

Key words: cytokines, innate immunity, sepsis, SOCS, STAT

Our environment contains a great variety of infectious microbes, which may be potentially destructive and can threaten our survival. As soon as microbes try to establish a site of infection, the host launches a complex defense system. Leukocyte infiltration into the site of infection and the subsequent activation of the cells are the fundamental host defenses during sepsis, during which infiltrating leukocytes inactivate and clear the invading pathogens

[1, 2]. Thus, inflammation is primarily a beneficial host response to infection. However, inflammation is also harmful depending on the circumstances, as an excessive and prolonged response can cause tissue damage, resulting in fatal organ failure [3, 4]. Therefore, the response must be under the strict control of endogenous mechanism (s). An imbalance in the cytokine response may allow infection to become established or may result in an uncontrolled systemic inflammatory response [5, 6].

Cytokines utilize complex signaling cascades to elicit their biological effects. Many of these cytokines exert their biological functions through the janus

kinase/signal transducer and activator of transcription (JAK/STAT) pathway [7, 8]. The importance of the JAK/STAT pathway in regulating cytokine signaling has been well established by the targeted disruption of genes encoding STATs [9]. On the other hand, suppressor of cytokine signaling (SOCS) proteins are negative regulators of cytokine signaling that act by inhibiting the JAK/STAT signal transduction pathway [10]. This review focuses on the biological significance of STAT/SOCS proteins during sepsis and septic shock.

Sepsis and Septic Shock

Sepsis is an infection-induced syndrome, and its clinical manifestations are usually the consequence of intense cellular interactions. Sepsis may initially be caused by a local infection, the frequent sites of which are the lungs, urinary tract, and abdomen [11, 12]. The first host response against invading pathogens involves the recruitment and activation of leukocytes at infectious foci, thus allowing these cells to successfully localize, kill and clear the pathogens. However, once the host fails to limit microbes to a local area, microbes may invade into bloodstream and their products then trigger an excessive and prolonged inflammatory response via the overzealous production of inflammatory mediators including cytokines, which can become pathologic, self-destructive, and, at times, can be more fatal than the original inciting pathogens [6, 11]. A typical example is systemic inflammatory response syndrome (SIRS) or multiple organ failure (MOF) [13].

Despite significant advances in the development of antibiotics, intensive care unit technology and mechanical ventilator support, sepsis and septic shock can be life-threatening conditions. The incidence of these conditions continues to increase [14], and the mortality rate has not significantly improved in the past 40 years [15]. The overall mortality rate of sepsis is approximately 25 to 35%, and patients with septic peritonitis have a higher mortality rate, between 60–80% [15, 16]. The limited therapeutic options are a direct reflection of our insufficient knowledge of the pathogenesis of sepsis and septic shock. The key to obtaining in-depth knowledge of the pathophysiological disorders is to analyze the molecular basis of sepsis using animal models of sepsis and septic shock. We employ, as an animal model of sepsis, a murine model of septic peritonitis induced by cecal ligation and puncture (CLP). CLP is a bacteria-infection-based sepsis model, as the host responds to the spillage of polymicrobial flora in the peritoneal cavity [17, 18]. CLP is physiologically relevant to human sepsis, as it possesses a number of the hallmarks of clinical sepsis with peritonitis associated with post-surgical or accidental trauma.

Type-1 and Type-2 Cytokine Response during Sepsis

Cytokine response is generally divided into 2 types based on the distinct cytokine secretion patterns. The response types are termed type-1 (IFNγ, TNFα) and type-2 (IL-4, IL-5, IL-13) responses [19]. Evidence indicates that the type-1 cytokine response is essential in the host defense against bacterial infection [20]. Recent studies have demonstrated in the CLP model that IL-12 (type-1 promoting cytokine) detected in the peritoneum is beneficial for clearing bacteria from the peritoneum possibly through the induction of IFNγ [21]. IFNγ primes/stimulates macrophages to increase their responsiveness to bacterial lipopolysaccharide (LPS), and induces antibacterial mediators such as nitric oxide and TNFα [22, 23]. TNFα augments the bactericidal activities of leukocytes, enhancing bacterial clearance in several bacterial infection models including CLP [21, 24]. Thus, the inflammatory mediator systems that result in eliciting and activating leukocytes appear to be essential to host defense in that they restrict the polymicrobial flora to a local area during infection. However, once bacteria invade into the body, type-2 cytokine plays a protective role in sepsis. We have found in the CLP model that IL-13 (a type-2 cytokine) is not detected in the peritoneum, but is found specifically in organs, and the tissue-specific IL-13 functions as an immunomodulator of MOF during sepsis [25]. IL-10 is another type-2 cytokine that has anti-inflammatory properties [26] and also plays a protective role during CLP by down-regulating inflammatory cytokines [27]. Thus, mice with septic peritonitis exhibit a mixed type-1 and type-2 cytokine response, leading to the hypothesis that a balanced type-1/type-2 cytokine response may be critical in host defense during sepsis.

STAT Proteins as Transcription Factors Mediating Cytokine Signaling

STATs consist of STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. Each member is activated by distinct cytokines. Cytokines bind to homodimeric or heterodimeric receptors, which bind JAK proteins. JAKs are activated by transphorylation and then phosphorylate cytokine receptors, allowing STATs to bind via SH2-phosphotyrosine interactions. STATs in turn are phosphorylated, and dimerization of activated STATs translates to the nucleus where STATs bind DNA and regulate gene expression (Fig. 1). STAT1, STAT3, STAT4 and STAT6 are essential in mediating responses to IFNγ, IL-10, IL-12 and IL-13, respectively [28].

Role of STATs during Sepsis and Septic Shock

All STATs have been targeted and the phenotypes have been reported. Studies utilizing gene-targeted mice allowed us to explore the biological functions of STATs [7, 9]. The phenotypes of mice lacking STATs in murine models of sepsis and septic shock are summarized in Table 1.

STAT1.   STAT1 mediates IFNγ signaling. IFNγ activates peritoneal macrophages, resulting in enhanced bacteria killing, and protects mice against lethal doses of *Listeria monocytogenes* infection [29, 30]. Studies using antibodies and gene-knockout mice demonstrated that a lack of IFNγ activities was deleterious to the defense against *Salmonella typhimurium* infection [31-33]. Thus, IFNγ plays crucial roles in host defense against intracellular pathogens. However, in a polymicrobial septic peritonitis model induced by CLP, neutralizing endogenous IFNγ conversely reduced the mortality that was associated with a decrease in HMGB1 release [34], a late mediator of endotoxin lethality [35]. The results indicate that IFNγ appears to play a deleterious role during the evolution of sepsis. In a different model of sepsis induced by endotoxin, IFNγR$^{-/-}$ mice as well as IFN$\gamma^{-/-}$ mice were resistant to the lethality relative to the wild-type (WT) mice, a result that was associ-

ated with reduced production of TNFα [36, 37]. Since IFNγ is mediated by STAT1, it is reasonable to speculate that STAT1 may play a role in endotoxin shock. Kamezaki *et al.* showed that STAT1$^{-/-}$ mice were moderately resistant to endotoxin shock [37]. STAT1 can be activated in the liver, kidney and small
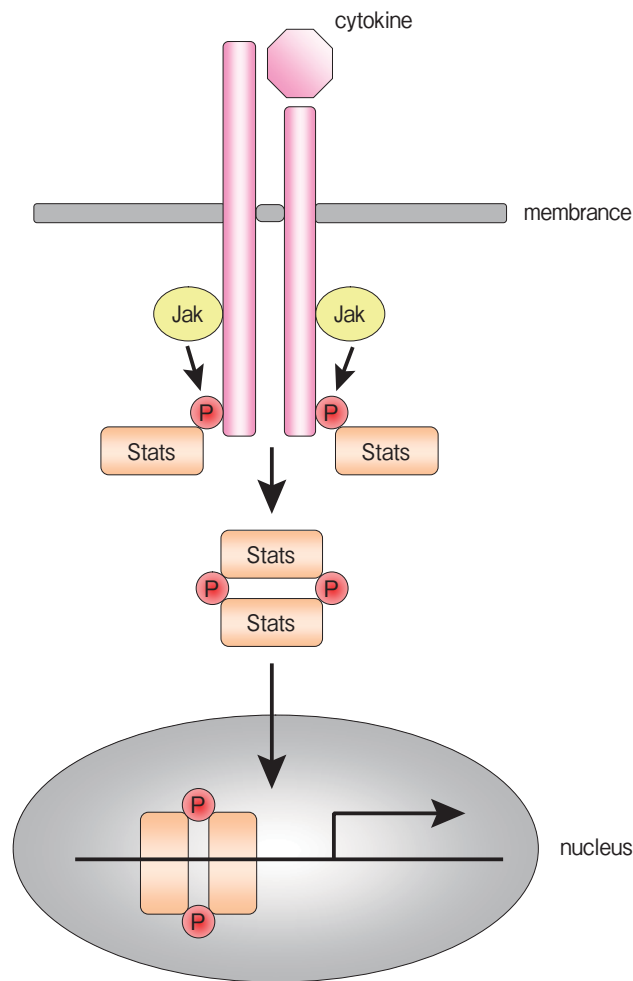

Fig. 1   Overview of cytokine signaling.

Table 1   Phenotype of mice lacking STATs in endotoxemia and septic peritonitis

STATs	Phenotype of Null Mice	References
STAT1	resistant to endotoxemia	[37]
STAT3*	susceptible to endotoxemia and septic peritonitis	[45, 46]
STAT4	susceptible to endotoxemia/resistant to septic peritonitis	[51, 52]
STAT6	susceptible to endotoxemia/resistant to septic peritonitis	[51, 52]

*conditional knockout in macrophages and neutrophils.

intestine during CLP [38], but the role of STAT1 in bacteria-based sepsis has not been fully determined.

STAT3.   STAT3 is a transcription factor that mediates IL-10, a powerful anti-inflammatory cytokine [26]. Enhanced inflammatory response was shown in IL-10$^{-/-}$ mice during infection [39–41]. Although STAT3$^{-/-}$ mice were embryonic lethal [42], conditional gene knockout mice allowed us to understand the role of STAT3 in immune response. Riley *et al.* has revealed that STAT3 deficiency in macrophages and neutrophils fails to respond to IL-10 and secretes high levels of TNFα after stimulation with IL-10 plus LPS [43], suggesting that STAT3 expressed in macrophages and neutrophils may negatively regulate inflammatory response. We have demonstrated that resident macrophages, but not other cell types, play a regulatory role in inflammation through the STAT3 signaling pathway, as mice with a cell-type specific disruption of the STAT3 gene in macrophages and neutrophils exhibited enhanced leukocyte infiltration concomitant with augmented production of inflammatory cytokines after intraperitoneal injection of thioglycollate. Adoptive transfer of resident macrophages from the conditional STAT3$^{-/-}$ mice into the control littermates resulted in an increased number of infiltrating leukocytes and elevated peritoneal levels of cytokines [44].

The conditional STAT3$^{-/-}$ mice were highly susceptible to endotoxemia due to increased levels of inflammatory cytokines including TNFα, IL-1β and IFNγ [45]. The mice also succumbed to septic peritonitis induced by CLP [46]. Although the bacterial load in the conditional STAT3$^{-/-}$ mice was comparable to that in the WT mice, the mice displayed excessive local and systemic inflammation, which was accompanied by substantial increases in the level of multiple cytokines such as TNFα, IL-1β, IL-6, IL-12 and IFNγ. Elevated serum amyloid A (SAA) and, in contrast, a decreased albumin level were found in conditional STAT3$^{-/-}$ mice. Both of these findings reflect the severity of the systemic inflammation. Hepatic and renal injury was significantly exacerbated in the mice [46]. Thus, mice with STAT3 deficiency in macrophages and neutrophils exhibited hypercytokinemia during CLP, leading to uncontrolled excessive local and systemic inflammation resulting in increased lethality. These results suggest that macrophage/neutrophil-specific STAT3 is crucial in not only regu-

lating local inflammation but also modulating multiple organ failure associated with systemic inflammation.

STAT4.   Mice lacking IL-12 show impaired Th1 differentiation, IFNγ production and cell-mediated immunity [47]. IL-12-induced increases in the production of IFNγ, in cellular proliferation and in natural killer (NK) cell cytotoxicity are abrogated in lymphocytes from STAT4$^{-/-}$ mice. STAT4$^{-/-}$ lymphocytes demonstrated a propensity towards the development of Th2 cells [48]. In addition, STAT4$^{-/-}$ mice were resistant to Th1-associated chronic diseases such as pulmonary granuloma and experimental autoimmune encephalomyelitis (EAE) [49, 50]. Thus, IL-12/IFNγ is essential in Th1 response, in which STAT4 plays an important role. In septic peritonitis induced by CLP, IL-12 was shown to enhance bacterial clearance partly via enhancing IFNγ production [21], which suggests that STAT4$^{-/-}$ mice would be susceptible to CLP due to an impaired bacterial clearance.

In contrast to this assumption, STAT4$^{-/-}$ mice were resistant to the CLP-lethality as compared to the WT mice. There were no differences in the numbers of infiltrating neutrophils and macrophages after CLP between STAT4$^{-/-}$ and WT mice, and STAT4$^{-/-}$ mice were burdened with a level of bacteria similar to that observed in WT mice [51]. Thus, the local response was not altered in STAT4$^{-/-}$ mice. The mechanism by which STAT4$^{-/-}$ mice were resistant to CLP appeared to be alleviated systemic organ damage. CLP resulted in increases in the serum level of aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN) and creatinine in WT mice. However, these levels were strongly decreased in STAT4$^{-/-}$ mice, and the levels were comparable to those in untreated mice. Hepatic inflammation was dramatically attenuated in STAT4$^{-/-}$ mice, as assessed by myeloperoxidase (MPO) level and histology. Tissue levels of type-2 cytokine IL-10 and IL-13 in STAT4$^{-/-}$ mice were significantly augmented in the liver, as compared to WT mice, whereas the levels of macrophage inflammatory protein (MIP)-2 and KC, CXC chemokines known to attract and activate neutrophils were markedly lower in the liver and kidney of STAT4$^{-/-}$ mice than those of WT mice. These results indicate that the cytokine profile in the organs during sepsis was altered in STAT4$^{-/-}$ mice in favor of anti-inflammatory properties, which may contribute to the improvement of organ injury, resulting in an

increased survival in STAT4$^{-/-}$ mice. On the other hand, Lentsch *et al.* reported in an endotoxemia model that STAT4$^{-/-}$ mice showed increased susceptibility to sepsis due to an elevated IL-12 level in the circulation [52]. The discrepancies may be explained by the substantial differences in the complexity between the infectious and noninfectious models.

STAT6.   Naive CD4^{+} T cells differentiate into Th2 cells via STAT6 when stimulated with IL-4 [53]. IL-13 is also capable of activating STAT6 [54], and the macrophage functions in response to IL-13 are impaired in STAT6$^{-/-}$ mice [55]. IL-4 and IL-13 are anti-inflammatory cytokines, inhibiting the production of pro-inflammatory cytokines such as TNFα, which are known to play a deleterious role in endotoxemia. STAT6$^{-/-}$ mice were highly susceptible to lethal endotoxemia, a result of the augmented production of pro-inflammatory cytokines and chemokines that can lead to increased organ inflammation and damage [52]. We have shown in the CLP model that IL-13 detected specifically in tissues protects mice from CLP-induced lethality by modulating inflammatory responses via the suppression of the overzealous production of inflammatory cytokines/chemokines [25]. These results led us to speculate that STAT6$^{-/-}$ mice would be susceptible to CLP due to an enhanced inflammatory response that causes severe organ damage.

Unexpectedly, survival rates after CLP in STAT6$^{-/-}$ mice were significantly higher than in WT mice [51]. Interestingly, the bacterial load recovered from the peritoneal cavity in STAT6$^{-/-}$ mice was significantly lower than that in WT mice. The number of neutrophils after CLP in STAT6$^{-/-}$ mice was significantly increased as compared to WT mice. These data suggest that the host defense at the site of infection was augmented in STAT6$^{-/-}$ mice. *In vitro*, no difference was found in the activation of leukocytes by measuring lysosomal enzyme release and superoxide anion (O$_2^-$) generation after stimulation with LPS or *E. coli*, suggesting that STAT6$^{-/-}$ mice cleared bacteria more effectively than WT mice based on the situation of the cells. Peritoneal levels of IL-12 and TNFα were significantly higher in STAT6$^{-/-}$ mice, as compared to WT mice. IL-12 and TNFα are capable of enhancing bacteria clearance in several infection models including septic peritonitis [21, 24]. Treatment of STAT6$^{-/-}$ mice with anti-IL-12 or anti-TNFα neutral-

izing antibodies increased the bacterial load in the peritoneum. Administration of anti-TNFα abrogated the increased number of neutrophils observed in STAT6$^{-/-}$ mice. Peritoneal levels of CCL22 (macrophage-derived chemokine/MDC) and CCL6 (C10), which are known to exhibit a strong type-1 response during CLP [17, 56], were also augmented in STAT6$^{-/-}$ mice. Altogether, these results indicate that STAT6$^{-/-}$ mice were resistant to CLP due to an altered cytokine profile in the peritoneum in favor of a type-1 response, possibly due to the type-1/type-2 cytokine balance. The enhanced type-1 response in STAT6$^{-/-}$ mice was also demonstrated in other studies. STAT6$^{-/-}$ mice were resistant to infection against *Leishmania mexicana* via the development of a type-1 cytokine response [57]. The induction of experimental autoimmune encephalomyelitis (EAE), a Th1-associated response, was exaggerated in STAT6$^{-/-}$ mice [49].

Negative Regulation of STATs by SOCS Proteins in Sepsis and Septic Shock

The JAK/STAT signal transduction pathway is negatively regulated by SOCS proteins [10, 58]. Recent studies indicate that SOCS proteins are implicated in a variety of immune and inflammatory diseases [8]. SOCS1$^{-/-}$ mice were highly sensitive to LPS-induced shock because of an increased serum level of TNFα [59, 60]. The introduction of SOCS1 inhibited LPS-induced NF-κB and STAT1 activation in macrophages. In addition, LPS tolerance, a refractory state to second LPS stimulation, was not observed in SOCS1$^{-/-}$ mice [59, 60]. Thus, SOCS1 negatively regulates LPS responses. Gene delivery of SOCS3 protected mice from lethal endotoxemia by decreasing the serum level of TNFα [61]. These results suggest that SOCS1 and SOCS3 may be new targets for the treatment of endotoxin-induced shock syndrome that occasionally occurs following infection.

Very recently, we demonstrated that mice with a cell-specific overexpression of SOCS5 in T cells (SOCS5Tg) are resistant to the lethality relative to the WT mice [62]. The bacterial burden in SOCS5Tg mice was significantly lower than in WT mice, and the accumulation of leukocytes was augmented in SOCS5Tg mice, which was accompanied by increased peritoneal levels of IL-12, IFNγ and TNFα.

In vitro bactericidal activities of macrophages and neutrophils were enhanced in SOCS5Tg mice. Both neutrophils and macrophages from WT mice showed enhanced bacterial killing activity when co-cultured with CD4$^+$ T cells from SOCS5Tg mice, relative to CD4$^+$ T cells from WT mice. Furthermore, the CLP-induced bacterial burden in T- and B-cell-deficient RAG-2$^{-/-}$ mice harboring SOCS5Tg-CD4$^+$ T cells was significantly reduced relative to the controls [62]. These findings provide evidence that SOCS5 in T cells affects innate immunity, and thus they highlight a novel role of T cells during sepsis. Further studies are necessary to elucidate the contribution of T cells to the initiation of innate immunity.

Concluding Remarks

The remedy for sepsis and septic shock remains unknown. Recent clinical trials aimed at modulating inflammatory response have failed or shown only modest clinical benefit. Although the local inflammatory response may be viewed as an unwelcome event following infection because of the unpleasant clinical symptoms (redness, heat, swelling and pain), the response is clearly a beneficial protective host response in the maintenance of health, which can be strengthened by STAT6 deactivation. However, excessive systemic inflammation is fatal to the host. Such inflammation can be weakened by STAT4 deactivation and in contrast STAT3 activation. Thus, therapeutic efforts aimed at regulating the STATs may represent a new therapeutic intervention for patients with sepsis or SIRS, which can be controlled by SOCSs. Innate immune response is tightly linked to acquired immune response, and STATs/SOCSs are essential to the linkage. Augmenting the innate responses by regulating STATs/SOCSs has the potential to promote immediate effects, as well as to deliver beneficial effects to the most beneficial downstream adaptive responses for defense against particular pathogens.

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