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Sepsis-induced SOCS-3 expression is immunologically restricted to phagocytes

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Abstract

We have shown that immune cells from septic mice exhibit a suppressed response to exogenous stimuli in vitro. The suppressors of the cytokine signaling (SOCS) family are proteins that block intracellular signaling and can be induced by inflammatory mediators. Therefore, we hypothesized that SOCS-3 is up-regulated in immune cells in response to a septic challenge induced by cecal ligation and puncture (CLP). Mice were subjected to CLP or sham-CLP, and 2–48 h later, the blood, thymus, spleen, lung, and peritoneal leukocytes were harvested and examined. SOCS-3 was undetectable in thymocytes or blood leukocytes. In contrast, SOCS-3 was up-regulated in the spleen, lung, and peritoneal leukocytes in a time-dependent manner. Further examination revealed that only the macrophages and neutrophils expressed SOCS-3. These data suggest that cytokines and bacterial toxins present during sepsis have the ability to suppress the cytokine and/or lipopolysaccharide response and the function of immune cells by up-regulating SOCS-3.

Keywords

macrophage; neutrophil; immunosuppression; CLP; mouse

INTRODUCTION

Sepsis and resulting multiple organ failure are a major cause of morbidity and mortality in the intensive care unit in patients who have undergone surgery or suffered trauma [1]. Clinically and in animal models, sepsis often manifests itself as a twostage process, physiologically and immunologically (reviewed in refs. [2,3]; in the immune system, there is an initial, exaggerated, systemic, inflammatory response that is followed by a state of generalized immune cell hyporesponsiveness, ref. [3] and references therein). In addition to bacterial products, cytokines are thought to play key roles in the modulation of sepsis [3–5], and their levels often reflect the severity of sepsis [6]. The balance between pro- and anti-inflammatory cytokines is crucial, demonstrated by the fact that in vivo inhibition of tumor necrosis factor α , interleukin (IL)-1 β (pro) [7], and IL-4 and IL-10 (anti) [8,9] improved survival from sepsis in animal models, and inhibition of IL-13 (anti) decreased the survival rate [10]. However, the efficacy of cytokine therapies in mouse models has not been successful in the clinical setting so far [7]. This suggests a further need to clarify our understanding of the complex process involved in the pathophysiology of sepsis.

In addition to the regulation of cytokine expression, cytokine activity is also regulated at the cellular level. A wide variety of signal-transduction pathways are activated in response to various inflammatory and/or infectious stimuli encountered during sepsis, and many of these

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can be regulated by the production of receptors and signaling proteins. Although little is known about mechanisms of negative regulation of cell activation, it has been shown that immune cells harvested from septic mice exhibit a suppressed response to exogenous stimuli in vitro (ref. [3] and references therein). Recently, a family of proteins that has the potential to negatively regulate cytokine-mediated responses during sepsis has emerged and is collectively known as the suppressors of cytokine signaling (SOCS) proteins (reviewed in refs. [11-13]). Although initially identified as inhibitors of the Janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, the SOCS proteins have also been shown to interfere with cell signaling by promoting the degradation of signaling proteins [14,15] as well as binding to a variety of signal transducers [11,16-18].

SOCS proteins are induced by a wide variety of stimuli, many of whose actions are inhibited by SOCS, thereby representing a negative-feedback control mechanism. Again, these proteins were first identified as being induced by cytokines that use the JAK-STAT pathway [12,14], but they can also be induced in a JAK-STAT-independent manner [19,20]. In regards to immunosuppression in sepsis, SOCS-1 and SOCS-3 are the only members of this family that appear to have roles in the immune system [11] and have been shown to be induced by numerous cytokines and inflammatory factors [such as lipopolysaccharide (LPS) and CpG-DNA], which are commonly present in septic animals [19-21]. Indeed, SOCS-3 has been found to be up-regulated in the liver of septic rats [22] and rats subjected to burn trauma [23]. Similarly, SOCS-1 has also been found to be up-regulated in the spleen in response to burn trauma [23]. Although the exact roles of the SOCS proteins in these models have not been fully elucidated, their expression has been linked to organ dysfunction.

A putative role for the SOCS proteins in the immunosuppression observed after sepsis has emerged via a variety of studies. As bacterial products such as LPS are abundant in septic animals, it is of interest that the SOCS proteins have been shown to be crucial mediators of LPS tolerance in vitro [24-26] and in vivo [25]. Also important in the immune response to sepsis, the SOCS proteins have been shown to play a role in the differentiation of T cells into the T helper cell type 1 (Th1; SOCS-1) and Th2 (SOCS-3) phenotypes [27]. Finally, the SOCS proteins have been implicated in the regulation of apoptosis through the regulation of members of the BCL-2 family [28,29]. We and others have shown that immune cells from septic patients and mice have increased levels of apoptosis (refs. [3,30] and references therein). Additionally, we have shown that macrophages isolated from septic mice are resistant to LPS-stimulated cytokine production [31] and that this suppression may be linked to their increased propensity to undergo apoptosis [32]. From these data, we proposed the hypothesis that SOCS protein expression (SOCS-1 and SOCS-3) is pathologically up-regulated in the immune cells of septic mice and contributes to the immunosuppression seen in these animals. To begin to examine their roles in the immune response to polymicrobial sepsis, we profiled the expression patterns of SOCS-3 and examined putative mediators of its up-regulation.

MATERIALS AND METHODS

Buffers and reagents

Hanks' balanced saline solution and phosphate-buffered saline (PBS) were obtained from Invitrogen (Grand Island, NY). RPMI 1640 was obtained from HyClone (Logan, UT). SOCS-3 (M-20) and horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Rat anti-mouse IL-4 monoclonal antibody (mAb; clone 11B.11) was obtained from the National Cancer Institute Biological Resources Branch Preclinical Repository (Rockville, MD). Rat anti-mouse IL-10 mAb (clone JES052A5) was obtained from R&D Systems (Minneapolis, MN). Rat immunoglobulin G (IgG) isotype control was obtained from Sigma Chemical Co. (St. Louis, MO). CD45/B220 mAb (clone RA-6B2) was obtained from PharMingen (San Diego, CA).

Mouse strains

Male mice were used in all studies. C3H/HeN (HeN, endotoxin-sensitive) mice were obtained from Charles River Laboratories (Wilmington, MA). C3H/HeJ (HeJ, endotoxin-tolerant) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were used at 6-8 weeks of age and ~20 g.

In vitro induction of SOCS-3

The macrophage cell line RAW264.7 was used as a positive control for SOCS-3 expression. For primary cells used in culture, 500 μ L sterile 3% thioglycolate was injected intraperitoneally (i.p.) 3 days before harvesting. Thioglycolate-induced peritoneal macrophages (TPM Φ) were obtained by lavage with 2×4 mL cold PBS. TPM Φ and RAW264.7 were incubated in RPMI 1640 containing 10% fetal calf serum (FCS; HyClone) $\pm$ 10 μ g/mL LPS (Sigma Chemical Co.) or 10:1 heat-killed *Escherichia coli* [#25922, American Type Culture Collection (ATCC), Manassas, VA] or *Staphylococcus aureus* (#25923, ATCC) for 4-24 h. The dose of LPS used is a standard dose used in our laboratory for in vitro stimulation [31,32]. *E. coli* and *S. aureus* were chosen as representative of Gram(-) and Gram(+) bacteria, as they are standard cells used for Toll-like receptor 4 (TLR4)- and TLR2-mediated responses, respectively, and each was shown to induce SOCS-3 in vitro in cell lines. The cells were then lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 10 mM NaPhosphate, 10 mM NaF, 1 mM NaOrthovanadate, 0.5% Triton X-100+Sigma's protease inhibitor cocktail) for 30 min on ice, and protein was quantitated using the Bio-Rad protein assay (Hercules, CA) and stored at -80°C until use.

Cecal ligation and puncture (CLP)

Mice were lightly anesthetized using Isoflurane (Abbot Laboratory, North Chicago, IL). A midline incision (1.5-2 cm) was made below the diaphragm, exposing the internal organs. The cecum was then ligated and punctured (CLP) with a 22-gauge needle in two places to induce sepsis. In the control animals (sham), the cecum was located but neither ligated nor punctured. The abdominal incision was then closed in layers with an Ethilon 6.0 suture, and the animal was resuscitated with 1.0 ml lactated Ringer's solution by subcutaneous injection. For antibody studies, anti-IL-4 (2 mg/mouse, ref. [8]) or anti-IL10 (250 μ g/mouse, ref. [9]) were injected i.p. 12 h post-surgery. Animals were allowed food and water ad lib.

Sample preparation

Post-surgery (2-48 h), the mice were killed, and the blood, thymus, lung, liver, peritoneal leukocytes, and spleen were collected. Blood leukocytes were obtained by dextran sedimentation of whole blood. Thymocytes and splenocytes were obtained by gently grinding the organs between ground glass slides. Contaminating red blood cells in all populations were lysed with hypotonic lysis. Cells were then lysed in lysis buffer for 30 min on ice. Liver and lung samples were homogenized in lysis buffer using a PowerGen 125 homogenizer (Fisher Scientific, Pittsburgh, PA). Protein content in the lysates was determined using the Bio-Rad protein assay.

Ex vivo separation of splenocytes and peritoneal leukocytes

Splenocytes harvested from mice 24 h post-CLP were separated into enriched populations via sequential adherence to uncoated and B220 antibody-coated plates (15 μ g/60 mm plate). Peritoneal leukocytes harvested from mice 24 h post-CLP were separated into macrophage- and neutrophil-enriched populations via adherence to plastic. For each population, cells were suspended in media + 10% FCS and cultured for 1.5-2 h on uncoated plates at 37°C. Nonadherent cells were collected (leukocytes) or transferred (splenocytes) to B220 antibody-coated plates and incubated for an additional 2 h. Adherent cells were collected by scraping.

We have consistently found that adherence to plastic results in populations that are ~95% macrophages [31,32] and have found minimal macrophage contamination in nonadherent cells (<10%) in the nonadherent (plastic) populations, as assessed by visual inspection of stained cytopins of the respective cell populations.

Western blot analysis

Thirty (peritoneal leukocytes and liver) or 50 (lung, spleen, thymus, and blood) µg protein was separated on 16% polyacrylamide gels and transferred to polyvinylidene difluoride according to the manufacturer's instructions (Novex, San Diego, CA). Membranes were then blocked with 5% milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 2 h at room temperature (RT). SOCS-3 antibody was added to blots, diluted 1:500 in 2% milk in TBST, and incubated overnight at 4°C. Membranes were washed 3× with TBST and incubated with a 1:750 dilution of HRP-conjugated secondary antibody for 1 h at RT. After washing 3× with TBST and 1× with TBS, proteins were visualized by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). Protein bands were quantitated by densitometry using SigmaGel software.

Statistical analysis

One-way ANOVA followed by Fisher's PLSD post-hoc analysis were used to find significant differences among groups. Statistical analysis was performed using SigmaStat software. Data were considered significant with $P < 0.05$.

RESULTS

SOCS-3 up-regulation by CLP is time- and tissue-dependent

To determine which populations exhibited an up-regulation of SOCS-3 in response to sepsis, mice were subjected to sham or CLP operations. Post-surgery (24 h), the lung, thymocytes, blood leukocytes, peritoneal leukocytes, splenocytes, and liver were examined for SOCS-3 expression. In all tissues tested, SOCS-3 was low-to-undetectable in the sham controls (Fig. 1). In agreement with previously published results [22], the liver exhibited a dramatic increase in SOCS-3 expression (data not shown). Similarly, SOCS-3 was readily detected in peritoneal leukocytes, splenocytes, and lung tissue (Fig. 1). In contrast, SOCS-3 was undetectable in peripheral blood leukocytes or thymocytes 24 h post-CLP (Fig. 1). With the possibility that SOCS-3 expression could be an early event in the blood or thymus, expression was also examined at an earlier time point; however, it was not detectable in either population at 4 h post-CLP (data not shown).

To further profile the expression of SOCS-3, the lung, spleen, and peritoneal leukocytes were harvested 2, 4, 12, 24, and 48 h post-CLP or sham operations to examine the expression of SOCS-3 over time. As expected, SOCS-3 was low-to-undetectable at all time points examined after the sham operation, suggesting that the trauma of the surgical procedure itself did not play a role in SOCS-3 expression, which was rapidly induced within 2-4 h in the spleen (Fig. 2, A and B) and lung (Fig. 2, A and C), reached maximal expression at approximately 12 h, and slowly decreased through 48 h. In contrast, SOCS-3 expression was not detected in the peritoneal leukocytes up to 12 h post-CLP but was significantly up-regulated by 24 h (Fig. 3), and like the lung and spleen, the expression was reduced by 48 h.

SOCS-3 expression is restricted by cell type

The samples obtained for the lung, spleen, and peritoneal leukocytes represent proteins contained within more than one cell type, and the SOCS proteins have been shown to be differentially regulated in a variety of cells. Therefore, it was of interest to determine which

cells within these populations expressed SOCS-3. Available reagents do not allow for this to be accomplished by immunohistochemistry or fluorescein-activated cell sorter analysis using cell type-specific markers. This limitation led to the use of in vitro adherence and antibody selection to produce populations that are enriched for macrophages, neutrophils, and B lymphocytes. As peritoneal leukocytes are primarily composed of macrophages and neutrophils, this population was separated by adherence to plastic. As can be seen in Figure 4A, SOCS-3 was induced in the adherent (macrophage, M Φ) and nonadherent (neutrophil, PMN) populations. Sequential adherence to plastic and B220 (B cell-specific marker) antibody-coated plates similarly separated splenocytes. Although SOCS-3 mRNA has been found to be up-regulated by LPS, CpG-DNA, and cytokines in several immune cell populations in vitro [12, 19-21], only the macrophage population in the spleen expressed SOCS-3 in response to CLP 24 h post-CLP (Fig. 4B). Similar results were obtained when splenocytes were examined 4 h post-CLP (data not shown).

Role of endotoxin in SOCS-3 expression

To begin to elucidate which factors induce SOCS-3 in vivo in response to CLP, the endotoxin-tolerant HeJ mouse strain was used to ascertain the role of endotoxin in the up-regulation of SOCS-3. Post-CLP (24 h), the lung, spleen, and peritoneal leukocytes were harvested and examined for SOCS-3 expression. SOCS-3 was up-regulated in each population in the HeN and HeJ mice (Fig. 5). Although expression levels in the lung and spleen do not vary between strains, the expression of SOCS-3 in the peritoneal leukocytes from the HeJ mouse was approximately half that observed in the HeN mouse. As SOCS-3 is still up-regulated in the HeJ mouse after CLP when compared with sham controls, TLR4-independent factors must up-regulate its expression. As CLP is polymicrobial by definition, Gram(+) factors are also present, which could induce SOCS-3. To test this, TPM Φ from HeN and HeJ mice were coinoculated with heat-killed *E. coli* and *S. aureus* for 4 h. As expected, SOCS-3 was up-regulated in TPM Φ from HeN incubated with *E. coli*, and this induction was ablated by the TLR4 mutation in the HeJ TPM Φ (Fig. 6). However, SOCS-3 expression was up-regulated in each population in response to *S. aureus* with no difference between HeN or HeJ, suggesting Gram(+) bacteria present after CLP may be responsible for the SOCS-3 induction seen in vivo in the HeJ mice.

Role of IL-4 or IL-10 in SOCS-3 expression

Two cytokines that induce SOCS-3 in vitro are IL-4 and IL-10 [12,20,24]. These two cytokines have also been found to play a significant role in CLP, as our laboratory has previously shown that neutralizing antibodies injected 12 h post-CLP significantly improved survival [8,9]. Therefore, the ability of these same neutralizing antibodies to affect the expression of SOCS-3 after CLP was examined. HeN mice were subjected to sham or CLP operations, and antibodies were injected 12 h post-surgery, a time point that not only provided a survival advantage [8, 9] but also correlates with the expression of these cytokines in CLP mice. As expected, neither antibody had an effect in sham animals (data not shown). Similarly, the antibody treatments had no effect on SOCS-3 expression in the lung, spleen, and peritoneal leukocytes harvested 24 h post-CLP (Fig. 7). As the neutralization effects of these antibodies have been documented in vivo and in vitro [8,9], our data suggest that their protective effect is not mediated by SOCS-3.

DISCUSSION

The SOCS proteins are an emerging family of regulatory proteins that inhibit cell signaling and possibly promote apoptosis, which are two aspects that directly contribute to the morbidity and mortality observed after sepsis. Therefore, the aim of this study was to profile the expression pattern of SOCS-3 during polymicrobial sepsis induced by CLP and to determine what factors may be involved in its expression. As expected, SOCS-3 expression was induced

by CLP; however, the timing and cell-specific nature are intriguing, not to mention the putative role(s) SOCS-3 plays in immunomodulation.

Previous reports have indicated that SOCS-3 is expressed in T cells/thymocytes, B cells, neutrophils, and macrophages by a variety of agents in vitro and in vivo [11,20,27,33]. However, in response to CLP, SOCS-3 appears only to be up-regulated in macrophages and neutrophils isolated from the peritoneal cavity and spleen (Fig. 4). The up-regulation of SOCS-3 in macrophages in the peritoneum and spleen suggests that the same is true for alveolar macrophages in the lung, but we are not able to confirm that with reagents available at this moment. The differences in our results and those previously published can be explained by a variety of factors. First, several of the previous studies used cell lines, and our differences may be a simple cell line versus primary cell dichotomy. Second, most studies only examined mRNA levels and may not directly reflect changes in protein levels, and data that address post-transcriptional regulation of SOCS proteins are limited. Finally, these studies were often the result of a single, direct, and potent stimulus. However, in CLP, the complexity of inflammatory mediators may differentially/coordinate regulate the expression of SOCS-3.

The complexity of the induction of SOCS-3 in response to sepsis is apparent as one considers the timing of induction in each population (Figs. 3 and 6) as well as the differential regulation of SOCS-3 expression by endotoxin (Fig. 5). We and others have shown a rapid induction (<4 h) of SOCS-3 in macrophages by LPS, CpG-DNA, *E. coli*, and *S. aureus* (Fig. 6) [19-21]. However, SOCS-3 is low-to-undetectable in macrophages or neutrophils until 24 h post-CLP (Fig. 3), although bacteria and phagocytes are present in high numbers in the peritoneum by 12 h. Although the expression of SOCS-3 could be a result of SOCS-3-expressing cells migrating into the peritoneum, the longevity of the cells and the strength of the signal suggest that SOCS-3 is up-regulated in existing cells. However, this is only speculation. The requirement for endotoxin signaling to obtain maximal SOCS-3 expression in peritoneal leukocytes (HeN vs. HeJ, Fig. 5) clearly demonstrates that several factors are influencing the expression of SOCS-3.

In contrast to the data obtained for the peritoneal leukocytes, SOCS-3 up-regulation in the lung and spleen is rapid (<4 h, Fig. 2, B and C). This is consistent with results obtained by i.p. injection of LPS [23] or CpG-DNA [19] in which SOCS-3 mRNA is up-regulated in the spleen within 2 h. However, their data does not address whether this is a direct or indirect effect. Our data demonstrate that the up-regulation of SOCS-3 in the spleen and lung does not require endotoxin signaling (Fig. 5). These data suggest that cytokines present early after the induction of sepsis are regulating SOCS-3 expression in these tissues. We have begun to address this issue using knockout mice and inhibitors, but our preliminary data remain inconclusive.

The biggest question we face is what the role of SOCS-3 is in sepsis. As SOCS-3 knockout mice display an embryonic, lethal phenotype [34], the question as to whether SOCS-3 expression is pathological or required to maintain a controlled immune response remains difficult to address. We have hypothesized that it is immunosuppressive in nature and therefore, not beneficial to the animal. Additionally, as the SOCS proteins have been implicated in the regulation of apoptosis-related proteins [28,29], and apoptosis is pathological in our model of sepsis (ref. [3] and references therein), the role of SOCS-3 as a pathological mediator is again suggested. However, Suzuki et al. [35] have recently shown that the inhibition of SOCS-3 in a mouse model of induced colitis results in a more severe disease. This suggests that SOCS-3 may provide a mechanism by which the immune system controls itself and protects the animal from a fulminant immune response. This scenario is similar to those results obtained by Matsukawa et al. [10] in which inhibition of the anti-inflammatory cytokine IL-13 decreased survival after CLP, possibly as a result of the overproduction of proinflammatory cytokines and chemokines. In contrast, Song et al. [8,9] have shown that the inhibition of the anti-

inflammatory cytokines IL-4 and IL-10 improves survival from sepsis, and we show here that this increased survival does not appear to involve changes in SOCS-3 expression (Fig. 7).

In summary, SOCS-3 has been found to be up-regulated in patients and animal models of burn trauma [23], interferon- γ -resistant myelogenous leukemia [36], stress-induced insulin resistance [15], colitis and Crohn's disease [35], and hemorrhagic shock (our unpublished results). Here, we have demonstrated that polymicrobial sepsis also induces SOCS-3 in macrophages and neutrophils in a time-, tissue-, and stimulus-specific manner. The specific role(s) SOCS-3 plays in the immunosuppression of sepsis as well as the expression/role of its counterpart SOCS-1 remain the primary focus of ongoing research. From these studies, we hope to gain a further understanding of the pathological aspects of sepsis and possibly provide information that will help in the discovery of new therapies to fight this syndrome.

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Fig. 1. SOCS-3 expression is site-specific. Blood (Blood Leuk.), spleen, lung, liver (Thymus), and peritoneal leukocyte (P. Leuk.) samples were examined for SOCS-3 expression 24 h post sham-CLP (Sham) or CLP by Western blot analysis. RAW264.7 (Raw) stimulated with LPS for 4 h was used as a positive control. Each lane represents samples taken from individual animals. Blots shown are representative of three individual experiments (three different groups of mice, two to three sham or CLP mice analyzed per group).

Fig. 2.

Time-dependent SOCS-3 expression in lung and spleen. SOCS-3 expression was examined over time in the lung and spleen by Western blot analysis (WB). Samples were collected 2, 4, 12, 24, and 48 h post-sham-CLP (Sham or S) or post-CLP (CLP or C). (A) Representative Western blots of SOCS-3. Protein bands were quantitated via densitometry and expressed as optical density (OD)/cm² for the spleen (B) and lung (C). Results are presented as mean $\pm$ SEM, n = 6. *, Time points where SOCS-3 expression after CLP is significantly greater than that measured in sham controls ($P < 0.05$).

Fig. 3. SOCS-3 expression is delayed in peritoneum. SOCS-3 expression was examined over time in the peritoneal leukocytes (P.Leuk.) by Western blot analysis. Samples were collected 4, 12, 24, and 48 h post-sham-CLP (Sham, S) or post-CLP (C). (A) Representative Western blot of SOCS-3. (B) Protein bands were quantitated via densitometry and expressed as OD/cm². Results are presented as mean $\pm$ SEM, n = 6. *, Time points where SOCS-3 expression after CLP is significantly greater than that measured in sham controls ($P < 0.05$).

Fig. 4.

SOCS-3 expression is limited to phagocyte populations. Splenocytes and peritoneal leukocytes were separated by adherence to determine which cell types within each population expressed SOCS-3. (A) Splenocytes were separated into macrophage (MΦ)-, B cell (B)-, and T cell (T)-enriched populations. (B) Peritoneal leukocytes were separated into MΦ- and neutrophil (PMN)-enriched populations. Each set (underlined groups) represents samples taken from individual animals. Blots shown are representative of three individual experiments (three different groups of mice, two to three sham or CLP mice analyzed per group).

Fig. 5. SOCS-3 induction by endotoxin is limited to the peritoneum. SOCS-3 expression was examined in the lung, spleen, and peritoneal leukocytes (P. Leukocytes) 24 h post-CLP by Western blot analysis. Induction of SOCS-3 via endotoxin/Gram(-) bacteria was examined using HeJ mice that express a mutant TLR4 and are, thus, endotoxin-tolerant. Expression was compared with that observed in their background controls, HeN. (A) Representative Western blots for each cell population. (B) Protein bands were quantitated via densitometry and expressed as OD/cm². Results are presented as mean $\pm$ SEM, n = 6. Gray bar/background denotes average level in sham controls. *, Expression levels that are significantly different than HeN controls ($P < 0.05$).

Fig. 6. Gram(+) and Gram(-) bacteria induce SOCS-3. The ability of heat-killed *E. coli* and *S. aureus* to induce SOCS-3 expression was examined in vitro. TPMΦ, obtained from endotoxin-sensitive HeN and endotoxin-tolerant HeJ mice, were incubated with bacteria for 4 h, and SOCS-3 expression was detected by Western blot analysis. Control samples received no bacteria. (A) Representative Western blot of SOCS-3 expression. (B) Protein bands were quantitated via densitometry and expressed as OD/cm². Results are presented as mean $\pm$ SEM, n = 5. *, Expression levels that are significantly different than HeN ($P < 0.05$).

Fig. 7.

Inhibition of IL-4 or IL-10 does not affect SOCS-3 expression, which was examined in the lung, spleen, and peritoneal leukocytes (P.Leukocytes) obtained from mice that were treated with neutralizing antibodies to IL-4 (α IL4) or IL-10 (α IL10) 12 h post-CLP. Control animals received rat IgG isotype-control antibody (rIgG). (A) Representative Western blots of SOCS-3 expression in each population. (B) Protein bands visualized by Western blot analysis were quantitated via densitometry and expressed as OD/cm². Results are presented as mean $\pm$ SEM, n = 6.