

Leishmania donovani-Induced Expression of Suppressor of Cytokine Signaling 3 in Human Macrophages: A Novel Mechanism for Intracellular Parasite Suppression of Activation

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Leishmania donovani protozoan parasites, the causative agent of visceral leishmaniasis, establish an infection partly by interfering with cytokine signaling in the host macrophages. Therefore, we investigated the expression of the suppressor of cytokine signaling (SOCS) genes in human macrophages infected with Leishmania donovani. The expression of SOCS3 mRNA was induced transiently after exposure to live or heat-killed parasites, but not purified lipophosphoglycan, while that of other SOCS genes remained unchanged. SOCS3 gene expression was not dependent on phagocytosis or on cytokines released by Leishmania donovani-infected macrophages, such as interleukin-1β or tumor necrosis factor alpha. In addition, Leishmania used a different signaling pathway(s) than bacterial lipopolysaccharide to induce SOCS3 mRNA, as indicated by the kinetics of induction and sensitivity to polymyxin B inhibition. Finally, phosphorylation of the STAT1 transcription factor was significantly reduced in Leishmania donovani-infected macrophages and required de novo transcription. The induction of SOCS3 provides a potent inhibitory mechanism by which intracellular microorganisms may suppress macrophage activation and interfere with the host immune response.

Leishmania donovani, a protozoan parasite, is the causative agent of visceral leishmaniasis, a fatal disease if left untreated that threatens millions of people living in or traveling to tropical and subtropical regions where leishmaniasis is endemic. Leishmania donovani has a dimorphic life cycle defined by a promastigote form within the sand fly vector and an amastigote stage present in the mammalian host. Upon infection, Leishmania donovani promastigotes are rapidly phagocytosed by and eventually establish a persistent infection within host macrophages (mφ) (1).

The initial host defense against Leishmania is executed by innate immunity, involving primarily mφ and to some extent Langerhans cells (4). To survive inside mφ and escape immunity, Leishmania has developed mechanisms that deactivate mφ immune functions, including the inhibition of the respiratory burst, inhibition of interleukin-12 (IL-12) and nitric oxide synthesis, and the downregulation of major histocompatibility complex (MHC) class II molecules, as well as promoting the synthesis of inhibitory cytokines like transforming growth factor β (TGF-β) and IL-10 (reviewed in reference 5). Cytokines, and especially gamma interferon (IFN-γ), are essential contributors to mφ activation to promote effective killing of the parasites.

The recent identification of the suppressor of cytokine signaling (SOCS/CIS/JAB/SSI) family (11, 18, 26) presents a new potential mechanism for the inhibitory effect of Leishmania on the cytokine activation of mφ. The expression of SOCS1 and SOCS3 in mφ is induced by multiple cytokines and growth factors (reviewed in reference 15), as well as lipopolysaccharide (LPS) (27) and gram-positive bacteria (28). While SOCS1 has been proposed to inhibit Janus kinase (JAK) activity by binding to the kinase activation loop (29), the mechanism of SOCS3 inactivation is still unclear. The association of SOCS3 with JAKs (2, 24) or with the phosphorylated receptor chain (16, 20, 24) has been postulated. In all cases, the signal transduction cascade is interrupted by lack of appropriate phosphorylation of STAT transcription factors, which inhibits the activation of mφ.

Because Leishmania donovani has been previously shown to interfere with cytokine signaling (references 3, 17, and 23 and references therein), it is important to investigate the level of expression of SOCS genes in Leishmania donovani-infected mφ. We report here that Leishmania promastigotes specifically induce the expression of SOCS3 mRNA in human mφ, while the expression of other SOCS genes remains unchanged. Possible mechanisms leading to the expression of SOCS3 in Leishmania donovani-infected mφ, as well as the effect on STAT1 phosphorylation, are also discussed.

MATERIALS AND METHODS

Parasite and bacterial strains. L. donovani (strains FDA97052 and MHOM/SD/62/LS-C121), Leishmania major (strain MHOM/IL/80/Friedlin), and Leishmania amazonensis (strain FDA99028) were maintained as promastigotes at 25°C as described previously (23). Heat-killed L. donovani parasites were prepared by incubating parasite suspensions at 56°C for 10 min. Parasites tested below the detection limits for endotoxin (<0.25 endotoxin unit/ml in the Limulus amebocyte lysate assay; Endosafe, Charleston, N.C.). Purified lipophosphoglycan (LPG) was kindly provided by David Sacks and used as indicated. Mycobacterium bovis (provided by Frank Collins), Listeria monocytogenes (provided by Karen Eikins), and fixed Staphylococcus aureus (Cowan 1 strain; Calbiochem, La Jolla, Calif.) were used at a mφ/bacterium ratio of 1:20, 1:200, and 2:10,000 (or 0.02%), respectively.

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Antibodies and reagents. Neutralizing antibodies to human IL-10 and IL-1β (Endogen, Rockford, Ill.), antibody to tumor necrosis factor alpha (TNF-α), and control isotype mouse immunoglobulin G1 (IgG1) (PharMingen, San Diego, Calif.) were used at 1 μg/ml. Rabbit polyclonal antibodies to STAT1 and phospho-STAT1 (Y701) (Cell Signaling Technology, Beverly, Mass.) and horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, Calif.) were used for standard Western blots. Recombinant human IFN-γ (rhIFN-γ) (Genzyme, Cambridge, Mass.), IL-1β, TNF-α, and IL-10 (Endogen) were used at 1 ng/ml. Latex beads, polymyxin B, and actinomycin D were obtained from Sigma (St. Louis, Mo.).

Culture, differentiation, and infection of human mφ. CD14+ monocytes were purified by elutriation of pheresed cells from healthy normal volunteer blood donors at the NIH Clinical Center Department of Transfusion Medicine. Monocyte-derived mφ were obtained by culturing monocytes for 7 days at 2 × 10^6 cells/ml in RPMI 1640 medium (Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal calf serum, 50 μg of gentamicin per ml, and 50 ng of mφ colony-stimulating factor per ml at 37°C in a humidified 5% CO₂ atmosphere. mφ were exposed for 45 min, when not otherwise indicated, to stationary-phase *Leishmania* promastigotes (mφ/parasite ratio of 1:20), heat-killed *L. donovani* (1:20), latex beads (0.005%,) or LPS (10 ng/ml). For the determination of STAT1

Molecular Biochemicals).

cription of multiprobe template sets using biotin RNA labeling mix (Roche Molecular Biochemicals). Biotinylated probes were size fractionated by electrophoresis in polyacrylamide gels (QuickPoint; Invitrogen, San Diego, Calif.), and transferred onto positively charged nylon membranes (Roche Molecular Biochemicals, Mannheim, Germany). Biotinylated probes were detected using streptavidin peroxidase and enhanced chemiluminescence (NorthWestern, Detroit, Mich.). Human SOCS, human CK-2b, or human CK-3 multiprobe RNA (20 to 100 ng). RNase-protected probes were size fractionated by electrophoresis in polyacrylamide gels (QuickPoint; Invitrogen, San Diego, Calif.), and transferred onto positively charged nylon membranes (Roche Molecular Biochemicals, Mannheim, Germany). Biotinylated probes were detected using streptavidin peroxidase and enhanced chemiluminescence (NorthWestern, Detroit, Mich.). Human SOCS, human CK-2b, or CK-3 multiprobe biotinylated RNA was obtained by transcrip-
tion of multiprobe template sets using biotin RNA labeling mix (Roche Molecular Biochemicals).

Cellular extracts and Western blot analysis. After infection, mφ (4 × 10^6 to 5 × 10^5) were washed with cold phosphate-buffered saline (PBS) containing 1 mM sodium orthovanadate and lysed in 100 μl of NuPAGE sample buffer (Invitrogen). Aprotinin (20 μl) were analyzed by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis in NuPAGE 4 to 12% Bis-Tris gels using RNA STAT-60 (Tel-Test, Inc., Friendswood, Tex.) according to the manufacturer’s instructions. RNase protection assay (RPA) (RiboQuant; PharMingen) was performed according to the manufacturer’s protocol. Briefly, equivalent amounts of RNA (5 to 10 μg) were hybridized overnight at 56°C with biotinylated human SOCS, human CK-2b, or human CK-3 multiprobe RNA (20 to 100 ng). RNase-protected probes were size fractionated by electrophoresis in polyacrylamide gels (QuickPoint; Invitrogen, San Diego, Calif.), and transferred onto positively charged nylon membranes (Roche Molecular Biochemicals, Mannheim, Germany). Biotinylated probes were detected using streptavidin peroxidase and enhanced chemiluminescence (NorthWestern, Detroit, Mich.). Human SOCS, human CK-2b, or CK-3 multiprobe biotinylated RNA was obtained by transcription of multiprobe template sets using biotin RNA labeling mix (Roche Molecular Biochemicals).

Flow cytometry (fluorescence-activated cell sorting [FACS]). After infection, mφ were washed with cold PBS, fixed, and stained at 4°C in PBS buffer containing 10% normal human serum (to block Fc receptors) and saturating concentrations of fluorescein isothiocyanate-conjugated anti-MHC class II antibodies (Caltag, Burlingame, Calif.) or a biotinylated antibody against IFN-γ receptor α chain (IFN-γRα), followed by phycoerythrin-conjugated streptavidin (PharMingen, Becton Dickinson, San Jose, Calif.). IgG2b isotypes were used to control for nonspecific binding. At least 10,000 events were collected and analyzed using a FACSscan flow cytometer and CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). The results are expressed in arbitrary units as the mean fluorescence index (MFI), where MFI = (geometric mean of the antibody/geometric mean of the IgG2b isotype).

RESULTS

Upon infection by *L. donovani*, human mφ transiently express SOCS3 mRNA. The recent discovery that the SOCS1 and SOCS3 genes are involved in the negative regulation of cyto-

kine signaling pathways prompted us to investigate their level of expression in *L. donovani*-infected mφ. Analysis by RPA of RNA from mφ infected for different periods with stationary-phase *L. donovani* promastigotes demonstrates increased SOCS3 mRNA expression (Fig. 1). The maximal level of SOCS3 mRNA was observed after 30 to 45 min of infection. Interestingly, *L. donovani* selectively induced SOCS3 mRNA expression in mφ, whereas other members of the SOCS family were apparently not affected up to 48 h postinfection (Fig. 1 and data not shown). *L. donovani* RNA tested negative for SOCS expression (data not shown), ruling out the possibility of a parasite contaminant. SOCS3 has been associated with the regulation of numerous signal transduction pathways, notably IFN-γ, IL-4, IL-6, IL-10, and IL-12. These cytokines are essential for mφ immune functions; therefore, blocking their action through SOCS3 expression may explain at least part of the downregulation of mφ activation following *Leishmania* infection.

Different *Leishmania* species, but not latex bead phagocytosis, induce the expression of SOCS3 mRNA in mφ. As shown in Fig. 2A, mφ infected with *L. major* or *L. amazonensis* also express SOCS3 mRNA, although the level was lower than that for mφ infected with the two *L. donovani* strains. To further determine whether SOCS3 gene expression is dependent on phagocytosis or requires active parasites, RNA from mφ exposed to latex beads or heat-killed *L. donovani* were analyzed for SOCS3 gene expression. As demonstrated in Fig. 2B, the phagocytosis of latex beads did not induce the expression of SOCS3, whereas SOCS3 mRNA was detected in response to live or heat-killed *L. donovani*, arguing for an inducing mechanism independent of the condition of the parasite. This latter observation suggests that parasite surface molecules might be involved as a triggering signal. Parasite LPG is the most abundant molecule on the surface of promastigotes; however, as

FIG. 1. *L. donovani* infection induces SOCS3 gene expression in mφ. mφ were exposed to stationary-phase *L. donovani* (*Ld*) promastigotes for the times indicated above the lanes. Total RNA was analyzed by RPA for expression of SOCS1 to SOCS6 and CIS, as well as L32 and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene. For a control, mφ were not infected with the *L. donovani* promastigotes (−). The positions of protected probes are indicated by the arrowheads.
shown in Fig. 2C, mφ did not express SOCS3 mRNA in response to stimulation with 10 to 1,000 ng of purified LPG per ml.

mφ exhibit differential SOCS3 gene expression in response to Leishmania or LPS stimulation. Gram-negative bacterial LPS and gram-positive L. monocytogenes were recently reported to induce the expression of SOCS3 in mouse mφ (27, 28), so it was of interest to compare SOCS3 gene expression in human mφ treated with either bacterial stimuli or L. donovani. While stimulation with M. bovis, gram-positive L. monocytogenes, fixed S. aureus, and gram-negative LPS resulted in strong and sustained SOCS3 gene expression after 2 h of treatment, L. donovani-infected mφ failed to show any increase in SOCS3 RNA at this late time point (Fig. 3A), in agreement with results presented in Fig. 1. The level of SOCS1 RNA was slightly increased in mφ exposed to bacterial stimuli but not affected in response to L. donovani compared to the uninfected control. In contrast, mφ exposed for 45 min to L. donovani showed the maximal level of SOCS3 RNA (Fig. 3B), whereas LPS treatment demonstrated only a marginal increase at the same time point (compare Fig. 3B with A). Furthermore, L. donovani-induced SOCS3 gene expression was resistant to polymyxin B treatment, whereas LPS-dependent induction was totally blocked by the drug (Fig. 3B). Together, these observations suggest that L. donovani targets other mφ receptor(s) or pathway(s) than LPS or the other bacteria do, resulting in differences in kinetics of SOCS3 gene expression and inhibitor sensitivity.

Leishmania-induced expression of SOCS3 in mφ is independent of cytokine activation. mφ infected by Leishmania have been reported to release cytokines such as TNF-α, TGF-β, IL-1, and IL-10 (reviewed in reference 5). Since most of these cytokines are also capable of stimulating SOCS3 gene expression, it was of interest to study whether it was the parasites or the cytokines produced by infected mφ that were responsible for the transient expression of SOCS3 mRNA. mφ exposed to L. donovani for various time periods were analyzed by RPA for cytokine gene expression, including IL-12, IL-10, IL-1α, IL-1β, IL-1R antagonist, IL-6, IL-18, TNF-α, TNF-β, lymphotoxin β, IFN-γ, IFN-β, TGF-β1, TGF-β2, and TGF-β3. Of all these cytokines, TNF-α and IL-1β mRNA were the only ones up-regulated during the first hour following infection with L. donovani promastigotes (data not shown). Within 15 min after L. donovani infection, SOCS3 mRNA, but not IL-1β or TNF-α, were detected (Fig. 4A), indicating that SOCS3 preceded cytokine gene expression. To further demonstrate that TNF-α and/or IL-1β was not responsible for the expression of SOCS3 mRNA, mφ were exposed to parasites in the presence of cytokine-specific neutralizing antibody or an unrelated IgG control. RNA from mφ infected with L. donovani demonstrated a consistent increase in SOCS3 mRNA, and neutralizing antibodies to IL-1β, TNF-α, or IL-10 were incapable of modulating L. donovani-induced SOCS3 gene expression (Fig. 4B). At the concentration used (1 μg/ml), the antibodies neutralized 1 to 10 ng of the corresponding cytokine per ml, as measured by inhibition of SOCS3 gene expression in cytokine-stimulated
mφ (data not shown). Together, these results rule out cytokine-induced SOCS3 expression in L. donovani-infected mφ and support the hypothesis of a direct signaling mechanism coming from the parasite itself.

**IFN-γ signal transduction is altered in Leishmania-infected mφ expressing SOCS3.** mφ immune functions primarily depend upon IFN-γ activation. IFN-γ signals through the JAK-STAT enzymatic cascade, resulting in the tyrosine phosphorylation of STAT1, translocation of the transcription factors to the nucleus, and initiation of gene transcription. Both SOCS1 and SOCS3 have been shown to inhibit the IFN-γ signaling pathway. To address whether the presence of SOCS3 in L. donovani-infected mφ affected the capability of mφ to respond to IFN-γ stimulation, the phosphorylation level of STAT1 was determined by Western blot analysis (Fig. 5A and B). Uninfected mφ responded to IFN-γ stimulation by specifically phosphorylating the tyrosine residue of STAT1 protein, as demonstrated by a phospho-specific antiserum to STAT1 (Y701). Analysis of protein extracts from mφ infected with L. donovani or heat-killed L. donovani, however, showed a time-dependent decrease in STAT1 phosphorylation in response to IFN-γ. Maximal inhibition (40 to 50%) was reached by 60 min postinfection with heat-killed L. donovani (Fig. 5A) and by 90 min with live parasites (Fig. 5B) and gradually returned to normal levels by 6 to 8 h (data not shown). Treating mφ with actinomycin D prior to infection with L. donovani to prevent transcription and further protein synthesis was enough to restore complete phosphorylation of STAT1 in response to IFN-γ (Fig. 5B). The expression of MHC class II in response to IFN-γ stimulation was strongly decreased in L. donovani-infected mφ, an effect that was maximal (65%) 4 h postinfection (Fig. 5C), confirming that the signal transduction pathway was affected. In addition, exposure of human mφ to L. donovani parasites resulted in reduced cell surface (Fig. 6) and total cellular content (data not shown) of the IFN-γRα. A maximal decrease of 72% was observed 6 to 8 h following infection (Fig. 6). The effect was transient and returned to normal levels by 24 h.

**DISCUSSION**

This study shows a novel association of a eukaryotic microorganism, the protozoan intracellular parasite L. donovani, with the induction of SOCS3 mRNA expression in infected human mφ. Two other species of Leishmania, L. major and L. amazonensis, which cause cutaneous infections in human hosts, also induced SOCS3 mRNA, although the levels of expression were lower than that in L. donovani-infected mφ. The list of pathogens shown to induce SOCS3 expression in mφ now includes L. monocytogenes (28), Leishmania spp., M. bovis, and S. aureus (this study), suggesting a generalized mechanism that initiates the suppression of mφ activation. Substantial differences were observed in the kinetics of SOCS3 gene expression and inhibition by polymyxin B when L. donovani and bacterial LPS stimuli were compared, suggesting that distinct pathways are used.

SOCS3 is part of a negative-feedback loop that regulates cytokine signaling. SOCS3 expression is dependent on STAT transcription factors and can be induced by several cytokines, including IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-11, IL-12, IL-13, IFN-γ, TNF-α (reviewed in reference 15), and type I interferons (8). Of these cytokines, we observed that the transcription of TNF-α and IL-1β genes was indeed induced in L. donovani-infected mφ, in agreement with previous studies (7), but this followed rather than preceded transcription of the SOCS3 gene. In addition, neither TNF-α nor IL-1β was responsible for the expression of SOCS3 mRNA in L. donovani-infected mφ, since neutralizing antibodies to these cytokines did not decrease the induction of SOCS3 gene expression in L. donovani-infected mφ. In addition, IL-10, which is also pro-

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**FIG. 4.** L. donovani-induced SOCS3 gene expression in mφ is independent of cytokine stimulation. (A) mφ were incubated for 45 min with L. donovani, and total RNA was analyzed by RPA for cytokine and SOCS3 expression. Relative RNA expression (compared to the total) is shown in arbitrary units (a.u.). (B) mφ were exposed (+) for 45 min to L. donovani (Ld) and neutralizing anti-IL-1β, anti-TNF-α, and anti-IL-10, or irrelevant IgG antibodies (10 μg/ml) in the presence of polymyxin B (10 μg/ml). Total RNA was analyzed by RPA for SOCS expression.
Produced by mφ infected with *Leishmania* (6) and which uses the JAK/STAT pathway, did not account for the induction of SOCS3 mRNA by *L. donovani*. On the basis of these observations, it is unlikely that cytokines mediate the induction of SOCS3 expression observed in *L. donovani*-infected cells. The hypothesis that a surface molecule of the parasite might be involved is supported by the observation that heat-killed *L. donovani* is as effective as the live parasite in activating SOCS3 mRNA expression, extending previous observations obtained with *Listeria*-infected mφ (28) to a eukaryotic organism. LPG is a highly abundant molecule present on the surface of *Leishmania* promastigotes and has been previously associated with mφ dysfunctions including protein kinase C activity (10) and IL-12 (p40) transcription (22). We reported previously that pretreatment of mφ with LPG had no inhibitory effect on STAT1 phosphorylation in response to IFN-γ stimulation (23). We show here that purified LPG does not induce the expression of SOCS3 RNA in human mφ as well, providing another link between the status of SOCS3 expression and the functionality of the IFN-γ signaling pathway. The parasite molecule(s) responsible for the transient expression of SOCS3 RNA in human mφ is yet to be discovered.

Compared with live *L. donovani*, heat-killed parasites induce stronger SOCS3 RNA expression and faster inhibition of STAT1 phosphorylation in response to IFN-γ stimulation in exposed mφ. This suggests that additional mechanisms may be involved. The observation reported by Dalpke et al. (9) that CpG DNA also induces SOCS3 expression may be another mechanism. Bacterial components inducing SOCS3 gene expression like CpG DNA and LPS signal through different mφ Toll-like receptors. Stoiber et al. (28) recently reported that the expression of SOCS3 in *L. monocytogenes*-infected mφ was

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**FIG. 5.** *L. donovani* infection inhibits mφ STAT1 tyrosine phosphorylation in response to IFN-γ stimulation. (A) mφ were infected for the indicated time with heat-killed *L. donovani* (hkLd) parasites before stimulation (+) with rhIFN-γ (1 ng/ml). Cellular extracts were analyzed by Western blotting for STAT1 phosphorylation. The membrane was incubated first with antiserum specific for phosphorylated STAT1 (Y701) (pY-STAT1) and then stripped and reprobed for STAT1 expression in each lane. (B) In a similar experiment using live *L. donovani* (Ld), mφ were incubated (+) with 1 μg of actinomycin D (Act.D) per ml prior to the infection where indicated. In panels A and B, mφ were not infected with *L. donovani* and not stimulated with IFN-γ (−) for a control. Autoradiograms were scanned, and bands were quantified using the NIH Image 1.60 software. Inhibition of phosphorylation was expressed as a percentage relative to the uninfected control and corrected to the level of STAT1 in each lane. (C) The induction of mφ MHC class II surface expression, in response to rhIFN-γ stimulation, was measured by FACS 24 h poststimulation.

**FIG. 6.** *L. donovani* infection decreases mφ IFN-γRα content. mφ were infected for the indicated time with *L. donovani* (Ld). mφ were further fixed, stained with an antibody specific for IFN-γRα, and analyzed by FACS for surface expression. The mean fluorescence index is shown in arbitrary units (a.u.).
not dependent on IL-10 but was dependent on p38 mitogen-activated protein kinase, suggesting a role for Toll-like receptors. We are therefore conducting studies to further characterize the possible role of mΦ Toll-like receptors in signaling for the induction of SOCS3 in L. donovani-infected mΦ.

IFN-γ signaling is essential for mΦ activation and elimination of invading parasites. In previous studies, Ray et al. (23) and others (19) reported reduced STAT1 phosphorylation in response to IFN-γ in L. donovani-infected human U937 cells and peripheral blood monocytes. This study suggests a role for SOCS3 as a possible regulatory mechanism induced by L. donovani to interfere with IFN-γ signaling in mΦ. This hypothesis is supported by earlier observations that SOCS3 partially reduced STAT1 phosphorylation and/or reporter gene activity in cell lines expressing normal levels of SOCS3 (28) or overexpressing SOCS3 (25, 27). SOCS3 has been shown to associate with tyrosine-phosphorylated receptor chains, thus inhibiting binding and phosphorylation of STATs (16, 20, 24). Furthermore, the expression of SOCS3 may explain the downregulation of the IFN-γRα reported in mΦ infected by L. donovani (23; this study) and M. avium (13), as SOCS3 can also interact with members of the ubiquitination protein family and targets receptors to proteosomal degradation (14, 30). The results presented in this study show that maximal IFN-γRα chain downregulation is achieved 6 to 8 h postinfection, which correlates with the time of peak SOCS3 protein expression reported by others (27, 28) and is associated with the proteosomal targeting of the receptor. SOCS3 can also bind to JAK1 (12) and JAK2 (2, 24), inhibiting the kinase activity, though this last assessment is still debated (21). If this last function is verified, the expression of SOCS3 could also explain the reduced JAK2 phosphorylation observed in L. donovani-infected U937 cells and human monocytes (19, 23). In a study involving mouse mΦ, Blanchette and coworkers demonstrated that L. donovani interferes with IFN-γ signaling by upregulating phosphatase SHP-1 activity, resulting in reduced phosphorylation of JAK2 (3). Whether SHP-1 plays a similar role in L. donovani-infected human mΦ remains to be determined.

Taken together, SOCS3 expression is involved in the regulation of a large number of cytokine signaling pathways. Transient induction of SOCS3 in response to infection may be a normal component of innate immunity; however, Leishmania might have adapted to profit from the initial mΦ unresponsiveness to cytokine activation. During these first few hours, promastigotes begin to transform into amastigotes, which are the stage ultimately responsible for causing infection and disease. The induction of SOCS3 by L. donovani provides a potential new mechanism for the suppression of mΦ activation during the initiation of an intracellular parasitic infection.

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