SOCS-1 Protects against *Chlamydia pneumoniae*-Induced Lethal Inflammation but Hampers Effective Bacterial Clearance

Tangbin Yang, Patrik Stark, Katrin Janik, Hans Wigzell and Martin E. Rottenberg

*J Immunol* 2008; 180:4040-4049; doi: 10.4049/jimmunol.180.6.4040

http://www.jimmunol.org/content/180/6/4040

---

**References**

This article cites 50 articles, 24 of which you can access for free at: [http://www.jimmunol.org/content/180/6/4040.full#ref-list-1](http://www.jimmunol.org/content/180/6/4040.full#ref-list-1)

---

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

---

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

---

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
SOCS-1 Protects against *Chlamydia pneumoniae*-Induced Lethal Inflammation but Hampers Effective Bacterial Clearance

Tangbin Yang,*† Patrik Stark, * Katrin Janik, ‡ Hans Wigzell,* and Martin E. Rottenberg2*

Suppressor of cytokine signaling 1 (SOCS1) plays a major role in the inhibition of STAT1-mediated responses. STAT1-dependent responses are critical for resistance against infection with *Chlamydia pneumoniae*. We studied the regulation of expression of SOCS1 and SOCS3, and the role of SOCS1 during infection with *C. pneumoniae* in mice. Bone marrow-derived macrophages (BMM) and dendritic cells in vitro or lungs in vivo all showed enhanced STAT1-dependent SOCS1 mRNA accumulation after infection with *C. pneumoniae*. Infection-induced SOCS1 mRNA levels were dependent on IFN-αβ but not on IFN-γ, T or B cells were not required for SOCS1 mRNA accumulation in vivo. Infection-induced STAT1-phosphorylation occurred more rapidly in SOCS1−/− BMM. In agreement, expression of IFN-γ responsive genes, but not IL-1β, IL-6, or TNF-α were relatively increased in *C. pneumoniae*-infected SOCS1−/− BMM. Surprisingly, *C. pneumoniae* infection-induced IFN-α, IFN-β, and IFN-γ expression in BMM were attenuated by SOCS1. *C. pneumoniae* infection of RAG1−/−/SOCS1−/− mice induced a rapid lethal inflammation, accompanied by diminished pulmonary bacterial load and increased levels of iNOS and IDO but not IL-1β, IL-6, or TNF-α mRNA. In summary, *C. pneumoniae* infection induces a STAT1, IFN-αβ-dependent and IFN-γ-independent SOCS1 mRNA accumulation. Presence of SOCS1 controls the infection-induced lethal inflammatory disease but impairs the bacterial control. *The Journal of Immunology, 2008, 180: 4040–4049.*

Immune and inflammatory systems are controlled by multiple cytokines, including interleukins and IFNs. Many cytokines exert their biological function through Janus kinases and signal transducers and activators of transcription factors. Suppressor of cytokine signaling (SOCS)3 are a family of intracellular proteins, several of which are regulators of cytokine homeostasis (1). There are eight SOCS family members. Gene deletion experiments have demonstrated that SOCS proteins have highly specific functions in vivo. Upon their translation in the cytoplasm, SOCS proteins function in a negative feedback loop to inhibit cytokine signaling by binding to either JAK or the receptor, and either inhibiting JAK activity directly, or targeting the receptor complex for ubiquitination and subsequent proteasome-mediated degradation.

SOCS1 and SOCS3 are among the best described SOCS molecules. Although SOCS1 plays important regulatory roles in many different cytokine pathways, a major role for SOCS1 in the inhibition of STAT1-mediated, IFN-α-β, and γ responses has been reported (2, 3). The vital importance of SOCS1 is stressed by the fact that SOCS1−/− mice die within 3 wk after birth with severe lymphopenia, necrosis of the liver, and mononuclear infiltration of diverse organs (4, 5). The neonatal defects exhibited by SOCS1−/− mice appear to be due to increased production of IFN-γ by T and NK T cells and to uncontrolled IFN-γ signaling in myeloid cells (4–6). Thus, SOCS1−/− mice show constitutive activation of IFN-γ-inducible genes (4). SOCS1 is also crucial in attenuating IFN-αβ signaling in vivo thus limiting host responses to viral infection (7).

Many cytokines induce expression of both SOCS1 and SOCS3 (8). Among these, IFN-γ induces expression of SOCS3 in STAT1 dependent and independent ways (9). Contrary to STAT1, which activates different immune genes, STAT3 inhibits NF-κB activation (10). Of interest, STAT3 is triggered by IL-6 and IL-10. However, SOCS3 impair STAT3-mediated IL-6 but not IL-10 responses (11–13).

The obligate intracellular Gram-negative bacterium *Chlamydia pneumoniae* is a common cause of high and low respiratory tract diseases and has been associated with development of atherosclerosis (14). After internalization into phagosomes, chlamydia avoid phagolysosomal fusion, and replicate intracellularly. IFN-γ is central in resistance to this pathogen, both in vivo and in vitro (15, 16). CD4+ and CD8+ T cells and myeloid cells are able to secrete IFN-γ and such IFN-γ-secreting cells are all needed for protection (17).

Macrophages infected with *C. pneumoniae* express IFN-γ, protecting the cells against chlamydial growth (18). Such bacterial infection-induced IFN-γ secretion is IL-12-independent, but requires IFN-αβ and STAT1 (19, 20). A protective role for IFN-α and IFN-γ in protection against *C. pneumoniae* is also evident during infection in vivo (21).

SOCS1 has been shown to be induced during infection with different mycobacterial species and *Borrelia burgdorferi* (22–24).
Moreover, in macrophages, SOCS-1 is induced by TLR ligands such as LPS and CpG-DNA (25, 26) and may cause hyporesponsiveness of these cells to cytokines such as IFN-γ after exposure to TLR ligands. More interestingly, absence of SOCS-1 results in hypersensitivity to LPS shock (27, 28). Although experiments using bacterial TLR ligands suggest the importance of SOCS1 in in vitro and in vivo infection with C. pneumoniae in vivo and in vitro in bone marrow-derived macrophages (BMM) and bone marrow-derived dendritic cells (BMDC).

We found that C. pneumoniae induced the expression of both SOCS1 and SOCS3 in vivo and in vitro in BMM and BMDC. Both IFN-α and IFN-γ were able to induce STAT1-mediated responses. Hence, SOCS1 is a main controller of STAT1-mediated responses, against dangerous infection-induced inflammatory responses. Be- roles: it impairs effective clearance of bacteria but protects the host of bacterial infections has not been studied.

Using bacterial TLR ligands suggest the importance of SOCS1 in hypersensitiveness to LPS shock (27, 28). Although experiments with TLR ligands are important in the study of SOCS1, SOCS1 deficiency in vivo suggested an impaired lethal inflammatory process, but at the same time hampered chlamydial control.

**Materials and Methods**

**Mice**

Mutant mouse strains with genomic deficiency in STAT1 (29), IFN-γR (30), IFN-αR (31), and RAG1 (32) were generated by homologous recombination in embryonic stem cells. Animals were bred and kept under specific pathogen-free conditions. Mice of the C57BL/6 background were bred and kept under specific pathogen-free conditions. Mice were bred and kept under specific pathogen-free conditions. Mice were bred and kept under specific pathogen-free conditions. Mice were bred and kept under specific pathogen-free conditions. Mice were bred and kept under specific pathogen-free conditions. Mice were bred and kept under specific pathogen-free conditions.

Bone marrow cells from uninfected STAT1−/− mice and wild type (WT) mice were harvested from the tibia and femur by flushing with cold PBS through the bone marrow cavities and RBC were lysed by hypotonic shock. To create bone marrow chimeras, WT and STAT1−/− mice were irradiated with 900 Gy and 4 h later inoculated in the tail vein with 2 × 10⁷ bone marrow cells from WT or STAT1−/− mice. Six weeks after reconstitution, mice were infected with C. pneumoniae. Mice were sacrificed 21 days after infection. Spleen cells from chimeric and control mice were obtained and the presence of total STAT1 was determined by Western blotting.

**Real time PCR**

Cytokine and hypoxanthine-guanine phosphoribosyltransferase (HPRT) transcripts in BMM at different time points after C. pneumoniae infection were quantified by real time PCR. Total RNA was transcribed to cDNA. The real time PCR was performed in duplicate 25 μl reactions containing Platinum SYBR Green aPCR Supermix-UDG (Invitrogen Life Technologies), 150 nM forward and reverse primers, and 0.5 μl of cDNA on an ABI Prism 7500 sequence detection system (Applied Biosystems). The following primer sequences were used: Sense SOCS1, 5′ GCT GTG CAG CAT TAA G 3′; Antisense SOCS1, 5′ CCA GAA GTG GGA ATC TC 3′; Sense SOCS3, 5′ TTC CCA TGC CGC TCA CA 3′; Antisense SOCS3, 5′ ACC ACC GAC CCC CAT AC 3′; Sense IFN-γ, 5′ GTC TCG CAC GTC TTC TCT CTC ATC TAT 3′; Sense IFN-γ, 5′ GAC ATG CAG ACC GTC TTC TCT CTC ATC TAT 3′; and Sense IP-10, 5′ GAC ATG CAG ACC GTC TTC TCT CTC ATC TAT 3′.

**Generation of mouse BMDC**

Mouse BMDC were differentiated as previously described (27). In brief, bone marrow was extracted from tibia and femur and cell suspensions cultured in IMDM (Cambrex) containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 ng/ml GM-CSF (PeproTech). Fresh medium and cytokine were replaced 3 days afterward. After 6 days of culture, loosely adherent cells were harvested and infected with C. pneumoniae as described for BMM.

**Infection and infectivity assay**

Mycoplasma-free C. pneumoniae isolate Kajaani 6 (34) was propagated in HEp-2 cells. Bacteria were stored in small aliquots in sucrose-phosphate-glutamate solution at −70°C until further use. The infectivity as measured by inclusion forming units (IFU) of bacterial preparation was determined in HEp-2 cells as described by guest on May 1, 2017 http://www.jimmunol.org/ Downloaded from
GCT GCC GTC ATT TTC TGC 3'; Antisense IP-10, 5' H11032
TCT CAC TGG
CCC GTC ATC 3'; Sense HPRT, 5' H11032
CCC AGC GTC GTG ATT AGC 3'; Antisense HPRT, 5' H11032
GGA ATA AAC ACT TTT TCC AAA TCC 3'.

Serial-fold dilutions of a cDNA sample were amplified to control amplification efficiency for each primer pair. Thereafter, the Ct values for all cDNA samples were obtained. HPRT was used as a control gene to calculate the Ct values for individual samples. The relative amount of cytokine/HPRT transcripts was calculated using the 2^-deltaCt method as described. These values were then used to calculate the relative expression of cytokine mRNA in uninfected and infected BMM.

Western blotting
Single-cell suspensions from control and C. pneumoniae-infected mice were lysed and separated on 10% separating/5% stacking SDS-polyacrylamide gels as described (15). Samples were then transferred onto nitrocellulose membranes (BioRad) by electroblotting at 100 V, 250 mA for 1 h. Immunostaining was performed using polyclonal rabbit anti-phosphorylated (Tyr 701) STAT1, total STAT1, phosphorylated IκB (1/1000 dilution; all from Cell Signaling Technology) or anti-actin (1/500 dilution; Sigma-Aldrich). Membranes were then washed and incubated with horseradish peroxidase (HRP)-conjugated polyclonal goat anti-rabbit Ig (1/2000 dilution; DakoCytomation) and developed using ECL-Plus (Amersham Pharmacia Biotech) and photographed using a Fuji intelligent dark box II digital camera.

Nitrite assay
Nitrite concentrations were measured in BMM culture supernatants using the Griess reagent in a previously described colorimetric assay (34). Aliquots (100 μl) of culture medium were mixed in 96-well plates with an equal volume of 0.5% sulfanilamide dihydrochloride and 0.05% naphthylethylenediamide dihydrochloride in 2.5% phosphoric acid and the absorbance (at 540 nm) was determined. Sodium nitrite, dissolved in DMEM,
A time points after infection with IFN-$\gamma$ induced SOCS1 and SOCS3 mRNA expression was studied. Total RNA was extracted from WT, IFN-$\alpha$-$\beta$R$^{-/-}$ BMM (A and B) or BMDC (C and D) at the indicated time points after infection with C. pneumoniae. The accumulation of SOCS1 (A and C) SOCS3 (B and D) and HPRT mRNA were measured by real time PCR. Comparable results were obtained in two separate experiments. The mean fold induction $\pm$ SEM of triplicate cultures is depicted. *, Differences with IFN-$\alpha$-$\beta$ R$^{-/-}$ cells are significant ($p < 0.05$ Student’s t test).

**Results**

**Expression of SOCS1 and SOCS3 mRNA during infection with C. pneumoniae of BMM and BMDC**

We first studied whether expression of SOCS1 and SOCS3 mRNA is increased in C. pneumoniae in vitro infected BMM and BMDC. Infection with C. pneumoniae of BMM or BMDC induced both SOCS1 and SOCS3 mRNA expressions (Fig. 1, A–D). Expression of SOCS1 and SOCS3 mRNA was dependent on STAT1 because no enhanced SOCS1 or SOCS3 mRNA levels were detected in STAT1$^{-/-}$ BMM or BMDC. Because C. pneumoniae infection of BMM induces the expression of IFN-$\gamma$, we tested whether IFN-$\gamma$ is mandatory for SOCS1 and SOCS3 expression. IFN-$\gamma$ was found nonessential for SOCS1 and SOCS3 expression because increased levels of SOCS1 and SOCS3 mRNA were detected in infected IFN-$\gamma^{-/-}$ BMM or BMDC (Fig. 1, E–H). Moreover, SOCS1 and SOCS3 mRNA levels in IFN-$\gamma^{-/-}$ BMM and BMDC were higher than in the WT controls. The mechanisms behind IFN-$\gamma$-mediated suppression of SOCS1 expression remain to be studied. Given that STAT1 activation also controls IFN-$\alpha$-$\beta$ signaling, the role of IFN-$\alpha$-$\beta$ in C. pneumoniae-infection-induced SOCS1 and SOCS3 mRNA expression was studied. IFN-$\alpha$-$\beta$ signaling plays a relevant role in SOCS1 mRNA expression because SOCS1 mRNA expression was diminished in C. pneumoniae-infected IFN-$\alpha$-$\beta$R$^{-/-}$ BMM and BMDC compared with controls (Fig. 2, A–D).

Because IFN-$\gamma$ is not required for SOCS1 expression during C. pneumoniae infection, we then tested whether IFN-$\gamma$ induces SOCS1 mRNA expression in BMM and BMDC. Coincubation of BMM or BMDC with IFN-$\gamma$ dramatically increased SOCS1 mRNA levels. The IFN-$\gamma$-induced SOCS1 mRNA expression is STAT1-dependent because no increase in SOCS1 levels was detected in STAT1$^{-/-}$ BMM or BMDC (Fig. 3, A–B). Incubation of BMM or BMDC with IFN-$\alpha$ did also induce STAT1-dependent SOCS1 mRNA expression (Fig. 3, C–D). SOCS3 mRNA was also induced by stimulation of BMDC or BMM with IFN-$\gamma$ or IFN-$\alpha$ (data not shown).

Thus, infection of BMM or BMDC with C. pneumoniae induces the expression of SOCS1 and SOCS3 genes in a STAT1-dependent manner. Infection-induced expression of SOCS1 is IFN-$\alpha$-$\beta$ dependent.

**Expression of SOCS1 and SOCS3 mRNA levels in lungs from infected mice**

We next studied whether SOCS1 mRNA levels increased in lungs of WT mice after intranasal infection with 10$^6$ C. pneumoniae. High levels of SOCS1 mRNA were noted in lungs at days 7 and 14 after infection (Fig. 4A), coinciding with high bacterial load and IFN-$\gamma$ mRNA expression (data not shown) (33). Levels of SOCS1 mRNA were normal at later time points after infection.

Next, we analyzed whether SOCS1 mRNA expression after infection with C. pneumoniae is dependent of STAT1. Lungs from STAT1$^{-/-}$ mice 7 days after infection with C. pneumoniae indeed showed impaired accumulation of SOCS1 mRNA, indicating that C. pneumoniae-induced SOCS1 mRNA accumulation is STAT1-dependent (Fig. 4B).

We then explored whether the STAT1-dependent expression of SOCS1 is mediated by hemopoietic and/or nonhemopoietic cells. Reciprocal bone marrow radiation chimeras between WT and STAT1$^{-/-}$ mice were generated by inoculation of bone marrow cells into irradiated recipients. STAT1 was detected by Western blotting in spleens from WT (donor)→ STAT1$^{-/-}$ (recipient) mice 8 wk after bone marrow cell transfer confirming repopulation
by inoculated stem cells (data not shown). Hemopoietic cells account for STAT1-dependent increased accumulation of SOCS1 mRNA, because WT→WT chimera and WT→STAT1−/− controls showed similar increased levels of SOCS1 mRNA, whereas no induction was detected in STAT1−/−→WT or WT→STAT1−/−→WT mice (Fig. 4C).

A possible need of T or B cells for SOCS1 mRNA expression in lungs from C. pneumoniae-infected mice was next analyzed. However, similar levels of SOCS1 and SOCS3 mRNA levels were detected in WT and RAG1−/− mice early after infection (Fig. 5, A and B). Lungs from RAG1−/− but not WT mice showed increased levels of these transcripts at late time points after infection (Fig. 5, A and B). Thus, T or B cells are not required for infection-induced increased accumulation of SOCS1 and SOCS3 mRNA.

Next, we explored whether IFN-γ signaling will induce expression of SOCS1 and SOCS3 mRNA during in vivo infection with C. pneumoniae. Lower levels of SOCS1 and SOCS3 mRNA in lungs of infected RAG1−/−/IFN-γ−/− were detected early after infection with C. pneumoniae compared with RAG1−/−/IFN-γ+/+ controls. However, later after infection, lungs from RAG1−/−/IFN-γ−/− and control mice showed similar SOCS1 and SOCS3 mRNA titers (Fig. 5, C and D). Thus, similar to the results obtained in vitro, IFN-γ is not essential for chlamydial infection-induced SOCS1 and SOCS3 mRNA accumulation.

In agreement with results obtained in vitro, IFN-αβ was required for increased expression of SOCS1 and SOCS3 mRNA in lungs of C. pneumoniae-infected animals (Fig. 5, E and F).

Thus, infection of mice with C. pneumoniae induces the pulmonary accumulation of SOCS1 mRNA. Hemopoietic cells accounted for the T and B cell independent, infection-induced SOCS1 expression in vivo. IFN-αβ and STAT1 but not IFN-γ were required for SOCS1 expression.

Role of SOCS1 in the control of infection with C. pneumoniae in BMM

The role of SOCS1 in the outcome of infection of BMM with C. pneumoniae was then studied. SOCS1−/− BMM were generated from RAG1−/−/SOCS1−/− mice whereas cells from RAG1−/−/SOCS1+/− mice were used to generate control macrophages. SOCS1−/− BMM showed faster STAT1 phosphorylation than control BMM after infection with C. pneumoniae (Fig. 6A), while an increase in total STAT1 levels was noted in mutant and control BMM 24 and 48 h after infection in agreement with previous reports (21). The expression of IFN-γ-dependent genes involved in bactericidal or bacteriostatic mechanisms such as IDO, iNOS, and LRG47 was also increased in C. pneumoniae-infected BMM SOCS1−/− mice compared with controls (Fig. 6, B–D).
The IFN-αβ- and IFN-γ-regulated chemokines CXCL9/monokine induced by IFN-γ (Mig) and CXCL10/IFN-γ-inducible protein 10 (IP-10) also showed higher expression in SOCS1−/− compared with SOCS1+/+ infected BMM (Fig. 6, E and F). SOCS1−/− BMM released higher levels of NO than control cells after infection with C. pneumoniae (Fig. 6J). In contrast, similar levels of IL1, IL6, or TNF-α were found in SOCS1−/− and control BMM after infection (Fig. 6, G–I). In agreement, mutant and control BMM showed similar kinetics of pIκB phosphorylation after infection with C. pneumoniae (Fig. 6K).
Because secretion of IFN-γ by C. pneumoniae-infected BMM is STAT1-dependent (21), we studied whether SOCS1 regulates IFN-γ expression in infected BMM. C. pneumoniae-infected SOCS1−/− BMM produced higher titers of IFN-γ than controls (Fig. 7B). Moreover, C. pneumoniae-infected SOCS1−/− BMM showed higher levels of IFN-β and IFN-α4 mRNA than control cells (Fig. 7, C and D).

We also analyzed whether SOCS1 regulates expression of SOCS3 in C. pneumoniae-infected BMM. Similar levels of SOCS3 were found in infected SOCS1−/− and WT BMM (Fig. 7A). Finally, the role of SOCS1 in the growth control of C. pneumoniae in BMM was investigated. C. pneumoniae levels in SOCS1−/− BMM were lower than that of controls (Fig. 7E).

Thus, SOCS1 regulates the infection-induced BMM expression of IFN-α, -β and –γ and of different IFN-responsive genes and plays a relevant role in the control of bacterial growth in BMM.

Role of SOCS1 in the outcome of infection with C. pneumoniae in vivo

All RAG1−/−/SOCS1−/− mice died or were moribund seven days after intranasal infection with 10⁶ C. pneumoniae, whereas RAG1−/− survived for >60 days after infection (26). Whereas lungs from RAG1−/− infected mice demonstrated an almost completely normal or slightly affected lung architecture (Fig. 8A), those from SOCS1−/− infected mice showed large areas of lung consolidation, large mononuclear infiltrates and bronchi often filled with inflammatory exudates containing polymorphs (Fig. 8B). However, SOCS1−/− mice showed 10-fold lower bacterial levels than controls at 6 days after infection, whereas similar bacterial levels were noted in lungs 3 days after infection (Fig. 8C). Levels of iNOS and IDO but not LRG47 mRNA were increased in lungs from RAG1−/−/SOCS1−/− infected mice (Fig. 9, A–C). The levels of Mig and IP-10 were also higher in lungs of RAG1−/−/SOCS1−/− compared with RAG1−/−/SOCS1−/− infected mice (Fig. 9, D and E). On the contrary, similar levels of IL-1β, IL-6, and TNF-α mRNA were found in lungs from RAG1−/−/SOCS1−/− mice infected with C. pneumoniae (Fig. 9, F–H). Also, similar levels of SOCS3 mRNA were detected in C. pneumoniae-infected RAG1−/−/SOCS1−/− and RAG1−/− mice (Fig. 9I). Different to the finding in BMM, comparable levels of IFN-γ mRNA were detected in RAG1−/−/SOCS1−/− and RAG1−/− infected animals at 5 days after infection (data not shown).
HPRT is depicted. The mean fold accumulation of the transcripts in relation to
significant (G/F, I), IL-6 (C), Mig (A), LRG47 (D), and HPRT mRNA measured by real
time PCR. The mean fold accumulation of the transcripts in relation to
HPRT is depicted. *, Differences with RAG1+/−/SOCS1+/− mice are signif-
icant (p < 0.05 Student’s t test).

Thus, SOCS1 protects the host from a lethal inflammatory pro-
cess but diminishes the efficiency of protective innate-immune
mechanisms. SOCS1 deficient C. pneumoniae-infected mice have
increased expression of IFN-responsive genes controlling micro-
bialic mechanisms and chemotaxis.

Discussion
We demonstrate that in vitro and in vivo infection with C. pneu-
moniae induces SOCS1 and SOCS3 mRNA accumulation. SOCS1
hampers a lethal inflammatory response in vivo but diminishes
the efficiency of protective innate immune responses.

SOCS-1 is induced by cytokines via the activation of STAT1
and downstream transcription factors such as IRF-1 (35). In addi-
tion, SOCS-1 can be induced independently of JAK-STAT path-
ways, as molecules that do not primarily use JAKs and/or STATs,
such as stem cell factor, TGF-β, insulin, and LPS all induce
SOCS1 expression (27). However, C. pneumoniae-induced
SOCS1 expression in BMM and BMDC was STAT1-dependent
(Fig. 1, A and C).

As expected, addition of IFN-γ or IFN-α dramatically increased
expression of SOCS1 mRNA levels in BMM and BMDC in a
STAT1-dependent manner. However, IFN-γ controlled SOCS1
mRNA levels early but not late after in vivo infection with C. pneumoniae (Fig. 5C). Moreover, SOCS1 mRNA accumulation
in BMM and BMDC was independent of IFN-γ signaling (Fig. 1, E and G). In vitro and in vivo infection with C. pneumoniae induced
an IFN-αβ-dependent SOCS1 mRNA accumulation (Fig. 2, A and
C and Fig. 5E). Infection of BMM with C. pneumoniae has been
shown to induce expression of IFN-αβ which will trigger STAT1-
dependent IFN-γ expression (18, 20, 21, 36). Such a pathway
could account for the redundancy of IFN-γ and the requirement
of IFN-αβ for SOCS1 mRNA expression during infection. The TLR-
MyD88 signaling has been shown to be required for IFN-αβ ex-
pression in Chlamydia infected cells, and is thus probably involved
in infection-induced SOCS1 expression (19, 20, 36).

Although SOCS1 expression in nonhemopoeic cells such as
neurons and fibroblasts has been reported (35, 37), SOCS1 defi-
ciency in the hemopoietic compartment is believed to be sufficient
to cause a SOCS1−/− disease, as transfer of SOCS1−/− bone
 marrow into irradiated JAK3-deficient recipients resulted in premature
lethality (5). In accordance, SOCS1 expression in C. pneumoniae
infected mice was dependent on presence of STAT1 in hemopoi-
etic cells (Fig. 4C).

STAT1 was also required for expression of SOCS3 mRNA ac-
cumulation during infection of BMM and BMDC with C. pneu-
moniae (Fig. 1, B and D). Previous reports have shown that STAT1
is primarily responsible for the induction of SOCS-3 in fibroblasts
and macrophages in response to IFN-γ (9, 38). Infection of mac-
rophyages with Listeria monocytogenes and Leishmania donovani
has also been shown to activate SOCS3 (39, 40).

We here demonstrated that SOCS1 controls BMM responses to
infection with C. pneumoniae. SOCS1−/− deficient BMM showed
higher levels of phosphorylated STAT1 and increased levels of
IFN-γ regulated molecules such as inducible NO synthase, IDO,

FIGURE 9. Role of SOCS1 in regulation of cytokine expression during
in vivo infection with C. pneumoniae. Total RNA was extracted from lungs
of individual RAG1−/−/SOCS1−/− and RAG1−/−/SOCS1+/+ mice (five
individuals per group) 6 days after infection with C. pneumoniae. The
accumulation of iNOS (A), IDO (B), LRG47 (C), Mig (D), IP-10 (E), IL-1β
(F), IL-6 (G), TNF-α (H), SOCS3 (I), and HPRT mRNA measured by real
time PCR. The mean fold accumulation of the transcripts in relation to
HPRT is depicted. *, Differences with RAG1−/−/SOCS1−/− mice are signif-
icant (p < 0.05 Student’s t test).

FIGURE 10. Regulation of expression and role SOCS1 expression in
BMM infected with C. pneumoniae. During macrophage infection with C.
pneumoniae SOCS1 and IFN-γ expression are induced in a STAT1 and IFN-
αβ-dependent manner (A). SOCS1 in turn will control the expression of and
the cellular responses to IFN-αβ and IFN-γ, inhibiting an effective clearance
of C. pneumoniae (B).
nary inflammation. Increased levels of Mig and IP-10 mRNA in *Leishmania major* show increased severity of cutaneous lesions in mice infected with *C. pneumoniae*. The SOCS proteins are indispensable for regulating many biochemical processes, including leukocyte homeostasis, glucose turnover, cell growth, and responses to pathogens. Further understanding of their roles in cytokine signal regulation is essential (50). Our data suggest that SOCS1 expression is induced by infection in a STAT-1 and IFN-αβ-dependent manner and may protect the host from inflammatory disease. However, SOCS1 also decreases the efficiency of protective innate immune mechanisms probably by hampering secretion of, and cellular responses to IFN-αβ and IFN-γ.

The SOCS proteins are indispensable for regulating many biochemical processes, including leukocyte homeostasis, glucose turnover, cell growth, and responses to pathogens. Further understanding of their roles in cytokine signal regulation is essential (50). Our data suggest that SOCS1 expression is induced by infection in a STAT-1 and IFN-αβ-dependent manner and may protect the host from inflammatory disease. However, SOCS1 also decreases the efficiency of protective innate immune mechanisms probably by hampering secretion of, and cellular responses to IFN-αβ and IFN-γ.

Acknowledgments
We are grateful to Prof. Andreas Klos, Department of Medical Microbiology, Medical School Hannover, Hannover, Germany, for comments. We thank Dr. T. Naka and T. Kishimoto for providing us SOCS1+/− mice. We thank Berit Olsson, Karolinska Institute, Stockholm, Sweden, for technical assistance.

Disclosures
The authors have no financial conflict of interest.

References

4048 ROLE OF SOCS1 DURING CHLAMYDIAL INFECTION

Downloaded from http://www.jimmunol.org/ by guest on May 1, 2017

Downloaded from http://www.jimmunol.org/ by guest on May 1, 2017


