Indirect induction of suppressor of cytokine signalling-1 in macrophages stimulated with bacterial lipopolysaccharide: partial role of autocrine/paracrine interferon-α/β

Ana CRESPO*†, Michael B. FILLA*, Stephen W. RUSSELL* and William J. MURPHY*[1]

*Wilkinson Laboratory of the Kansas Cancer Institute and the Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, 1008 WHW, 3901 Rainbow Boulevard, Kansas City, KS 66160-7184, U.S.A., and †Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Rua Roberto Frias s/n, 4200 Porto, Portugal

It has previously been reported by us that a brief prior exposure of mouse bone marrow culture-derived macrophages to bacterial lipopolysaccharide (LPS) resulted in a dramatic reduction in their ability to produce NO in response to a subsequent stimulus with either interferon-γ (IFN-γ) or IFN-γ plus LPS. We show here that this brief exposure to LPS results in an impaired response to subsequently added IFN-γ. A 2–4 h pretreatment with LPS leads to a dramatic reduction in the IFN-γ-induced DNA-binding of the transcription factor, signal transducer and activator of transcription 1α (STAT1α). This loss in ability to activate STAT1α temporally correlates with the LPS-induced accumulation of mRNA encoding the suppressor of cytokine signalling-1 (SOCS-1). However, LPS does not directly induce the synthesis of SOCS-1. Rather, LPS induces the synthesis of autocrine/paracrine factors that are the true mediators of SOCS-1 induction. IFN-α/β is one of these mediators, but plays only a partial role in the induction of SOCS-1 because neutralization of LPS-induced IFN-α/β production incompletely inhibits the induction of SOCS-1. We show that mouse IFN-β directly induces the synthesis of SOCS-1, without the need for prior protein synthesis, and does so with faster kinetics than does LPS. Our results are consistent with the non-specific nature of LPS-induced tolerance and provide a mechanistic insight into nonspecificity; LPS indirectly induces the synthesis of a protein mediator, SOCS-1, which inhibits the signalling that is induced by IFN-γ.

Key words: cellular activation, nitric oxide, signal transduction, tolerance/suppression.

INTRODUCTION

Bacterial lipopolysaccharide (LPS) elicits a multitude of effects on the immune system and other organ systems of many vertebrates (reviewed in [1]). Systemic release of LPS induces the synthesis of numerous cytokines (including, for the purpose of our discussion, the interferons), growth factors, eicosinoids, and reactive oxygen and reactive nitrogen species [2]. While LPS may play a direct role in the induced synthesis of a number of these molecules, many of the biological effects of LPS are indirectly elicited by these secondary mediators, especially proinflammatory agents such as tumour necrosis factor-α [3], interleukin-1 (IL-1) [4], interferon-γ (IFN-γ) [5] and IL-8 [6].

Prior exposure to sublethal quantities of LPS, however, results in decreased responsiveness to a second challenge with LPS, a phenomenon referred to as 'LPS tolerance' [7]. Tolerance can be observed within hours of primary exposure to LPS and leads to a suppressed ability to produce many of the proinflammatory agents mentioned in the preceding paragraph [8]. Using whole animal models, the principal cellular mediator of tolerance has been shown to be the monocyte/macrophage lineage [9]. In fact, numerous reports support the conclusion that both macrophage cell lines [10–14] and *ex vivo* isolates of normal macrophages [12,13,15–19] can be rendered tolerant to LPS after their *in vitro* exposure to it. What is also clear is the fact that primary exposure to LPS renders macrophages tolerant not only to LPS, but to other proinflammatory mediators (see [8,9] and the Discussion section in this paper).

It has been reported by us [16] and by other investigators [12], that a brief prior exposure of mouse macrophages to LPS suppresses their responsiveness (as measured by NO production) to subsequent stimulation with either IFN-γ alone, or IFN-γ plus LPS. The goal of the work reported here was to determine whether LPS pretreatment of macrophages might lead to a suppression of cellular signalling initiated by IFN-γ. Our studies reveal that LPS treatment of mouse macrophages induce the synthesis of suppressor of cytokine signalling-1 (SOCS-1). SOCS-1 has been shown to interfere with IFN-γ signalling by inhibiting the IFN-γ receptor-directed tyrosine phosphorylation events that occur after cellular binding of IFN-γ [20]. We show that LPS does not, however, effect the direct induction of SOCS-1 mRNA synthesis. Rather, LPS first induces autocrine/paracrine mediators, including IFN-α/β, that are subsequently responsible for the induction of SOCS-1. These results provide molecular evidence for the ability of LPS to indirectly induce the negative regulatory components of distinct signalling pathways.

EXPERIMENTAL

Cells, culture medium and reagents

Male C3H/HeN mice were obtained from the National Cancer Institute (Bethesda, MD, U.S.A.). Mice were used at 6–9 weeks

Abbreviations used: BMCDM, bone marrow culture-derived macrophages; CHX, cycloheximide; EMSA, electrophoretic mobility-shift assay; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; LPS, bacterial lipopolysaccharide; SOCS, suppressor of cytokine signalling; STAT, signal transducers and activators of transcription.

[1] To whom correspondence should be addressed (e-mail bmurphy@kumc.edu).

of age to isolate bone marrow culture-derived macrophages (BMCDM), as described previously [21]. BMCDM were grown in Eagle's modified minimal essential medium supplemented with 2 mM glutamine (Flow Laboratories, McLean, VA, U.S.A.), 100 units/ml penicillin, 100 μg/ml streptomycin (Pfizer, New York, NY, U.S.A.), 15 mM Hepes (Sigma), 10 % fetal bovine serum (Hyclone, Logan, UT, U.S.A.), 5 % equine serum (Hyclone), and L-929 cell-conditioned medium (as a source of colony-stimulating factor-1). Cells were collected after 10–14 days of culture by cold PBS treatment as described in [22], centrifuged (4 °C, 250 × ***g***, 10 min), and resuspended in medium without L-929 cell-conditioned medium, and were incubated for 24 h at 37 °C in 5 % CO_2 in air before treatments.

All culture media tested negative for detectable endotoxin by *Limulus amebocyte* lysate assay (Associates of Cape Cod, Woods Hole, MA, U.S.A.), at a sensitivity of 0.05 ng/ml. LPS, the lipid A-rich fraction II of phenol-extracted *Escherichia coli* O111 : B4, was purchased from List Biological Laboratories (Campbell, CA, U.S.A.). Recombinant murine IFN-γ (specific activity of 1.27×10^6 units/mg) was provided by Schering-Plough Corporation (Bloomfield, NJ, U.S.A.) through the American Cancer Society (Atlanta, GA, U.S.A.). Rabbit anti-mouse-IFN-α/β serum and normal rabbit serum were obtained from Access BioMedical (San Diego, CA, U.S.A.). Cycloheximide (CHX) was purchased from Sigma.

Electrophoretic mobility-shift assay (EMSA)

BMCDM were treated as indicated in the legend to Figure 1. Following treatment, nuclear extracts were prepared as described by Gao et al. [23,24]. The sequence of the synthetic oligonucleotide (Life Technologies) used for EMSA, which corresponded to the IFN-γ-activated site ('GAS') of the inducible NO synthase (*iNOS*) gene, was 5′-CTTTTCCCCTAACAC-3′. The pair of complementary oligonucleotides were end-labelled, using T4 polynucleotide kinase, and annealed as described in [25]. EMSA reactions were performed as described in [23,24]. A 20 μl aliquot of each reaction mixture was loaded on to a 5 % polyacrylamide gel containing 50 mM Tris/HCl, 2 mM EDTA, and 0.38 M glycine. Electrophoresis was carried out at 350 V and 4 °C for 1.5 h.

Northern-blot analysis

Monolayers containing approx. 1.7×10^7 BMCDM were prepared on 100-mm diameter cell-culture plates. After treatment (as indicated in the Figure legends), total RNA was isolated using TRI Reagent (Sigma) following the manufacturer's protocol. A 10 μg portion of total RNA from each sample were electrophoresed on a 1 % formaldehyde–agarose gel, blotted on to a Nytran nylon membrane by capillary transfer for 3 h using a TurboBlotter kit (Schleicher and Schuell, Keene, NH, U.S.A.), and baked at 80 °C for 1 h. Membranes were prehybridized for 30 min at 55 °C with QuickHyb solution (Stratagene, La Jolla, CA, U.S.A.). The cDNA probe encoding mouse SOCS-1 (generously provided by Dr Douglas Hilton, The Walter and Eliza Hall Institute for Medical Research, Parkville, Victoria, Australia) was radiolabelled using the Multiprime DNA labelling system (Amersham International). Heat-denatured probe (2.5–4×10^7 c.p.m.) and 100 μg of hearing sperm DNA were then added to the prehybridization solution. Hybridization was performed at 55 °C for 1 h, followed by four washes of 15 min each at 50 °C (twice with 0.30 M NaCl/0.03 M sodium citrate/0.1 % SDS, and twice with 0.03 M NaCl/3 mM sodium citrate/0.1 % SDS). The membranes were then subjected to autoradiography in cassettes with intensifying screens at − 70 °C. To ensure that equal amounts of RNA were examined, blots were stripped and then rehybridized with a radiolabelled cDNA probe encoding human glyceraldehyde-3-phosphate dehydrogenase. Figures 2(A) and 5(A) demonstrate that BMCDM mRNA for glyceraldehyde-3-phosphate dehydrogenase was induced by both LPS and IFN-β respectively, at time points exceeding approx. 4 h. However, at shorter time intervals, mRNA encoding this enzyme provided an adequate control for mRNA loading in Northern blots.

RESULTS

Prior exposure of BMCDM to LPS inhibits the activation of transcription factor STAT1α by IFN-γ

Previous work from our laboratory [16] showed that a brief prior exposure of mouse macrophages to LPS for 2–4 h, followed by the addition of IFN-γ for 2 h, resulted in a dramatic decrease in NO production when compared with concurrent stimulation with LPS plus IFN-γ for 2 h (NO was measured 16 h after removal of LPS and IFN-γ by washing). To determine the effect of prior exposure to LPS on IFN-γ signalling, we pretreated BMCDM with LPS for 2 or 4 h, followed by washing and a subsequent 30 min treatment with LPS plus IFN-γ. Nuclear extracts were then prepared and assayed for binding of the IFN-γ-activated transcription factor, STAT1α, to the IFN-γ-activated site located in the enhancer of the mouse *iNOS* gene. As shown in Figure 1, this pretreatment protocol resulted in a dramatic, time-dependent suppression in the binding of STAT1α to the iNOS IFN-γ activated site. An average of three independent experiments revealed that STAT1α binding was reduced by 27 % after a 2 h pretreatment with LPS, and by 56 % after a 4 h pretreatment.

LPS induces the accumulation of SOCS-1 mRNA

To determine the molecular basis of LPS inhibitory effects on the subsequent stimulation by IFN-γ, we examined whether LPS could induce members of the SOCS family of suppressors during the 2–4 hour pretreatment time span. Using probes for different members of the SOCS family of inhibitors, preliminary Northern-blot analysis of total RNA isolated from LPS-stimulated and -unstimulated cell cultures revealed that SOCS-1 mRNA was induced by this treatment (results not shown).

To further elucidate the mechanisms of SOCS-1 induction by LPS, we first performed time-course and dose-dependency

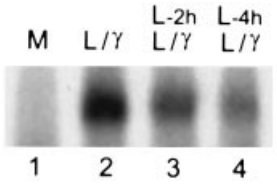

Figure 1 Brief prior exposure of macrophages to LPS suppresses the IFN-γ-induced activation of STAT1α binding to the iNOS IFN-γ activated site

Two cultures of BMCDM were incubated for 30 min with either medium alone (lane 1) or medium containing 1 ng/ml LPS + 100 units/ml IFN-γ (lane 2). An additional set of two cultures were preincubated for either 2 h (lane 3) or 4 h (lane 4) in medium containing 1 ng/ml LPS. These latter two cultures were then washed, and fresh medium containing 1 ng/ml LPS + 100 units/ml IFN-γ was added for an additional 30 min. Nuclear extracts were then prepared from each culture and EMSAs were performed using 10 μg of each nuclear extract and an oligonucleotide probe containing the IFN-γ activated site of the *iNOS* gene, as described in the Experimental section.

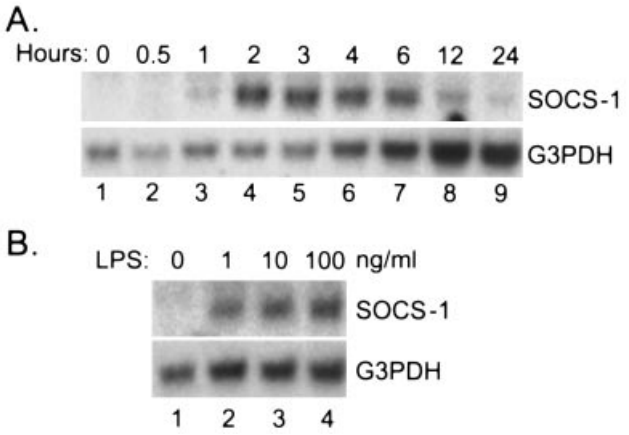

Figure 2 Time-course and dose-response analysis of the effects of LPS on SOCS-1 mRNA expression

(**A**) Total RNA was isolated from BMCDM incubated for 0–24 h in medium containing 100 ng/ml of LPS. Equal amounts of total RNA (10 μg) were electrophoretically size-separated, transferred to nylon membranes, and hybridized with a ^{32}P-labelled mouse SOCS-1 cDNA probe. After autoradiography, the nylon membrane was stripped and reprobed with a ^{32}P-labelled human glyceraldehyde-3-phosphate dehydrogenase probe. The result shown is representative of three independent experiments. (**B**) BMCDM were cultured in medium alone (lane 1) or medium containing LPS at concentrations of either 1 ng/ml (lane 2), 10 ng/ml (lane 3), or 100 ng/ml (lane 4). Total RNA was isolated after 2 h of stimulation. A 10 μg aliquot of total RNA was analysed by Northern blot as described in (**A**). The results shown are representative of three independent experiments.

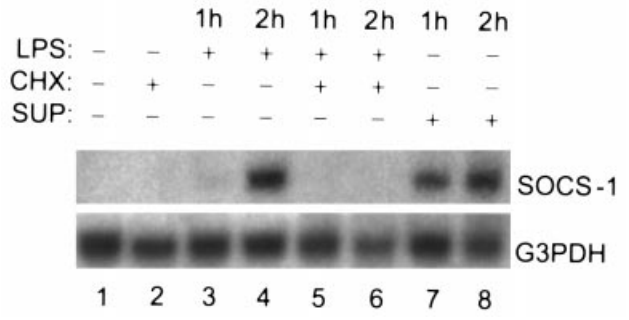

Figure 3 Indirect nature of LPS-induced SOCS-1 mRNA induction

BMCDM cultures were pre-incubated for 30 min in medium alone (lanes 1, 3 and 4) or medium containing 3 μg/ml of CHX (lanes 2, 5 and 6). Medium was then replaced with medium alone (lane 1), or medium containing either CHX alone (lane 2), 100 ng/ml of LPS alone (lanes 3 and 4), or LPS plus CHX (lanes 5 and 6). Cultures were subsequently incubated for the times indicated at the top of the Figure. Cultures represented by lanes 7 and 8 were incubated for the times indicated with cell culture supernatant (SUP), which had been transferred from primary BMCDM cultures that were treated for 2 h with 100 ng/ml of LPS. A 10 μg aliquot of total RNA, prepared from each culture, was subjected to Northern blotting as described in the legend to Figure 2.

studies. Figure 2(A) shows the time course of SOCS-1 induction in BMCDM treated with 100 ng/ml of LPS. Accumulation of SOCS-1 mRNA was evident after 1 h of cellular exposure to LPS. Maximal accumulation was observed after 2–3 h of stimulation, with mRNA levels decreasing thereafter. Accumulation of mRNA occurred in a dose-dependent fashion, as shown in Figure 2(B). The induction of SOCS-1 mRNA accumulation was observed with as little as 1 ng/ml LPS, and reached a maximum with 100 ng/ml.

Induction of SOCS-1 by LPS requires the synthesis of an autocrine/paracrine factor

It has been reported that SOCS-1 is induced by a variety of cytokines, including IL-6 [26], IFN-γ [27], and IL-4 [26,28]. Each of these cytokines signal via the Janus kinase (JAK)/STAT pathway of tyrosine kinases/transcription factors. It has been postulated that induction of SOCS-1 by these cytokines precipitates a negative-feedback loop, which tempers the strength and duration of signalling by these cytokines [28]. Because LPS does not itself elicit signalling via the JAK/STAT pathway, but does induce the *de novo* synthesis of several cytokines that do signal via the JAK/STAT pathway, we wished to determine whether LPS exerted a direct or indirect effect on the expression of SOCS-1 mRNA. To this end, we first determined the effect of the protein synthesis inhibitor, CHX, on LPS-induced accumulation of SOCS-1 mRNA. As shown in Figure 3, CHX alone did not induce the accumulation of SOCS-1 mRNA (lane 2). When BMCDM were co-incubated in the presence of LPS plus CHX for either 1 h (Figure 3, lane 5) or 2 h (lane 6), accumulation of SOCS-1 mRNA was completely abolished, in comparison with that induced by LPS alone (lanes 3 and 4).

To verify LPS-induced secretion into the culture medium of an autocrine/paracrine factor responsible for SOCS-1 induction, we also performed a culture supernatant-transfer experiment. Primary cultures of BMCDM were incubated for 2 h in the presence of 100 ng/ml of LPS, after which the culture supernatants were removed, centrifuged, and applied to fresh, secondary BMCDM cultures. RNA was extracted from these secondary cultures after 1 or 2 h of exposure and examined by Northern-blot analysis. As can be seen in Figure 3, expression of SOCS-1 mRNA could be detected after 1 h of exposure to the transferred culture supernatant (lane 7). By comparison, LPS-induced accumulation of SOCS-1 mRNA in primary cultures was undetectable after 1 h of treatment (Figure 3, lane 3). Taken together, the CHX and supernatant-transfer experiments demonstrated that LPS induced the synthesis in primary BMCDM cultures of a protein mediator that, when applied to secondary BMCDM cultures, induced a faster rate of accumulation of SOCS-1 than LPS alone.

Induction of SOCS-1 by LPS requires, at least in part, the autocrine/paracrine activity of IFN-β

Previous reports from our group demonstrated that LPS induces in mouse macrophages the production of IFN-β, which acts as a co-inducer, along with LPS, of the production of NO [29,30], and as an activator of the transcription factor, STAT1α [24]. To test the possibility that the autocrine/paracrine factor responsible for SOCS-1 induction consisted of IFN-β, we assessed the ability of LPS to induce SOCS-1 mRNA accumulation in the presence of anti-IFN-α/β serum or control antiserum. The dilution of anti-IFN-α/β serum used was determined in preliminary experiments to be that which neutralized 95% of the nitrite induced by treatment of mouse peritoneal macrophages with LPS. We have previously shown that LPS-induced production of nitrite in mouse peritoneal macrophages was completely dependent upon the autocrine/paracrine production of IFN-β [24,29]. As shown in Figure 4, the LPS-induced accumulation of SOCS-1 mRNA was suppressed by the presence of anti-IFN-α/β serum (lane 6) when compared with the accumulation observed after treatment with LPS alone (lane 4) or with that observed after treatment with LPS in the presence of a similar dilution of rabbit pre-immune serum (lane 5). In comparison with cultures treated with LPS alone, treatment in the presence of pre-immune serum resulted in a 20% reduction in SOCS-1 mRNA induction by LPS (averaged from two independent experiments). Using this

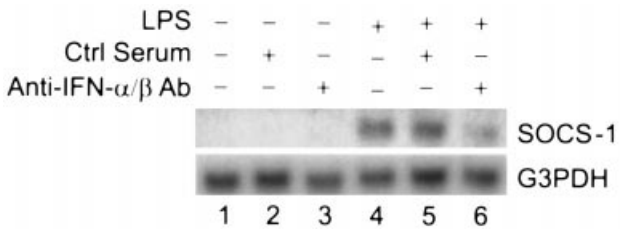

Figure 4 Effect of neutralizing anti-IFN-α/β antibodies on LPS-induced SOCS-1 mRNA accumulation

BMCDM were cultured for 3 h in medium alone (lane 1), or in medium containing either control serum (used at a 1:330 dilution, lane 2), anti-IFN-α/β serum (used at a 1:330 dilution, lane 3), LPS at 100 ng/ml (lane 4), LPS plus control serum (lane 5), or LPS plus anti-IFN-α/β serum (lane 6). A 10 μg aliquot of total RNA, prepared from each culture, was analysed by Northern blotting as described in the legend to Figure 2.

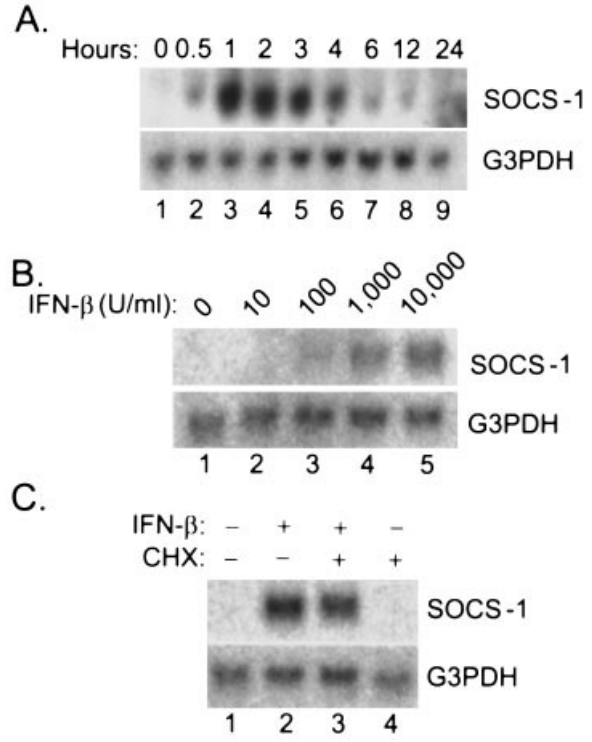

Figure 5 Analysis of SOCS-1 mRNA induction by IFN-β

(**A**) Total RNA was isolated from BMCDM treated for 0–24 h in medium containing 1000 units/ml of IFN-β. Equal amounts of total RNA (10 μg) were analysed as described in the legend to Figure 2. (**B**) BMCDM were cultured in medium alone (lane 1) or in medium containing IFN-β at concentrations of either 10 units/ml (lane 2), 100 units/ml (lane 3), 1000 units/ml (lane 4), or 10000 units/ml (lane 5). Total RNA was isolated from BMCDM after 1 h of stimulation and 10 μg aliquots were analysed by Northern blotting as described in the legend to Figure 2. (**C**) BMCDM were preincubated for 30 min in either medium alone (lanes 1 and 2) or in medium containing 3 μg/ml of CHX (lanes 3 and 4). Culture medium was then replaced with medium alone (lane 1), or with medium containing either 1000 units/ml of IFN-β alone (lane 2), IFN-β plus CHX (lane 3), or CHX alone (lane 4). Cultures were subsequently incubated for one additional hour. A 10 μg aliquot of total RNA, prepared from each culture, was subjected to Northern-blot analysis as described in the legend to Figure 2.

latter level of SOCS-1 induction as a control, the anti-IFN-α/β antibodies reduced LPS-induced expression in these two experiments by an average of 46%. Thus suppression of SOCS-1 mRNA accumulation was not complete in the presence of anti-IFN-α/β serum.

IFN-β induces SOCS-1 mRNA accumulation with faster kinetics than LPS, and in a manner independent of new protein synthesis

To provide further evidence that IFN-β induces the accumulation of SOCS-1 mRNA in BMCDM, we performed time-course and dose-dependency experiments. BMCDM were treated with 1000 units/ml of IFN-β for the times indicated in Figure 5(A), followed by preparation of RNA for Northern-blot analysis. As demonstrated in the Figure, accumulation of SOCS-1 mRNA was detectable after as little as 0.5 h of exposure to 1000 units/ml of IFN-β (Figure 5, lane 2). Accumulation reached a maximum at approx. 1 h of stimulation (Figure 5, lane 3), after which it gradually declined until it was undetectable at 24 h of stimulation (lane 9). As expected, the time course of induction of SOCS-1 mRNA by IFN-β was shifted to early time points, when compared with the time course of induction by LPS.

As shown in Figure 5(B), the accumulation of SOCS-1 mRNA could be detected after BMCDM were treated with as little as 100 units/ml of IFN-β. Maximal accumulation was detected using 10000 units/ml of IFN-β. This observation is consistent with the results of other experiments performed by us, which demonstrated maximal production of nitrite by BMCDM, using LPS plus 10000 units/ml of IFN-β (results not shown).

To verify that accumulation of SOCS-1 mRNA was a direct effect of IFN-β stimulation, we performed cellular treatments in the presence and absence of CHX, as described above for LPS. As shown in Figure 5(C), when BMCDM were incubated with both IFN-β and CHX, the level of induced SOCS-1 mRNA accumulation was equivalent to that observed using IFN-β alone (compare lane 3 with lane 2). Again, CHX alone had no effect on the expression of SOCS-1 mRNA (Figure 5, lane 4). Thus IFN-β effects the direct induction of SOCS-1 mRNA accumulation without a need for prior protein synthesis.

DISCUSSION

Numerous reports confirm the non-specific nature of LPS-induced tolerance. While prior exposure to LPS induces suppressed responsiveness to subsequent challenge with LPS, suppressed responsiveness can also be observed when the second challenge consists of either IFN-γ [12,16,31], IL-1β [8], toxic shock syndrome toxin-1 [8], tumour necrosis factor-α [31], PMA [31,32], zymosan [33], or gram positive bacterial components [33]. The results presented here are important because they demonstrate, at the molecular level, that prior exposure of mouse macrophages to LPS can effect the induction of the SOCS-1 protein, which in turn inhibits signalling via the IFN-γ pathway.

Our results also underscore the observation that many of the cellular and organismal effects of LPS are not directly due to the influence of LPS itself. Rather, secondary-response modifiers, induced by LPS, are often the true mediators of LPS action. For example, antibody-directed neutralization of IL-1β [4] and tumour necrosis factor-α [3] can mitigate some of the effects of LPS in human beings. In the case presented here, LPS induces the synthesis of IFN-α/β, which works in autocrine/paracrine fashion to induce the accumulation of SOCS-1 mRNA.

It is noteworthy that antisera to IFN-α/β do not completely abolish the induction of SOCS-1 mRNA accumulation, even though this concentration of antibody nearly completely inhibits nitrite production and autocrine/paracrine IFN-β-induced signalling in mouse peritoneal macrophages. This would indicate that other autocrine/paracrine factors induced by LPS may also play a role in the induction of SOCS-1 mRNA synthesis. Indeed,

LPS is known to induce in mouse macrophages a number of cytokines that have a demonstrable effect on the induction of SOCS-1, including: IL-6, IL-10, IL-12, and colony-stimulating factors [26]. Thus induction of SOCS-1 mRNA accumulation by LPS is likely to be due to the combined effects of multiple autocrine/paracrine factors.

The question of the importance of LPS-induced, secondary-response modifiers to the induction of tolerance is an important one. Evidence would suggest that the production of tolerance is, in fact, an autocrine/paracrine effect [34,35] that may partly involve IL-10 and transforming growth factor-β [35]. Furthermore, it has been shown that pretreatment of human macrophages with both LPS and IFN-γ prevents the acquisition of the tolerant state that is induced by LPS alone. Pretreatment with LPS and IFN-β, on the other hand, promotes acquired tolerance [11]. From other studies it is clear that IFN-β by itself also induces a state of suppressed macrophage responsiveness to subsequent stimulation with LPS and IFN-γ, whereas pretreatment with IFN-γ does not [22]. Thus IFN-β may also play an important role in the production of LPS-induced tolerance.

In addition to the induction of SOCS-1 reported here, it has recently been shown that LPS can induce the synthesis of SOCS-3 in mouse macrophages [36], as well as in human and rat macrophages [37]. LPS-induced synthesis of SOCS-3 mRNA in mouse macrophages, unlike that of SOCS-1, does not require *de novo* protein synthesis [36]. Overexpression in mouse macrophages of either SOCS-1 [38] or SOCS-3 [36] results in a suppression of IFN-γ-induced signalling. Furthermore, studies of SOCS-1-deficient mice [39] have demonstrated an essential role for this suppressor protein in protection against the pathologic effects of unrestrained IFN-γ action. Thus LPS induces the synthesis of two key regulators of IFN-γ action by distinct mechanisms.

The control of host responsiveness to LPS is of paramount importance in order to protect against unrestrained inflammatory processes that may result in tissue destruction, organ dysfunction, and, in severe cases, death [1,40]. That prior exposure to low doses of LPS itself partially protects the host from subsequent challenge with greater quantities of LPS is of significant value if the molecular mechanisms that govern this protection can be uncovered and exploited. In this regard, it is important to distinguish between the direct effects of LPS and those effects that are caused by secondary agents, the synthesis of which are induced by LPS.

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