Role of Host Protein Tyrosine Phosphatase SHP-1 in 
Leishmania donovani-Induced Inhibition of 
Nitric Oxide Production

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In order to survive within the macrophages of its host organism, the protozoan parasite Leishmania inhibits a number of critical, gamma interferon (IFN-γ)-inducible, macrophage functions, including the generation of nitric oxide. We have previously shown that the protein tyrosine phosphatase SHP-1 (Src-homology 2 domain containing phosphatase-1) is activated during Leishmania infection and plays an important role in both the survival of Leishmania within cultured macrophages and disease progression in vivo by inhibiting nitric oxide production. Here we use a SHP-1−/− macrophage cell line derived from motheaten mice to address the mechanisms by which SHP-1 prevents IFN-γ-dependent nitric oxide production during Leishmania donovani infection. We show that Leishmania inhibits nitric oxide production in response to IFN-γ poorly in SHP-1-deficient macrophages. This correlates with the inability of Leishmania to alter JAK2 and mitogen-activated protein kinase extracellular signal-regulated kinase 1 and 2 (ERK1/2) phosphorylation and to prevent nuclear translocation of transcription factors NF-κB and AP-1, although the latter two to a lesser extent. Surprisingly, Leishmania inactivated the transcription factor STAT1 to a similar extent in SHP-1-deficient and wild-type macrophages, so STAT1 is not necessary for nitric oxide production by infected macrophages. Overall, this study demonstrates that induction of SHP-1 by Leishmania is vital for inhibition of nitric oxide generation and that this inhibition occurs through the inactivation of JAK2 and ERK1/2, and transcription factors NF-κB and AP-1.

In order to survive and propagate within the macrophages of its host, the protozoan parasite Leishmania must inhibit a number of important macrophage functions. In particular, it must prevent the production of highly toxic nitric oxide (NO) in response to gamma interferon (IFN-γ). IFN-γ treatment of normal macrophages activates the JAK2-STAT1 and mitogen-activated protein kinase (MAPK) intracellular signaling pathways, resulting in induction of the type 2 (inducible) nitric oxide synthase (iNOS) gene and NO production (1, 5, 8, 20). Leishmania-infected macrophages, however, are unable to produce NO in response to IFN-γ (11, 18, 25, 26, 41, 46). We along with others have shown that this is largely a result of activation of macrophage protein tyrosine phosphatases (PTPs), in particular the PTP SHP-1 (Src-homology 2 domain containing phosphatase-1 [PTP1C]). Macrophages infected with Leishmania in vitro have elevated SHP-1 activity as well as total PTP activity, resulting in widespread dephosphorylation of high-molecular-weight proteins (2). Furthermore, infection causes colocalization of SHP-1 and JAK2 and prevents tyrosine phosphorylation of JAK2 in response to IFN-γ (2). In contrast, macrophages derived from SHP-1−/− mice show elevated iNOS induction and NO generation and are more efficient at killing Leishmania (13). This is reflected in vivo by increased NO generation and reduced parasite load in both SHP-1-deficient mice and mice treated with chemical PTP inhibitors (13, 30, 35). In this study we have used SHP-1−/− macrophages to investigate the intracellular signaling mechanisms by which elevated SHP-1 activity in infected macrophages results in decreased iNOS induction. We found that, unlike normal macrophages, SHP-1−/− macrophages activate JAK2, extracellular signal-regulated kinase 1 and 2 (ERK1/2), and the transcription factors NF-κB and AP-1 following IFN-γ treatment, even when infected with Leishmania, indicating that Leishmania-activated SHP-1 inhibits NO production by inhibiting these intermediates.

MATERIALS AND METHODS

Materials. Isotopes (γ-32P) were obtained from NEN Life Science Products, Inc. (Boston, MA). Recombinant murine IFN-γ (stock; 1 × 105 U/ml) was purchased from Life Technologies, Inc. The anti-phosphotyrosine (clone 4G10), anti-phospho-JAK2, and anti-JAK2 antibodies were obtained from Upstate Biotechnology Inc. (Lake Placid, NY); anti-phospho-ERK1/2 and anti-ERK1/2 antibodies were from Cell Signaling Technologies (New England Biolabs, Beverly, MA); and anti-iNOS was from Cedarlane (Hornby, ON, Canada). Oligonucleotides containing STAT1, NF-κB, and AP-1 consensus binding sequences came from Santa Cruz Biotechnology, Inc.

Cell culture. Leishmania donovani strain 2211 promastigotes were grown at 25°C and maintained by biweekly transfers in SDM-79 culture medium as described previously (35). The generation of murine bone marrow-derived macrophage bulk cell lines me (SHP-1−/−) and LMme (littermate me) or clones me-3 (SHP-1−/−) and LM-1 (littermate) has been previously described (13). Macrophages were grown in Dulbecco’s modified Eagle’s medium (Gibco-BRL, Canada) supplemented with 10% fetal calf serum (HyClone) plus penicillin (100
for 5 min at 4°C, the supernatants were stored at 6°C. Incubation at 4°C on a shaking platform for 15 min. After centrifugation at 12,000
NaCl, 1 mM EDTA, 1 mM EGTA, and 1 mM dithiothreitol, and 1 mM PMSF) and were resuspended in 50
HEPES (pH 7.5), 0.1% 2-mercaptoethanol, 2 mM pNPP, and 10
M Na3VO4, a PTP inhibitor (Sigma), was added to the reaction mixture containing 20 mM Tris-HCl (pH 8.0), 0.14 M NaCl, 10% glycerol (vol/vol), 1% NP-40 (vol/vol), 1 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors (40 μg/ml aprotinin and 20 μg/ml leupeptin). The lysates (20 μg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membranes (Millipore). Membranes were blocked in Tris-buffered saline-0.1% Tween containing 5% nonfat dry milk for 1 h. For anti-phospho-JAK2, anti-JAK2, anti-phospho-ERK1/2, and anti-ERK1/2 rabbit polyclonal antibodies, membranes were blocked in Tris-buffered saline-0.1% Tween containing 5% nonfat dry milk for 1 h, washed, and incubated with primary antibody overnight. After the washing step, membranes were incubated with anti-rabbit or anti-mouse horseradish peroxidase-conjugated antibody (NE Life Science Products) for 1 h, and proteins were visualized with the use of a Renaissance Western Blot Chemiluminescence Reagent Plus detection system (NE Life Science Products). Where quantification was necessary, films were scanned, and band intensity was measured using Quantity One (Bio-Rad). The change in phosphorylation following IFN-γ treatment is expressed as the relative increase (n-fold) normalized to total protein band intensity.

PTP activity measurement. Total macrophage protein lysates were collected by adding 50 μl of lysis buffer containing 50 mM Tris (pH 7), 0.1 mM EDTA, 0.1 mM EGTA, 0.1% 2-mercaptoethanol (vol/vol), 1% NP-40, and protease inhibitors (40 μg/ml aprotinin and 20 μg/ml leupeptin) to cell monolayers after three washes with PBS. PTP activity was determined by evaluating the capacity of macrophage lysates to hydrolyze para-nitrophenyl phosphate (pNPP; Sigma) (2, 23). The assays were conducted in 200 μl of reaction mixture containing 50 mM HEPES (pH 7.5), 0.1% 2-mercaptoethanol, 2 mM pNPP, and 10 μg (20 μl) of lysate. Assay plates were incubated at 37°C for up to 1 h. PTP activity was monitored by measuring the absorbance at 405 nm. To verify the assay specificity for PTPs, Na3VO4, a PTP inhibitor (Sigma), was added to the reaction mixture at a final concentration of 1, 5, 10, or 25 μM.

NO generation. Macrophages were seeded in 24-well plates (4 × 104 cells/well) and infected with Leishmania for 6 h. After washings and rest, recombinant murine IFN-γ was added to the medium (100 U/ml) (Life Technologies Inc.) for 18 to 24 h. NO levels were evaluated by measuring the accumulation of nitrates in the culture medium as described previously (35).

Electrophoretic mobility shift assay (EMSA). Cells (2 × 104 to 4 × 104) were treated according to the experiments described above and then washed three times with PBS. The last wash was done using ice-cold PBS. After centrifugation, cells were resuspended in 400 μl of ice-cold buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, and 0.5 mM PMSF), and incubated on ice for 15 min. A total of 25 μl of 10% RPEPAL [octylphenyloctethoxetanol (Sigma)] was then added, and tubes were vortexed for 10 s and then centrifuged at maximum speed for 30 s. Nuclear fractions were resuspended in 50 μl of ice-cold buffer C (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, and 1 mM PMSF) and incubated at 4°C on a shaking platform for 15 min. After centrifugation at 12,000 × g for 5 min at 4°C, the supernatants were stored at −70°C until further use. Six micrograms of these nuclear protein extracts was mixed with a γ-32P-labeled oligonucleotide containing a consensus binding sequences for STAT1, NF-κB, or AP-1. Complexes were then resolved by electrophoresis on a 4% nondenaturing polyacrylamide gel. The gel was dried and visualized by autoradiography. The consensus sequences for NF-κB, STAT3, and AP-1 were 5′-AGT-TGA-GGG-G GC-TGC-CGC-AGG-C3′, 5′-AAG-TAT-TTC-CAG-TAT-ATT-ACT-CT A3′, and 5′-GCC-TTG-ATG-ACT-CAG-CGG-GAA-3′, respectively.

RESULTS

Role of SHP-1 in inhibition of nitric oxide production. To understand the role played by SHP-1 in the inhibition of NO generation, we compared the response to IFN-γ of immortalized macrophages derived from the bone marrow of SHP-1−/− (motelecan) mice with that of phenotypically normal macrophages from heterozygous littermates. As we have previously reported (13), SHP-1-deficient macrophages had a greater capacity than littermate macrophages to express iNOS and produce NO upon IFN-γ stimulation (Fig. 1A). Release of NO was measured by the Greiss reaction (mean ± standard error of the mean of one out of three independent experiments performed in triplicate). Differences in NO induction between IFN-γ-stimulated cells and their reciprocal control and infected cells were all significant. (B) iNOS protein in the cells was measured by Western blotting. Nil, uninfected macrophages; Ld, macrophages infected with L. donovani.

FIG. 1. IFN-γ-induced NO generation by SHP-1-deficient and littermate macrophages following Leishmania infection. Macrophages were infected for 6 h before being stimulated with IFN-γ (100 U/ml). (A) Release of NO was measured by the Greiss reaction (mean ± standard error of the mean of one out of three independent experiments performed in triplicate). Differences in NO induction between IFN-γ-stimulated cells and their reciprocal control and infected cells were all significant. (B) iNOS protein in the cells was measured by Western blotting. Nil, uninfected macrophages; Ld, macrophages infected with L. donovani.
high-molecular-weight proteins (2). In order to determine the role played by SHP-1 in this process, we compared tyrosine phosphorylation in SHP-1-deficient macrophages with that in littermate controls, following infection with *L. donovani*. In accordance with our earlier study, dephosphorylation occurred rapidly in littermate macrophages, with almost no tyrosine-phosphorylated proteins larger than 90 kDa detectable 3 h postinfection (Fig. 2A). Infection of SHP-1-deficient macrophages, however, resulted in a much smaller reduction in tyrosine phosphorylation, indicating that SHP-1 plays a critical role in *Leishmania*-induced dephosphorylation. Similarly, total PTP activity was increased fourfold by *Leishmania* in littermate cells but only twofold in SHP-1-deficient cells (Fig. 2B). This non-SHP-1 PTP activity may account for the residual inhibition of IFN-γ-stimulated NO production in SHP-1−/− cells (Fig. 1). In both cell lines, PTP activity was maximal around 1 h postinfection and was stable until the end of the experiment at 6 h. The addition of sodium orthovanadate (Na3VO4, a PTP inhibitor) to the reaction mixture inhibited the PTP activity measured in both cell types in a dose-dependent manner (data not shown), confirming the specificity of the assay. In addition, we have recently obtained data suggesting that *Leishmania* parasites can also induce the PTP PTP-1B and others (M.A. Gomez and M. Olivier, unpublished). Taken together, these data suggest that SHP-1 accounts for ∼50% of the PTP activity induced by *Leishmania* but is responsible for nearly all of the resulting dephosphorylation of high-molecular-weight proteins.

**Effect of SHP-1 deficiency on IFN-γ-dependent phosphorylation events.** We next looked at the role of SHP-1 in inhibiting the intracellular signal transduction events that link IFN-γ to induction of iNOS. As shown in Fig. 3A, and consistent with our previous study (2), IFN-γ produced a rapid but transient phosphorylation of high-molecular-weight proteins in littermate macrophages. In the absence of SHP-1, not only was there a greater increase in phosphorylation stimulated by IFN-γ but also this increase was continued for at least 30 min, consistent with a role for SHP-1 in limiting the response to IFN-γ. When cells were infected with *Leishmania* before stimulation, IFN-γ was unable to cause tyrosine phosphorylation of high-molecular-weight proteins in littermate macrophages. In SHP-1−/− macrophages, however, there was a marked increase in tyrosine phosphorylation, indicating that the inhibition of tyrosine phosphorylation by *Leishmania* is dependent on SHP-1. It is interesting, however, that the IFN-γ-dependent high-molecular-weight tyrosine phosphorylation was somewhat transient in infected SHP-1−/− macrophages, suggesting that other PTPs induced by *Leishmania* are able to compensate to some extent.

We next looked at the effect of SHP-1 deficiency on three second messengers implicated in iNOS expression, JAK2 (which is larger than 100 kDa) and the closely related MAPKs ERK1 and 2, using antibodies specific for the active (phosphorylated) forms. All are phosphorylated in response to IFN-γ, and this phosphorylation is inhibited by prior infection with *Leishmania* in littermate macrophages (2, 33) (Fig. 3B and C). However, infection does not inhibit phosphorylation in SHP-1-deficient cells, indicating that *Leishmania* prevents the activation of JAK2 and ERK1/2 through a SHP-1-dependent mechanism. Furthermore, in the absence of IFN-γ, there was more phosphorylated ERK1/2 in SHP-1−/− macrophages than in littermate cells, consistent with a role for SHP-1 in limiting ERK activation.

**Role of SHP-1 in the inactivation of STAT1 during L. donovani infection.** We next looked for targets of JAK2 and ERK1/2 that could mediate induction of iNOS in SHP-1−/− cells. The transcription factor STAT1 is directly phosphory-
lated by JAK2 on tyrosine 701 (19). This allows it to dimerize and translocate to the nucleus, where it activates transcription of a number of genes, including iNOS (1, 10). Furthermore, there is evidence that phosphorylation of serine 727 by ERK1/2 is necessary for maximal activation (1, 44). Since STAT1 is essential for the induction of iNOS by IFN-γ in macrophages and activation of neither JAK2 nor ERK1/2 is inhibited by Leishmania SHP-1-deficient macrophages, we investigated whether a failure to inhibit STAT1 could account for the inability of Leishmania to inhibit NO production in SHP-1-deficient cells. As expected, an EMSA showed that IFN-γ induced the nuclear localization and DNA binding of STAT1 in both littermate and SHP-1-deficient macrophages. Surprisingly, however, neither cell type retained this ability following prior infection with Leishmania (Fig. 4). This means that the NO production seen in infected SHP-1−/− cells cannot be due to activation of STAT1α by JAK2 or ERK. Furthermore, the fact that SHP-1 accounts for ~50% of Leishmania-induced phosphatase activity (Fig. 2B) and yet STAT1 activity is completely inhibited in infected SHP-1−/− cells suggests that inhibition of STAT1 is an entirely SHP-1-independent process.

Role of SHP-1 in the inactivation of NF-κB and AP-1 during L. donovani infection. While STAT1 has been shown to bind the iNOS promoter and appears to be necessary for maximum induction by stimuli such as IFN-γ (1, 15, 42), other transcription factors are also important (12, 24, 27, 28, 47, 48). Induction of iNOS by IFN-γ in infected SHP-1−/− macrophages could therefore be due to compensation by NF-κB and/or AP-1 for the absence of STAT1. Both of these transcription factors are phosphorylated by the ERK1/2 pathway and are inhibited by Leishmania infection (17), making them possible targets of SHP-1-mediated inhibition. We therefore investigated the DNA-binding activities of NF-κB and AP-1 following infection of SHP-1-deficient cells. As shown in Fig. 5, both are inhibited by Leishmania in littermate cells, but the inhibition is weak in the absence of SHP-1. Furthermore, both transcription factors responded to IFN-γ, even in Leishmania-infected SHP-1−/− macrophages. This suggests that IFN-γ may induce iNOS via NF-κB and AP-1 in these cells.

DISCUSSION

Inhibition of macrophage nitric oxide production is critical for the survival of Leishmania parasites. We have previously obtained evidence in vivo that Leishmania achieve this inhibition by activating host PTPs, in particular SHP-1 (13, 30, 35). In this study, we have used a SHP-1-deficient macrophage cell line to specifically address the role of SHP-1 and the signaling mechanisms that are employed to achieve this inhibition.

Our first striking observation was that SHP-1-deficient mac-
phages, in the absence of any stimulus, released a higher level of NO into the supernatant in comparison with littermate cells and responded more strongly to IFN-γ stimulation. This is in accordance with our previous report (13) and indicates that SHP-1 acts as a negative regulator of NO production, even under resting conditions. After Leishmania infection, littermate macrophages were unable to produce NO when stimulated with IFN-γ, as previously observed (3, 6, 41). In contrast, the absence of SHP-1 allowed infected cells to generate NO in the presence of IFN-γ. This result demonstrates once again the importance for Leishmania to activate SHP-1 in the host cell. Indeed, infected SHP-1-deficient cells produced as much NO as uninfected, littermate cells and thus could be as microbicidal. This correlates with previous experiments showing that the inability of the parasite to survive in SHP-1-deficient macrophages is due to NO production (13). We could thus speculate that, during the initial events of infection in vivo, the ability of Leishmania promastigotes to activate macrophages SHP-1 precludes these cells from responding to IFN-γ secreted by nearby NK cells. This then greatly reduces their antileishmanial activity and allows silent progression of the infection and establishment of the disease. We therefore studied the consequences of this SHP-1 activation for intracellular phosphorylation events.

We observed that the general dephosphorylation of high-molecular-weight protein tyrosyl residues seen in infected macrophages (2) did not occur in SHP-1−/− macrophages. Furthermore, the phosphorylation of high-molecular-weight proteins in response to IFN-γ was not only greater in SHP-1−/− cells but was also observed even in Leishmania-infected cells, although to a reduced extent. This reduction may be due to activation of other PTPs or dual specificity (threonine/tyrosine) phosphatases by Leishmania (9, 32, 36, 45, 49). This interpretation is supported by the PTP assays, which showed a twofold increase in PTP activity in SHP-1−/− macrophages following infection, compared with the fourfold in their littermate equivalents. Identification of these other phosphatases will be of considerable interest, not least because in our NO production assays, Leishmania was still able to cause a twofold inhibition of IFN-γ-dependent NO production in the absence of SHP-1. Among candidate PTPs, PTP-1B has been shown to modulate IFN-γ signaling by dephosphorylating JAK2 and TYK2 (31); evaluation of its role during Leishmania infection may be very worthwhile. In fact, as mentioned earlier, we have recently obtained evidence that macrophage PTP-1B is rapidly induced upon Leishmania (Gomez and Olivier, unpublished data).

SHP-1 has been shown to be a negative regulator of both the JAK2-STAT1 and MAPK ERK1/2 pathways (21, 22, 37, 38).
Since both pathways are important for IFN-γ-mediated NO production (reference 1 and references therein) and both are modulated following Leishmania infection (2, 29, 33, 34) and since SHP-1 has been shown to associate with JAK2 following infection (2), we addressed the role of Leishmania-activated SHP-1 in suppressing these two pathways. We found that infection inhibited phosphorylation (activation) of both JAK2 and MAPK ERK1/2 in response to IFN-γ/H9253 in littermate but not SHP-1/H11002/H11002 cells. This suggests that SHP-1 is necessary for the inhibition of these pathways.

In the case of MAPK ERK1/2, we show for the first time that its IFN-γ-induced phosphorylation is inhibited during Leishmania infection. Two previous studies have observed altered ERK phosphorylation in Leishmania-infected macrophages stimulated with either phorbol myristate acetate (32) or lipopolysaccharide (LPS) (29). It therefore seems that altered IFN-γ responses in macrophages during Leishmania infection are dependent not only on inhibition of the JAK2/STAT1 pathway but also on inhibition of some ERK-dependent pathways. It is also important to note that studies of the role of ERK in iNOS expression and NO generation have generally been performed in cells treated with LPS or IFN-γ and LPS together, not with IFN-γ alone. This study supports our report, which highlighted the involvement of ERK1/2 in IFN-γ-dependent signaling leading to NO generation (1). Furthermore, it demonstrates that SHP-1 plays a major role in the inhibition of ERK1/2 phosphorylation during Leishmania infection, which is consistent with previous findings showing that SHP-1 activity toward ERK was increased in Leishmania-infected macrophages (32). Thus, ERK inactivation by SHP-1 activated by Leishmania seems to lead to inhibition of NO production by macrophages. It is likely that other JAK2- and ERK-regulated functions are also manipulated by Leishmania via SHP-1.

Activated JAK2 phosphorylates STAT1 on tyrosine 701 (19), allowing its dimerization and nuclear localization. ERK-dependent phosphorylation of serine 727 appears to be necessary for maximal transcriptional activity (1, 44). Furthermore, Leishmania infection has been shown to lead to defective phosphorylation ofSTAT1 in phorbol myristate acetate-differentiated U937 cells, and this may have been SHP-1 dependent (33). Nuclear localization and DNA binding were not directly addressed in this latter study; here we show that, even in the absence of SHP-1, basal STAT1 nuclear activity is severely inhibited by Leishmania, and there is no detectable activation by IFN-γ. This is consistent with our recent description of a protein kinase C- and proteasome-dependent degradation of STAT1 during Leishmania infection, which does not involve PTPs (14).

In addition to STAT1, transcription factors NF-κB and AP-1 contribute to iNOS induction (24, 27, 47, 48). Here, we show that both transcription factors are inhibited by Leishmania in littermate cells and that IFN-γ is unable to rescue this inhibition. However, inhibition of NF-κB and AP-1 is dependent on SHP-1, and both transcription factors respond to IFN-γ in the
absence of SHP-1, even in infected cells. Furthermore, we observe higher basal NF-κB and AP-1 activities in unstimulated, uninfected cells, consistent with SHP-1’s being a negative regulator of these transcription factors under normal physiological conditions.

Reduction of AP-1 and NF-κB DNA binding activities by *Leishmania* has been previously reported in unstimulated cells (17, 40), consistent with our observations in lintermate cells in the absence of IFN-γ. Furthermore, infection has been shown to prevent degradation of IκB, and thus, presumably, the activation of NF-κB, in response to LPS (40). Repression of NF-κB and AP-1 activity has been linked to activation of ceramide synthesis by the lipophosphoglycans on the surface of the parasite, which leads to inhibition of ERK phosphorylation (17). PTP activity appears to be increased by exogenous ceramide, which also inhibits phosphorylation of ERK1/2 in response to LPS (16). Like *Leishmania*-dependent ERK1/2 dephosphorylation, dephosphorylation in response to exogenous ceramide is prevented by sodium vanadate, a PTP inhibitor (16). Furthermore, NO production in response to LPS can be restored in infected macrophages by inhibitors of ceramide generation, with a corresponding reduction in parasite survival (16). It is therefore possible that increased ceramide in infected cells causes activation of SHP-1, resulting in the prevention of IFN-γ-stimulated NO release via dephosphorylation of ERK1/2 and consequent nonresponsiveness of NF-κB and AP-1. However, the activation of PTP by *Leishmania* is very rapid, detectable within a few minutes (2), whereas studies of the role of ceramide addressed much later time points when feedback and other secondary mechanisms may greatly increase the complexity (16, 17). While it is therefore premature to assume a causal link, a more detailed study of the upstream signaling events leading from contact with the parasite to SHP-1 activation and the role played by ceramide will be of very great interest.

Taken together, the data in this study highlight the role of SHP-1 as a negative regulator of IFN-γ-dependent gene expression during *Leishmania* infection. Activation of SHP-1 by *Leishmania* inhibits JAK2 and ERK2 and their downstream transcription factors NF-κB and AP-1. This, in turn, results in suppression of iNOS induction and NO generation following IFN-γ stimulation. However, SHP-1 is not the only macrophage signaling intermediate exploited by *Leishmania*. SHP-1−/− macrophages still show some increase in PTP activity and inhibition of NF-κB and AP-1 during infection, indicating a role for other PTPs in suppressing the response to IFN-γ. Furthermore, inhibition of STAT-1 appears to be entirely independent of PTPs (14). It is important to note that, while we have only addressed iNOS induction in this study, IFN-γ induces a large number of macrophage genes that are important for mounting an effective immune response, and many of these are repressed by *Leishmania*, such as interleukin-12 and major histocompatibility complex class II (4, 7, 39, 43). It is likely that the mechanism we have described here will also be important for repression of many of these genes.

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**REFERENCES**


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