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IFN-γ primes macrophages for antimicrobial activity, increased killing of intracellular pathogens, and Ag processing and presentation to lymphocytes by cooperating with a second signal (provided by LPS or endogenous TNF-α) to promote increased proinflammatory cytokine production, NO production, and MHC class II expression. Macrophage-stimulating protein (MSP) suppresses NO production by activated peritoneal macrophages in vitro. Furthermore, targeted deletion of the receptor for MSP, stem cell-derived tyrosine kinase receptor (STK/RON), resulted in increased production of NO by activated macrophages both in vitro and in vivo. Here we demonstrate that expression of STK in RAW264.7 cells resulted in suppression of NO production following IFN-γ stimulation. Experiments with neutralizing Abs to IFN-γ as well as knockout studies have confirmed an essential role for IFN-γ signaling pathway in the activation of macrophages and, ultimately, host resistance to infection (1–8). Many of these biological responses to IFN-γ stimulation are mediated by, among other things, increased proinflammatory cytokine production, NO production and MHC class II expression following IFN-γ stimulation. The presence of the STK receptor did not significantly alter the expression of the IFN-γ receptor, STAT1 phosphorylation, or the up-regulation of IFN response factor-1 expression following IFN-γ stimulation. However, nuclear translocation of NF-κB following stimulation of RAW cells with IFN-γ and LPS was reduced in the presence of the MSP/STK signaling pathway. These results suggest that the negative regulation of macrophage responses by MSP/STK occurs at least in part via inhibition of costimulatory signals, resulting in NF-κB activation, that cooperate with IFN-γ to promote activation. The Journal of Immunology, 1999, 163: 6606–6613.

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† Abbreviations used in this paper: IRF, IFN response factor; ICSBP, IFN consensus sequence binding protein; iNOS, inducible NO synthase; MSP, macrophage-stimulating protein; PI3-kinase, phosphoinositide 3-kinase; NCS, newborn calf serum; DM, double mutant; STK/RON, stem cell-derived tyrosine kinase receptor.
α-chain and a transmembrane β-chain with intrinsic tyrosine kinase activity. The biological functions of MET and STK/RON are mediated primarily by a two-tyrosine multifunctional docking site in the C-terminal tail of the respective receptors that has been shown to associate with a number of SH2-containing signaling molecules, such as phospholipase C-γ, PI3-kinase, Shc, and Grb2 upon ligand stimulation (24, 25), possibly through the adaptor protein, Gab1 (26). MET is broadly expressed, and knockout studies have revealed an essential role for MET in liver and muscle development (27–29). In contrast, STK is expressed relatively late during embryogenesis, and its expression in the adult is restricted primarily to areas of the central nervous system, specialized epithelium, and macrophages (30, 31).

STK is not expressed at detectable levels on circulating monocytes or bone marrow–derived macrophages, but is up-regulated following the migration of these cells to the peritoneal cavity during an inflammatory response (32). In addition to peritoneal macrophages, expression of STK has been detected on some populations of tissue–resident macrophages, such as osteoclasts, where MSP induces bone resorption (33), but has not been shown on others, such as resident macrophages of the lung. We have recently demonstrated STK expression in Kupffer cells in the liver, where it also appears to have a role in regulating NO production (P. H. Correll et al., manuscript in preparation). It has been suggested that the differences in STK expression in tissue–resident macrophages may account for differences in susceptibility of tissues to inflammatory damage. The presence of potential NF-κB, IFN-γ response elements (γIRE), and Sp-1 sites in the STK/RON promoter suggest a mechanism for regulation of expression in response to inflammation (34). Thus, regulation of STK expression may act as a negative feedback mechanism to protect host tissues from excessive inflammatory damage during an immune response.

Activated macrophages from mice carrying targeted mutations in STK produce elevated levels of NO both in vitro and in vivo, rendering the mice more susceptible to endotoxic shock (35, 36). These data suggest that STK is an important regulator of macrophage activity. Following stimulation, the production of NO was determined by assaying culture supernatants for NO2−, a stable reaction product of NO with molecular oxygen. Briefly, 100 μl of culture supernatant was reacted with an equal volume of Griess reagent (1% (w/v) sulfanilamide/0.1% (w/v) naphthylethylenediamine dihydrochloride/2.5% (w/v) H3PO4) at room temperature for 10 min. The absorbance at 550 nm was determined. All measurements were performed in triplicate. The concentration of NO2− was calculated by comparison with a standard curve prepared using NaN3.

RT-PCR

The iNOS expression in resting and IFN-γ-activated RAW cells was analyzed by RT-PCR. Briefly, 1 × 106 cells were activated with 10 U/ml IFN-γ for 0, 2, 4, 6, and 24 h, after which they were harvested for RNA isolation using the guanidinium thiocyanate method. Reverse transcription was conducted for 15 min at 42°C using random hexamers. Conditions for PCR are as follows: 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. The primers were: NOSIIA, 5′-ATGCGCAACATGAGTCGCCCATC-3′; and NOSIII-B, 5′-GCTTGTGTCACAGAAGTCTC-3′. Conditions for IRF-1 and IRF-2 were 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. The primers were: IRF-2S, 5′-TTAGGACGTTCATGCTGAC-3′; IRF-2A, 5′-CTCCCCATGTTGCCGAGTAC-3′; IRF-1S, 5′-TGAACCCGTGGACTAGATG-3′; and IRF-1A, 5′-TATGCCTGTGGTGAATG-3′.

Immunoprecipitation

Cells (107) were lysed in 0.8 ml of cold lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA (pH 7.4), 1% Nonidet P-40, 0.25% deoxycholate, 20 mM β-glycerol phosphate, 1 mM Na3VO4, 1 mM PMSF, 1 μg/ml leupeptin, and 2 μg/ml aprotinin) for 15 min, followed by centrifugation at 10,000 rpm for 10 min. Seven hundred microliters of supernatant was then incubated with 2.5 μg of Ab and 30 μl of 50% protein A beads (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. The agarose solution was then centrifuged for 4 min at 10,000 rpm at 4°C. The pellet was washed with lysis buffer three or four times, resuspended in 20 μl of 2.5% SDS sample buffer, boiled for 5 min, and centrifuged at 10,000 rpm at 4°C for 5 min, and supernatants were loaded onto a 10% SDS-PAGE gel.

Western blot analysis

After incubation with the indicated stimuli, cells (1 × 106 cells/100-mm plate) were washed in PBS and lysed in 1 ml of boiling lysis buffer (1% SDS, 1.0 mM sodium orthovanadate, and 10 mM Tris, pH 7.4). The protein contents were determined using the DC protein assay kit (Bio-Rad, Richmond, CA). Absorbance was measured at 750 nm with a Beckman DU530 spectrophotometer (Palo Alto, CA). Proteins were mixed with 5× SDS sample buffer, SDS-PAGE, using 12.5% bis-acrylamide for the separation gel and transfer of protein, was performed with a MiniProtein II Cell (Bio-Rad) at 65 V for 15 min, then at 150–160 V for 1 h. Nitrocellulose membranes were washed in ddH2O briefly, then equilibrated in Transblotting buffer (48 mM Tris 39 mM glycine, and 20% methanol) for 5 min. The gels were washed in ddH2O for 5 min, then in Trans-Blotting buffer for 15 min. Transblotting was performed using Trans-Blot SD SemiDry Trans-Cell (Bio-Rad) for 20–30 min at 15 V according to the manufacturer’s instructions. The blots were placed in 1% blocking buffer (BM POD Kit, Roche Molecular Biochemicals, Indianapolis, IN) for 1 h at room temperature for blocking of nonspecific binding. Primary Abs in 0.5% blocking buffer were incubated with blots 1 h or overnight at 4°C, then the blots were incubated with peroxidase-conjugated secondary Abs. Chemiluminescence substrates (BM POD Kit) were used to reveal positive bands. The bands were exposed on x-ray films. The Abs were iNOS/NSOS type II (Transduction Laboratories, Lexington, KY); ICSPB (C-19), IRF-1 (M-20), and p65 (Santa Cruz Biotechnology); STAT-1 (C-termius; Transduction Laboratories); and anti-phosphotyrosine (Upstate Biotechnology, Lake Placid, NY).

Flow cytometric analysis

Cells were harvested and washed with PBS, and 1 × 106 cells/100 μl were resuspended in PBS/2% newborn calf serum (NCS) on ice. Fc receptors were blocked using 1 μg/ml of anti-mouse CD32/16 (FcγRIIb receptor; Pharmingen) for 5 min on ice, followed by incubation in the presence of primary Ab for 30–60 min on ice. Expression of STK was detected using 2 μl of biotinylated 2B (0.3 μg/ml) anti-STK (provided by T. Suda, Kumamoto University) followed by three washes in PBS/2% NCS and a 30–to 60-min incubation in the presence of 1 μl of PE-conjugated streptavidin. The levels of IFN-γR on the cell surface were determined using FITC-conjugated anti-CD119 (Pharmingen). Cells were washed in 4 ml of PBS/2% NCS, resuspended in 1 ml of PBS/2% NCS with 10 μg/ml propidium iodide, and analyzed by flow cytometry (XL; Coulter, Hialeah, FL).
The supernatant were stored at 4°C. Briefly, 10 ml Promega Gel Shift Assay System according to the manufacturer’s instructions. Tissue extraction by centrifugation at 13,000 g for 15 min at 4°C, Nonidet P-40 was added to reach a 0.5% concentration. The tubes were gently vortex mixed for 15 s, and nuclei were sedimented by centrifugation at 8000 g for 15 min. The pellet was resuspended in 100 µl of buffer A supplemented with 20% glycerol and 0.4 M KCl. Incubation with 5 µg of pMinosGluc or control pGL2-Basic (Promega) using Lipofectamine (Life Technologies). After an overnight recovery, cells from each individual transfection were split into two dishes, an untreated control and an experimental dish treated with 100 U/ml IFN-γ and 0.1 µg/ml LPS. Following activation for 18 h, cells were lysed, and luciferase activity was measured.

Electrophoretic mobility shift assay

To prepare nuclear extracts, 1 × 107 cells were washed twice with ice-cold PBS and harvested in 0.4 ml of buffer A (10 mM HEPES (pH 7.9), 1 mM EDTA, 1 mM EGTA, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml aprotinin, 10 μg/ml leupeptin, 2 μg/ml 1-chloro-3-tosylamido-7-amino-2-heptaneamine, 5 mM NaF, 1 mM Na3VO4, and 10 mM Na2MoO4). After 10 min at 4°C, Nonidet P-40 was added to reach a 0.5% concentration. The nucleotides with 100 µl of buffer A supplemented with 20% glycerol and 0.4 M KCl. Incubation was continued for 30 min at 4°C with gentle vortexing. Nuclear proteins were extracted by centrifugation at 13,000 g for 15 min, and aliquots of the supernatant were stored at −80°C. EMSA was conducted using the Promega Gel Shift Assay System according to the manufacturer’s instructions. Briefly, 10 µg of nuclear extracts were used in the reaction mixture. The reactions were incubated at room temperature for 20 min, then analyzed by electrophoresis on a 6% nondenaturing polyacrylamide gel (NOVEX, San Diego, CA) at 100 V for 1 h. The running buffer was 0.5X TBE. The gel was transferred to 3 MM Whatman paper (Clifton, NJ) by drying under vacuum at 60°C and then exposed at −70°C to x-ray film. Competition with unlabeled oligonucleotide or nonspecific nucleotide (AP2) was performed using a 50-fold excess of DNA in the binding reaction. For supershift, 1 μl of anti-p65 (Santa Cruz Biotechnology) was added to the binding reaction.

Results

Expression of STK in RAW cells

To examine the role of STK in macrophage activation, we transfected RAW264.7 cells with a eukaryotic expression vector containing the STK cDNA (25). The transfectants were cloned by limiting dilution, and the expression of STK in individual clones was verified by flow cytometry using an mAb to the extracellular domain of STK (32). STK is not expressed on the surface of mock-transfected cells, but is easily detectable on the surface of the transfected clones (Fig. 1). Three clones expressing high levels of STK (STK-2, STK-13, and STK-14) were used for all subsequent experiments. As a control, we also transfected the STK cDNA containing Y to F mutations in the two tyrosines comprising the multifunctional docking site in the C-terminal tail (25). Three clones (DM-1, DM-2, and DM-4) expressing levels of receptor comparable to the STK clones were chosen (data not shown). All experiments shown here were performed in the presence of serum or 100 ng/ml recombinant MIP. Similar results were observed in either case.

STK suppresses NO production in RAW 264.7 cells

Previously, we have shown that peritoneal macrophages from mice lacking a functional STK receptor produce elevated levels of NO in vitro following stimulation with IFN-γ (35). To determine whether activation of the STK pathway in RAW264.7 cells resulted in the suppression of NO production, we measured the levels of NO in the supernatant of transfected RAW cells following

FIGURE 1. Expression of STK in RAW 264.7 cells. RAW264.7 cells were transfected with a eukaryotic expression vector containing the STK cDNA, and individual clones were analyzed by flow cytometry. The cells were stained with a biotinylated monoclonal anti-STK Ab followed by PE-conjugated streptavidin. Three clones shown here (STK2, STK13, and STK14), expressing high levels of STK receptor on the cell surface, were used for all subsequent experiments.

FIGURE 2. Suppression of NO production by STK. A, RAW, STK-2, STK-13, and STK-14 cells (3 × 105 cells/well) were stimulated with the indicated concentration of IFN-γ for 24 h, following which the level of NO in the supernatant was determined. B, RAW, STK13, and three clones transfected with a mutated STK cDNA (DM-1, -2, and -4) were stimulated with 100 U/ml IFN-γ for 24 h, and the levels of NO in the supernatant were determined using the Greiss reagent. All assays were performed in triplicate.
24 h of stimulation with various doses of IFN-γ. The results from these experiments demonstrate a suppression of NO production in all three transfected clones following IFN-γ stimulation (Fig. 2A).

The NO production by all clones was completely suppressed in the presence of the NO inhibitor Nα-glycyl-L-arginine (L-NMMA) (data not shown). Previous studies with MET and STK/RON, in which the two tyrosines in the C-terminal tail of the receptor were converted to Phe, suggest that these sites are required for receptor function both in vitro and in vivo (25, 27). Therefore, we tested the effects of the double mutant (DM) on its ability to suppress NO production by the RAW cells. The levels of NO produced by three independent clones expressing the mutated STK were not statistically different from those produced by the untransfected RAW cells following 24 h of stimulation with 100 U/ml IFN-γ (Fig. 2B).

To determine whether this suppression of NO production by the IFN-γ-activated macrophages was due to a decrease in the transcriptional activation of iNOS, we examined the levels of iNOS mRNA following activation. RAW, STK2, STK13, and STK14 cells were stimulated with 10 U/ml IFN-γ for the indicated times from 0–24 h. Following stimulation, the cells were harvested, and RNA was extracted for RT-PCR analysis. A, To determine the levels of iNOS mRNA following activation, RAW, STK2, STK13, and STK14 cells were stimulated with 10 U/ml IFN-γ for 6 h, following which protein was extracted, and Western blot analysis was performed. B, To determine the levels of iNOS protein following activation, RAW, STK2, STK13, and STK14 cells were stimulated with 10 U/ml IFN-γ for 6 h, following which protein was extracted, and Western blot analysis was performed. C, RAW and STK2 cells were transiently transfected with a reporter construct containing luciferase driven by the murine iNOS promoter. The transfected cells were left unstimulated or were stimulated with 100 U of IFN and 0.1 μg/ml LPS and assayed for luciferase activity 18 h later. All assays were performed in triplicate. The data shown are the averages of two independent experiments.

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RNA by RT-PCR at various time points following IFN-γ stimulation. We consistently observed a significant decrease in the levels of iNOS mRNA in the transfected clones at 6 and 24 h after IFN-γ stimulation (Fig. 3A), suggesting that the decrease in NO production observed in these clones is due to lower levels of iNOS mRNA. This decrease in iNOS mRNA levels in the STK-transfected clones was reflected by a decrease in iNOS protein 6 h following stimulation with 10 U/ml IFN-γ (Fig. 3B). To quantitate the difference in transcriptional activation of iNOS, we performed transient transfections using a luciferase reporter driven by the murine iNOS promoter. Results from these studies consistently demonstrated a 10- to 20-fold increase in iNOS reporter activity in RAW cells following stimulation with IFN-γ and LPS compared with a significantly lower (2- to 3-fold) increase in the presence of STK (Fig. 3C).

The MSP/STK does not regulate receptor proximal events in the IFN-γ signaling pathway

The regulation of IFN-γ responses by MSP/STK could occur at the level of expression of the IFN-γ receptor or at the level of IFN-γ response genes. To determine whether the suppressive effect of the MSP/STK pathway on IFN-γ activation of macrophages occurs at the level of the IFN-γ receptor, we examined the level of the IFN-γ receptor on the transfected macrophages before (Fig. 4A) and after (Fig. 4B) stimulation with IFN-γ. Although the levels of the IFN-γ receptor were not significantly different between the transfected and untransfected cells, the level of CD119 expression on the surface of the macrophages was significantly lower in the transfected cells compared with the untransfected cells (Fig. 4B).

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receptor on one of the clones appeared slightly lower following IFN-γ stimulation, overall there were no significant differences in the levels of IFN-γ receptor expression on untransfected cells vs cells transfected with STK. These results suggest that the decrease in responsiveness of the cells expressing STK to IFN-γ is not due to decreased expression of the IFN-γ receptor.

The activation of macrophages by IFN-γ results in rapid phosphorylation of the transcriptional regulator, STAT-1, by Janus kinases 1 and 2. Phosphorylated STAT-1 then forms a homodimer and is translocated to the nucleus, where it binds to consensus γ activation sequence (GAS) elements present in IFN-γ-regulated promoters. To determine whether the activation of STAT-1 by IFN-γ is altered in the presence of the MSP/STK signaling pathway, we examined the phosphorylation status of STAT-1 in the transfected cells following stimulation with IFN-γ in the presence of MSP for 30 min (Fig. 5). Results from these studies failed to demonstrate a significant difference in the phosphorylation of STAT-1 in the presence or the absence of STK, suggesting that STAT-1 phosphorylation is not a target of MSP/STK-mediated suppression of macrophage activation in response to IFN-γ.

The up-regulation of iNOS expression is regulated in part by the IRF family of transcriptional regulators. Therefore, we measured the levels of IRF-1, IRF-2, and ICSBP mRNA (Fig. 6A) and protein (Fig. 6B) in the transfected cells before and after IFN-γ stimulation. IRF-1 and ICSBP expressions are up-regulated following IFN-γ treatment. These findings are supported by the results in Fig. 6, which demonstrate increased expression of IRF-1 and ICSBP upon IFN-γ treatment. However, our data suggest that the up-regulation of IRF-1 (mRNA and protein) and ICSBP (protein) is unaffected by the presence of STK (Fig. 6, A and B). Furthermore, the level of IRF-2 (mRNA), which is constitutively expressed in macrophages, is also unaltered by the expression of STK (Fig. 6A). These results are confirmed by gel-shift analysis, which demonstrates similar DNA binding by IRF-1 in RAW and STK2 cells (Fig. 6C).

We did, however, observe a slight decrease in IRF-1 activity in response to IFN-γ in RAW/STK cells (Fig. 6D), suggesting that other signaling pathways may be affected by STK, which alter the

![FIGURE 5. STK does not affect IFN-γ-mediated phosphorylation of STAT-1. To determine the ability of IFN-γ to activate STAT-1 in the presence of the MSP/STK signaling pathway, we starved RAW, STK2, STK13, and STK14 cells for 4 h, followed by activation with 100 U/ml IFN-γ in the presence of 100 ng/ml MSP. Following 30 min of stimulation, cells were harvested, and STAT-1 was immunoprecipitated. The blot was probed with an anti-phosphotyrosine Ab (upper panel), stripped, and re-probed for STAT-1 (lower panel).](http://www.jimmunol.org/)

![FIGURE 6. STK does not affect the up-regulation of IRF-1 expression. A. To determine the levels of IRF-1 and IRF-2 mRNA in activated cells, RAW, STK2, STK13, and STK14 cells were stimulated with 10 U/ml IFN-γ for 2 h, cells were harvested for RNA extraction, and RT-PCR was performed. Results are representative of three individual experiments. B. To determine the levels of IRF-1 and ICSBP protein in activated cells, RAW, STK2, STK13, and STK14 cells were stimulated with 10 U/ml IFN-γ for 6 h, following which protein was isolated for Western blot analysis. Results are shown for RAW and STK2 cells. C. RAW and STK2 cells were stimulated with 100 U/ml IFN-γ for 6 h, following which nuclear extracts were harvested, and gel-shift analysis was performed. Lane 1, Negative control; lane 2, unstimulated RAW; lane 3, unstimulated STK2; lane 4, stimulated RAW; lane 5, stimulated STK2; lane 6, stimulated RAW and specific competitor; lane 7, stimulated RAW and nonspecific competitor. D. RAW, STK2, STK13, and STK14 cells were transiently transfected with a reporter construct containing an IRF-1 response element driving expression of luciferase and stimulated with 10 U/ml IFN-γ for 24 h, following which luciferase activity was measured. All experimental values are the average of triplicate transfections.](http://www.jimmunol.org/)

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and STK-transfected cells with IFN-α. The up-regulation of iNOS expression in response to IFN-α is mediated by NF-κB and STAT-1 activation. As shown in Fig. 7A, nuclear translocation of NF-κB following IFN-α and LPS stimulation was reduced in the clones expressing STK compared with that in the untransfected RAW cells. Furthermore, gel-shift analysis of nuclear extracts from these cells demonstrated decreased binding of NF-κB to its consensus sequence in the presence of the MSP/STK signaling pathway (Fig. 7B). Finally, the increase in NF-κB activation was verified by transient transfection assays. Transfection of the STK-expressing clones with a luciferase reporter containing two adjacent NF-κB binding sites from the HIV long terminal repeat resulted in decreased luciferase activity compared with that of the parental cell line (Fig. 7C). These data suggest that activation of cells with IFN-α and LPS in the presence of activated STK results in reduced activation of NF-κB. The reduction in NF-κB activity may be at least partially responsible for the suppression of macrophage activation by the MSP/STK signaling pathway.

**Discussion**

The data presented here confirm the role of the MSP/STK signaling pathway in the regulation of macrophage activation and provide us with an in vitro model with which to examine the biochemical mechanism behind the action of MSP in this system. Previous work has shown that MSP/STK regulates the expression of iNOS in response to IFN-α and/or LPS (15, 35, 37). We have also used these cell lines as well as STK-deficient mice to demonstrate decreased MHC class II expression and IL-6 production in the presence of activated STK following macrophage activation with IFN-α and LPS (data not shown). Taken together, our data point to a broader role for the MSP/STK pathway in regulating macrophage activation and the resulting proinflammatory signals. The cell lines described here accurately reflect the role of STK in primary cells and, therefore, will be a valuable tool, along with the STK-deficient mice, with which to dissect the MSP/STK signaling pathway and its regulation of IFN-α signaling in a biologically relevant system.

IFN-α primes macrophages through the activation of the latent transcription factor, STAT-1. STAT-1, in turn, cooperates with NF-κB (induced by the costimulatory signal) in the transcriptional activation of IRF-1 (38, 39). The pivotal role of the IRF family of transcriptional regulators in the regulation of a number of IFN-α-responsive genes has recently emerged. IRF-1 plays a central role in the transcriptional regulation of iNOS (40), supported by the
presence of two adjacent IRF-1 response elements in the iNOS promoter (41), and MHC class II, through the transcriptional activation of class II trans-activator (CIITA) expression (42–44). In addition, a role for IRF-1 in the transcriptional regulation of the IL-6 promoter has recently been demonstrated, which also involves cooperation between IRF-1 and NF-κB (45, 46). The data presented here demonstrate that although STK inhibits IRF-1-mediated responses, STK-mediated suppression does not occur through negative regulation of STAT-1 phosphorylation or up-regulation of IRF-1 expression following IFN-γ stimulation. However, we observed a slight decrease in IRF-1 activity in RAW/STK cells in response to IFN-γ, suggesting that MSP/STK may inhibit the trans-activation capacity of IRF-1 via changes in post-translational modification.

Alternatively, our data suggest that the negative regulation of macrophage activation by the MSP/STK signaling pathway occurs at least in part through the suppression of NF-κB activation. Results with the mutant STK clones, suggest that this inhibition by MSP/STK is mediated primarily by the multifunctional docking site in the C-terminal tail of STK. Recent work by Chen et al. has shown that suppression of iNOS expression by STK can be inhibited by wortmannin (37), suggesting a role for PI3-kinase in this process. Furthermore, treatment of LPS-activated RAW cells with wortmannin resulted in inhibition of iNOS expression, accompanied by prolonged activation of NF-κB. These data suggest that PI3-kinase is a negative regulator of NF-κB activation in macrophages in response to LPS (47). Taken together with the studies presented here, we propose a model in which STK expression is up-regulated following macrophage activation, resulting in the activation of PI3-kinase, which, in turn, results in the negative feedback inhibition of iNOS expression through inhibition of NF-κB activation. Future work will be aimed at identifying the mechanism by which STK mediates suppression of NF-κB and to determine whether other signals implicated in macrophage activation are affected by the MSP/STK signaling pathway.

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