The Protein-tyrosine Phosphatase SHP-1 Associates with the Phosphorylated Immunoreceptor Tyrosine-based Activation Motif of FcγRIIa to Modulate Signaling Events in Myeloid Cells*

Received for publication, May 14, 2003, and in revised form, June 24, 2003 Published, JBC Papers in Press, June 27, 2003, DOI 10.1074/jbc.M305078200

Latha P. Ganesan, Huiqing Fang, Clay B. Marsh, and Susheela Tridandapani‡

From the Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, The Dorothy M. Davis Heart and Lung Institute, and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

FcγRIIa is a low affinity IgG receptor uniquely expressed in human cells that promotes phagocytosis of immune complexes and induces inflammatory cytokine gene transcription. Recent studies have revealed that phagocytosis initiated by FcγRIIa is tightly controlled by the inositol phosphatase SHIP-1, and the protein-tyrosine phosphatase SHP-1. Whereas the molecular nature of SHIP-1 involvement with FcγRIIa has been well studied, it is not clear how SHP-1 is activated by FcγRIIa to exert its regulatory effect. Here we report that FcγRIIa clustering induces SHP-1 phosphatase activity in THP-1 cells. Using synthetic phosphopeptides, and stable transfectants expressing immunoreceptor tyrosine-based activation motif (ITAM) tyrosine mutants of FcγRIIa, we demonstrate that SHP-1 associates with the phosphorylated amino-terminal ITAM tyrosine of FcγRIIa, whereas the tyrosine kinase Syk associates with the carboxyl-terminal ITAM tyrosine. Association of SHP-1 with FcγRIIa ITAM appears to suppress total cellular tyrosine phosphorylation. Furthermore, FcγRIIa clustering results in the association of SHP-1 with key signaling molecules such as Syk, p85 subunit of PtdIns 3-kinase, and p62dok, suggesting that these molecules may be substrates of SHP-1 in this system. Finally, overexpression of wild-type SHP-1 but not catalytically deficient SHP-1 led to a down-regulation of NFκB-dependent gene transcription in THP-1 cells activated by clustering FcγRIIa.

The abbreviations used are: ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; PtdIns, phosphatidylinositol; EGFP, enhanced green fluorescent protein; SH2, Src homology domain 2.

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engagement of the SH2 domains with cognate phosphopeptides has been shown to activate the phosphatase. Enzyme activity of SHP-1 is further enhanced by phosphorylation of tyrosines (Tyr-356 and Tyr-366) in the COOH-terminal region (29). The significance of the regulatory role of SHP-1 in the hematopoietic system is best exemplified in mice homozygous for motheaten (me/me) or motheaten viable (mev/mev) mutations (30–32). The mev mice do not express any SHP-1 protein, whereas the mev/mev mice express inactive splice variants of SHP-1. Both of these results suggest that signaling events initiated by the association of SHP-1 with the above molecules upon Fc systems (33–35). Likewise, our current studies demonstrate with and dephosphorylates Syk, p85, and p62dok in other cell mutants of SHP-1 have demonstrated that SHP-1 associates with and ity, whereas Syk associates with the COOH-terminal ITAM tyrosine of SHP-1. Previous studies using substrate-trapping mutants of SHP-1 have demonstrated that SHP-1 associates with and dephosphorylates Syk, p85, and p62dok in other cell systems (33–35). Likewise, our current studies demonstrate association of SHP-1 with the above molecules upon FcyRIIIA clustering suggesting that SHP-1 may dephosphorylate these molecules to down-regulate related signaling pathways. Consistent with this notion, analysis of functional consequence of SHP-1 phosphatase activity during FcγRIIIA signaling demonstrated that overexpression of wild-type SHP-1 but not catalytically deficient (D419A) SHP-1 (a kind gift from Dr. J. C. Edberg (University of Alabama) (36). COS-7 cells were transfected as previously described (37), with slight modifications. To measure phosphatase activity associated with FcγRIIIA, SHP-1, Syk, p85, p62dok, and Syk, these proteins were immunoprecipitated from resting and activated (FcγRIIIA clustering)/THP-1 cells. Immunoprecipitations with control antibodies were done in lyses of cells stimulated for 7 min. The immunoprecipitates were washed six times in wash buffer (10 mM Tris, pH 7.4), and subsequently incubated with tyrosine phosphopeptide substrate (RRLLDEAEYAAPG) (Upstate Biotechnology) in 10 mM Tris, pH 7.4, for 30 min. Reaction was stopped with 100 µl of malachite green solution, incubated for a further 15 min, and the absorbance was measured at 630 nm. All assays were performed at least three times and the values obtained were plotted as mean ± S.D.

Transfection of THP-1 Cells and Luciferase Assays—For analysis of SHP-1 influence on NFκB transcriptional activity, THP-1 cells were transfected by electroporation (310 V, 950 µF; Bio-Rad Gene Pulser II) with 5 µg of wild-type SHP-1 or catalytically deficient (D419A) SHP-1 (a kind gift from Dr. R. Siraganian) (38), 1 µg of NFκB-luc plasmid, and 0.5 µg of pEGFP to normalize for transfection efficiency. Transfectants were harvested 24 h later, activated by clustering FcγRIIIA by methods described above for 6 h at 37 °C. The cells were lysed in 100 µl of cell culture lysis reagent (Promega). Luciferase activity was measured using the Promega luciferase assay reagent. Data are represented as graphs indicating the % increase in NFκB activity in cells activated by clustering with FcγRIIIA over those that were not activated. Data points are expressed as mean ± S.D. of three independent experiments. Statistical analysis was performed by Student’s t test.

Transfection of COS-7 Cells—COS-7 cells were transfected as previously described (39). Briefly, cells were grown on culture dishes until they were 60–70% confluent. Plasmids encoding wild-type SHP-1 and D419A SHP-1 were mixed with pEGFP to normalize for transfection efficiency. Transfectants were harvested 24 h later and analyzed for expression of the transfected cDNAs by Western blotting whole cell lysates and SHP-1 immunoprecipitates from the transfectants were assessed for phosphatase activity as described above.

GFP-SHP-1 Construct—Wild-type SHP-1 cDNA in pSVL vector was obtained from Dr. R. Siraganian, and subcloned into pEGFP vector (Clontech). Expression of GFP-SHP-1 was first confirmed by transfecting COS-7 fibroblasts with either empty pEGFP vector or GFP-SHP-1 constructs, and subsequent Western blotting with anti-SHP-1 antibody.

Transfection of P388D1 Cells and Confocal Microscopy—P388D1 cells stably expressing human FcγRIIIA were transfected with GFP-SHP-1 plasmids using LipofectAMINE, as described above for COS-7 cells. Cells were harvested 24 h post-transfection, serum starved, and stimulated by clustering FcγRIIIA for 5 min. Resting and activated cells were fixed in 1% paraformaldehyde, cytofunct onto glass slides, and stained with Hoechst nuclear stain. Slides were then mounted using mounting media (Molecular Probes) and analyzed by confocal microscopy using a Zeiss LSM510 multiphoton confocal microscope.

RESULTS

SHP-1 Is Activated by FcγRIIIA Clustering—To assess whether SHP-1 is activated by FcγRIIIA, THP-1 cells were stimulated by clustering FcγRIIIA with Fab fragments of the receptor-specific monoclonal antibody IV.3, followed by secondary cross-linking with goat F(ab')2 fragments of anti-mouse Ig antibody. SHP-1 was immunoprecipitated from resting and activated cells and analyzed first, for phosphatase activity (Fig. 1A) and second, for tyrosine phosphorylation by Western blotting (Fig. 1B). The use of Fab(Fab')2 fragments of the clustering antibodies precludes the engagement of other FcγR present on the THP-1 cells by IgG ligand interaction ensuring that the resultant signals are emanating from FcγRIIIA alone. In Fig. 1A, SHP-1 phosphatase activity was measured in THP-1 cells

**MATERIALS AND METHODS**

**Cells, Antibodies, and Reagents—**THP-1 cells were obtained from ATCC and cultured in RPMI supplemented with 10% fetal bovine serum. P388D1 transfectants expressing human FcγRIIIA were a generous gift from Dr. J. C. Edberg (University of Alabama) (36). COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. Anti-FcγRIIIA antibody IV.3 was obtained from Medarex (Annandale, NJ). Rabbit polyclonal SHP-1, p85, Syk antibodies, and mouse monoclonal anti-phosphosynase antibody and phosphatase assay kits were purchased from Upstate Biotechnology (Charlottesville, VA).

**Immunoprecipitation and Western Blotting—**THP-1 cells and transfected P388D1 cells were activated by clustering FcγRIIIA with Fab(ab')2 fragments of monoclonal antibody IV.3 and goat Fab(‘)l anti-mouse Ig secondary antibody. Resting and activated cells were lysed in TN1 buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 10 mM Na3P04, 10 mM NaF, 1% Triton X-100, 125 mM NaCl, 10 mM NaVO4, 10 µg/ml each aprotinin and leupeptin), and postnuclear lysates were incubated overnight with the antibody of interest and protein G-agarose beads (Invitrogen) or goat anti-mouse Ig covalently linked to Sepharose, depending on the antibody. Immunoprecipitations with control antibodies were performed in lyses of cells stimulated for 3 min. Immune complexes bound to beads were washed in TN1 and boiled in SDS sample buffer (60 mM Tris, pH 6.8, 2.3% SDS, 10% glycerol, 0.01% bromphenol blue, and 1% 2-mercaptoethanol) for 5 min. Proteins were separated by SDS-PAGE, transferred to nitrocellulose filters, probed with the antibody of interest, and developed by enhanced chemiluminescence.

**Analysis of FcγRIIIA Expression by Flow Cytometry—**P388D1 transfectants were tested for expression of FcγRIIIA by incubating with Fab fragments of anti-FcγRIIIA monoclonal antibody IV.3, at a concentration of 10 µg/ml for 30 min at 4 °C. The cells were washed and incubated with fluorescein isothiocyanate-labeled goat F(ab')2 anti-mouse Ig secondary antibody for 30 min at 4 °C. Cells were subsequently washed, fixed in 1% paraformaldehyde, and analyzed by flow cytometry on an Elite EPICS fluorescence-activated cell sorter (Coulter, Hialeah, FL). Data from 10,000 cells per condition were recorded to yield the percentage of cells expressing receptors.

**Phosphatase Assays—**Phosphatase assays were performed as described previously (37), with slight modifications. To measure phosphatase activity associated with FcγRIIIA, SHP-1, Syk, p85, p62dok, and Syk, these proteins were immunoprecipitated from resting and activated (FcγRIIIA clustering)/THP-1 cells. Immunoprecipitations with control antibodies were done in lyses of cells stimulated for 7 min. The immunoprecipitates were washed six times in wash buffer (10 mM Tris, pH 7.4), and subsequently incubated with tyrosine phosphopeptide substrate (RRLLDEAEYAAPG) (Upstate Biotechnology) in 10 mM Tris, pH 7.4, for 30 min. Reaction was stopped with 100 µl of malachite green solution, incubated for a further 15 min, and the absorbance was measured at 630 nm. All assays were performed at least three times and the values obtained were plotted as mean ± S.D.
activated for the various time points indicated in the figure. Results indicate that SHP-1 phosphatase activity is induced by FcγRIIa clustering and the activity peaks around 7 min post-stimulation. Previous studies have indicated that the enzyme activity of SHP-1 is enhanced upon tyrosine phosphorylation of SHP-1 (29). The results shown in Fig. 1B demonstrate that SHP-1 is tyrosine-phosphorylated upon FcγRIIa clustering. A reprobe of the same membrane with anti-SHP-1 antibody in the lower panel indicates equal loading of SHP-1 in all lanes. The last lane marked “C” is a control immunoprecipitate with normal rabbit IgG.

To further confirm that the clustering antibodies used do not engage other FcγR expressed on THP-1 cells, specifically FcγRIIb, which bears a high level homology with FcγRIIa in the extracellular domain, binding of Fab fragments of monoclonal antibody IV.3 to FcγRIIb was analyzed. For this THP-1 cells were subjected to immunoprecipitation with Fab fragments of FcγRIIa-specific monoclonal antibody IV.3, intact IV.3 antibody, and pan-FcγRII antibodies KB61 and AT10. The ability of the antibodies to immunoprecipitate FcγRIIb was tested by Western blotting with FcγRIIb-specific antibody 163 (upper panel). Parallel samples were analyzed by Western blotting with FcγRIIa-specific antibody 260. D, P388D1 mouse macrophages stably expressing human FcγRIIa were transiently transfected with GFP-SHP-1, activated by clustering FcγRIIa for 5 min, and analyzed by confocal microscopy.

SHP-1 Translocates to the Membrane upon FcγRIIa Clustering—To test whether FcγRIIa clustering resulted in membrane translocation of SHP-1, GFP-SHP-1 constructs were generated and transiently transfected into P388D1 mouse macrophage cells stably expressing human FcγRIIa. Cells were stimulated for 5 min by clustering FcγRIIa and analyzed by confocal microscopy. Results indicated that SHP-1 is distributed in the cytoplasm in resting cells and translocates to the membrane in cells activated by clustering FcγRIIa (Fig. 1D). In parallel samples transfected with EGFP alone, no movement of GFP was observed in activated cells compared with resting cells (data not shown).

SHP-1 Down-regulates FcγRIIa Function

Fig. 1. SHP-1 is activated by FcγRIIa clustering in THP-1 cells. A, THP-1 cells were activated by clustering FcγRIIa for the time points indicated in the figure. SHP-1 was immunoprecipitated from resting and activated samples, and assayed for phosphatase activity by measuring the release of phosphate from a phosphopeptide substrate. Values plotted in the graph were obtained by subtracting the values in resting samples. The graph represents the mean ± S.D. of values from three independent experiments. B, SHP-1 immunoprecipitates from resting and activated THP-1 cells were analyzed by Western blotting with anti-phosphotyrosine antibody (upper panel). The lower panel is a reprobe of the same membrane with anti-SHP-1 antibody. These results are representative of three independent experiments. C, THP-1 cells were subjected to immunoprecipitation with Fab fragments of FcγRIIa-specific monoclonal antibody IV.3, intact IV.3 antibody, and pan-FcγRII antibodies KB61 and AT10. The ability of the antibodies to immunoprecipitate FcγRIIb was tested by Western blotting with FcγRIIb-specific antibody 163 (upper panel). Parallel samples were analyzed by Western blotting with FcγRIIa-specific antibody 260. D, P388D1 mouse macrophages stably expressing human FcγRIIa were transiently transfected with GFP-SHP-1, activated by clustering FcγRIIa for 5 min, and analyzed by confocal microscopy.
SHP-1 Down-regulates FcγRIIa Function

Association with FcγRIIa—FcγRIIa by Western blotting with the FcγRIIa-specific antibody 260. Results indicated that SHP-1 associates with FcγRIIa upon activation (Fig. 2A, upper panel). No association was detectable in resting cells. The same membrane was reprobed with anti-SHP-1 antibody to ensure equivalent loading of SHP-1 in all lanes (lower panel).

As a second approach to confirm association of SHP-1 with FcγRIIa, the receptors were immunoprecipitated from resting and activated THP-1 cells and subjected to a phosphatase assay along with a phosphopeptide substrate. The amount of free phosphate released was detected by the addition of malachite green. Results are expressed as picomole of phosphate released by immunoprecipitates from activated cells after subtracting the phosphate released was detected by the addition of malachite green. Results are expressed as picomole of phosphate released by immunoprecipitates from resting and activated THP-1 cells and analyzed for association with SHP-1 by Western blotting. The results shown in Fig. 3A, upper panel, indicate that the phosphorylated NH₂-terminal ITAM tyrosine, but not the COOH-terminal tyrosine, efficiently bound SHP-1. SHP-1 did not associate with the non-phosphorylated peptide (lane 1). In contrast, parallel experiments analyzing the binding properties of the peptides demonstrated that the peptide phosphorylated on the COOH-terminal ITAM tyrosine is functional and is able to associate with Syk (Fig. 3A, middle panel) and p85 (Fig. 3A, lower panel). These latter findings are consistent with earlier reports demonstrating that the COOH-terminal ITAM tyrosine of FcγRIIa is sufficient for association with Syk (40), and that p85 associates with both NH₂- and COOH-terminal ITAM tyrosines of FcγRIIa.

Because the above experiments were performed with synthetic peptides, we next asked whether the native FcγRIIa receptor would likewise demonstrate the differential ITAM tyrosine requirement for association with SHP-1 and Syk. For these experiments we used P388D1 mouse macrophage transfectants stably expressing single ITAM tyrosine mutants of human FcγRIIa. The P388D1 transfectants were activated by clustering FcγRIIa, the receptors were immunoprecipitated from resting and activated cells and analyzed by Western blotting for co-precipitating SHP-1 (Fig. 3B, upper panel) or Syk (Fig. 3B, lower panel). Results indicated that SHP-1 failed to associate with FcγRIIa when the NH₂-terminal ITAM tyrosine was mutated to phenylalanine (Y252F). However, the Y252F receptor displayed efficient binding to Syk. These results are consistent with the above peptide binding experiments.

To assess the signaling outcome of the ITAM tyrosine mutations, we compared the ability of these mutated receptors versus the wild-type receptor to induce signaling. For this we first...
ensured that the transfected receptors were expressed to comparable levels by flow cytometry (Fig. 4A). The transfected cells were stimulated by clustering FcγRIIa. FcγRIIa was immuno-precipitated from resting and activated cells and analyzed for tyrosine phosphorylation. Results indicated that all three receptors are capable of being tyrosine phosphorylated (Fig. 4B, upper panel). As might be expected the single ITAM tyrosine mutants displayed lower phosphorylation levels than the wild-type receptor. A reprobe of the membrane demonstrated equivalent receptor expression in the transfectants (Fig. 4B, lower panel). The reduced signal seen with anti-FcγRIIa antibody in the activated lane is because of the fact that the anti-FcγRIIa blotting antibody often displays lower efficiency of detection of the phosphorylated FcγRIIa in a reprobe. We, and others, have previously reported this property of the anti-FcγRIIa blotting antibody (19, 42).

We next analyzed total cellular tyrosine phosphorylation in the transfectants stimulated by FcγRIIa clustering (Fig. 4C). Results indicated that, clustering of the NH2-terminal ITAM tyrosine mutant leads to enhanced overall cellular tyrosine phosphorylation in comparison to clustering of the wild-type receptor (lane 4 versus lane 1). In contrast, mutation of the COOH-terminal ITAM tyrosine completely abrogated overall cellular phosphorylation. These observations are consistent with the notion that SHP-1 associates with the NH2-terminal ITAM tyrosine to down-modulate tyrosine phosphorylation events, and that Syk associates with the COOH-terminal ITAM tyrosine to become activated and lead to the phosphorylation of signaling proteins in the cell.

SHP-1 Associates with p85, Syk, and p62dok during FcγRIIa Signaling—The activation of SHP-1 during FcγRIIa signaling suggests that SHP-1 causes dephosphorylation of tyrosine-phosphorylated proteins. Numerous previous studies have identified the association of SHP-1 with tyrosine-phosphorylated signaling molecules, the subsequent dephosphorylation of these molecules, and down-regulation of the related signaling pathways (43). Drawing from these previous studies, we next analyzed whether SHP-1 associated with the tyrosine kinase Syk, the p85 adapter molecule of PtdIns 3-kinase, and the Ras GAP-binding protein p62dok during FcγRIIa signaling. Thus, THP-1 cells were activated by clustering FcγRIIa for various time points. SHP-1 was immunoprecipitated from resting and activated THP-1 cells and analyzed by Western blotting for the presence of co-precipitating Syk (Fig. 5A), p85 (Fig. 5B), and p62dok (Fig. 5C). As seen in the figure, SHP-1 associated with the above molecules in an activation-dependent manner. The membranes were reprobed with anti-SHP-1 antibody to ensure equal loading in all lanes. To further analyze whether active
SHP-1 is associated with Syk, p85, and p62dok, phosphatase assays were performed on the respective immunoprecipitates from resting and activated THP-1 cells. Consistent with association of SHP-1 protein, results indicated that phosphatase activity was present in Syk, p85, and p62dok immunoprecipitates (Fig. 5D). In control experiments, no association of SHP-1 phosphatase activity was observed in Erk immunoprecipitates from activated THP-1 cells (data not shown). Taken together these data suggest that SHP-1 may dephosphorylate the above molecules to down-regulate activation events induced by FcγRIIa clustering.

**SHP-1 Down-regulates FcγRIIa-mediated Function**—In recent reports we, and others, have demonstrated that FcγRIIa clustering results in the activation of NFκB-dependent gene transcription (19, 21, 44). These activation events are subject to regulation by the inositol phosphatases SHIP-1 and SHIP-2, presumably as a result of the consumption of the lipid products of PtdIns 3-kinase and the downstream signaling thereof. Our present studies demonstrate that SHP-1 associates with the p85 subunit of PtdIns 3-kinase, suggesting that SHP-1 may modulate PtdIns 3-kinase activity. Therefore, we next asked whether SHP-1 also played a role in modulating NFκB-dependent gene transcription initiated by FcγRIIa clustering. In these experiments we used wild-type and catalytically inactive (D419A) SHP-1 constructs, which we first expressed in COS-7 fibroblasts by transient transfection and analyzed for SHP-1 protein expression and enzyme activity. The results shown in Fig. 6B demonstrate that both wild-type and D419A SHP-1 are expressed efficiently from these plasmids. COS-7 fibroblasts do not express any endogenous SHP-1 as is seen from the absence of SHP-1 in the mock-transfected cells (lane 1). Shown in Fig. 6B, lower panel, is the phosphatase activity of these two SHP-1 proteins expressed in COS-7 cells.

Having ensured that we could achieve appropriate protein expression from these constructs, we then transiently transfected THP-1 cells with plasmids encoding the NFκB binding element coupled to a luciferase gene (NFκB-luc) either alone or with an excess of wild-type SHP-1 or D419A SHP-1. The cells were harvested 24 h post-transfection, activated by clustering FcγRIIa, and NFκB-dependent luciferase expression was assayed in a luciferase enzyme assay. Results from three independent experiments are shown in Fig. 6A. Overexpression of wild-type SHP-1 completely abrogated NFκB-dependent luciferase induction. In contrast, overexpression of the catalytically inactive D419A SHP-1 resulted in enhanced luciferase induction. These data demonstrate that SHP-1 negatively regulates FcγRIIa-mediated biological outcomes in human myeloid cells.

**DISCUSSION**

The human-specific FcγRIIa is a low affinity IgG receptor that has several unique features to it. In addition to being the most widely expressed IgG receptor, it also contains an unusually lengthy ITAM in its cytoplasmic domain. Mutational analyses of the cytoplasmic domain of FcγRIIa have identified specific amino acid motifs that are important for the phagocytic process. For example, mutation of either of the two tyrosine residues within the ITAM of FcγRIIa have been reported to severely abrogate intracellular calcium mobilization and phagocytosis (36, 45). An additional tyrosine residue located NH2-terminal to the ITAM also becomes phosphorylated upon receptor clustering and plays a role in FcγRIIa-mediated activation (45). More recent studies have identified an LTL motif in the cytoplasmic domain of FcγRIIa that is involved in the formation of phagosomes (46, 47). Thus the cytoplasmic domain of FcγRIIa is made up of a complex set of signaling motifs that are not yet fully explored.

Once FcγRIIa receptors are clustered the Src family of tyrosine kinases phosphorylate tyrosine residues in the cytoplasmic domain of FcγRIIa (6). Phosphorylation of the ITAM promotes recruitment and activation of Syk, followed by the phosphorylation of multiple cytosolic signaling proteins. Unlike its T cell homolog ZAP-70 that requires both of its tandem SH2 domains to be engaged by phosphorylated ITAMs to be activated, single SH2 domain engagement is sufficient for Syk...
activation (48). Accordingly, the results shown in Fig. 3 demonstrate that the COOH-terminal ITAM tyrosine of FcγRIIa is necessary and sufficient for Syk association. Interestingly, there was constitutive Syk association with Y252F FcγRIIa, at a time when no tyrosine phosphorylation of the receptor was detectable (Fig. 4D). These results suggest that perhaps mutation of tyrosine 252 might lead to a non-SH2-dependent association of Syk with Y252F FcγRIIa. Additional studies are needed to define the nature of this novel interaction.

Recent studies have revealed that FcγRIIa clustering not only initiates activating events, but it also induces negative regulatory events such that the resultant biologic outcome is tempered. Thus, FcγRIIa recruits the inositol phosphatases SHP-1 and SHP-2 to modulate signaling events (19–21). In a transfected COS-7 fibroblast model the protein-tyrosine phosphatase SHP-1 and SHP-2 were shown to modulate signaling events (19–21). In a transfected COS-7 fibroblast model the protein-tyrosine phosphatase SHP-1, a fibroblast transfectant was harvested 24 h later, activated by clustering FcγRIIa for 5 h, and analyzed for luciferase activity. Results from three experiments are shown as mean ± S.D. of percent increase of luciferase activity in the activated samples over that in resting samples. B, COS-7 cells were transfected either with empty vector or plasmids encoding the wild-type SHP-1 or D419A SHP-1. Whole cell lysates were analyzed by Western blotting for the expression of the transfected SHP-1 (upper panel). SHP-1 was immunoprecipitated from the transfected cells and assessed for phosphatase activity (lower panel).

Fig. 6. SHP-1 negatively regulates NFκB-dependent gene transcription in THP-1 cells in response to FcγRIIa clustering. A, THP-1 cells were transfected with plasmids encoding the NFκB-binding element coupled to a luciferase gene (NFκB-luc), along with wild-type or D419A SHP-1. Transfectants were harvested 24 h later, activated by clustering FcγRIIa for 5 h, and analyzed for luciferase activity. Results from three experiments are shown as mean ± S.D. of percent increase of luciferase activity in the activated samples over that in resting samples. B, COS-7 cells were transfected either with empty vector or plasmids encoding the wild-type SHP-1 or D419A SHP-1. Whole cell lysates were analyzed by Western blotting for the expression of the transfected SHP-1 (upper panel). SHP-1 was immunoprecipitated from the transfected cells and assessed for phosphatase activity (lower panel).
