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Differential Regulation of the Alpha/Beta Interferon-Stimulated Jak/Stat Pathway by the SH2 Domain-Containing Tyrosine Phosphatase SHPTP1

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Interferons (IFNs) induce early-response genes by stimulating Janus family (Jak) tyrosine kinases, leading to tyrosine phosphorylation of Stat transcription factors. Previous studies implicated protein-tyrosine phosphatase (PTP) activity in the control of IFN-regulated Jak/Stat signaling, but the specific PTPs responsible remained unidentified. We have found that SH2 domain-containing PTP1 (SHPTP1; also called PTP1C, HCP, or SHP) reversibly associates with the IFN-α receptor complex upon IFN addition. Compared with macrophages from normal littermate controls, macrophages from *motheaten* mice, which lack SHPTP1, show dramatically increased Jak1 and Stat1α tyrosine phosphorylation, whereas Tyk2 and Stat2 activation is largely unaffected. These findings correlate with selectively increased complex formation on a gamma response element, but not an IFN-stimulated response element, in *motheaten* macrophages. Our results establish that SHPTP1 selectively regulates distinct components of Jak/Stat signal transduction pathways in vivo.

Interferons (IFNs) were the first of many cytokines and growth factors that were found to stimulate the expression of early-response genes by inducing the tyrosine phosphorylation of SH2 domain-containing transcription factors (subsequently termed Stats) (20, 21). Tyrosine phosphorylation of Stat proteins induces them to form homodimeric and/or heterodimeric complexes, which translocate to the nucleus and interact with similar elements in a variety of enhancers to promote transcription. Janus family protein-tyrosine kinases (PTKs) are integral components of the signaling cascades regulated by cytokines that activate Stats (17).

Recent work in many laboratories has identified several downstream components of IFN-α/β signaling. Two subunits (α and β) of the IFN-α/β receptor (IFNα/βR) have been molecularly cloned (28, 44). Binding of IFN-α/β to its receptor results in rapid activation of the Janus PTKs Jak1 and Tyk2. This results in tyrosyl phosphorylation of both Stat1α (p91) and Stat2 (p113) and the formation of at least two transcription factor complexes. One complex, composed of a heterotrimer of Stat1α, Stat2, and the DNA-binding component p48, binds to IFN-stimulated response elements (ISREs) (12, 13, 30), whereas Stat1α homodimers bind to gamma response region (GRR) elements or the IFN-γ activation sequence (14, 29). Substantial genetic and biochemical evidence indicates that activation of Janus PTKs precedes and is necessary for Stat tyrosyl phosphorylation and transcription complex formation. However, little is known about the molecules and mechanisms required for termination of IFN signal transduction.

Several reports have implicated protein-tyrosine phosphatases (PTPs) in both positive (signal-enhancing) and negative (signal-terminating) regulation of IFN signaling (6, 14, 15). Preincubation of cell homogenates with PTP inhibitors prevents activation of Stat1 by both IFN-α and IFN-γ (6, 14). These data suggest that at least one PTP is required to initiate IFN signaling but not to maintain its activity. Incubation of a variety of cells with vanadate also induces tyrosine phosphorylation of the Janus PTKs, as well as Stat1α, such that Stat complexes can form and bind to enhancers required for IFN-induced gene expression (15, 16). Thus, a PTP(s) also may negatively regulate IFN signal transduction. Since PTP inhibition results in IFN-independent activation of Jak/Stat family members, these results further suggest that a PTP(s) may be intimately associated with proximal components of this pathway. However, the specific PTPs required for activation and termination of IFN signaling pathway have remained unidentified.

SH2 domain-containing PTPs (SHPTPs) are attractive candidates for such regulatory molecules. Two mammalian SHPTPs have been identified (reviewed in references 9 and 26). SHPTP2 (11), also known as Syp (8), PTP1D (46), and PTP2C (1), is expressed ubiquitously and is the homolog of *Drosophila* Csw (32). Genetic analysis indicates that Csw is required for signaling by Torso (32) and other *Drosophila* receptor tyrosine kinases (31). Likewise, vertebrate SHPTP2 has been implicated in positive signaling by the insulin receptor (49), the epidermal growth factor receptor (49), and the fibroblast growth factor receptor (38). SHPTP2 also is tyrosine phosphorylated upon stimulation of appropriate cells with ciliary neurotrophic factor/interleukin-6 (IL-6)/leukemia-inhibitory factor/oncostatin M (2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 (47), or erythropoietin (EPO) (39), although its role in these cytokine pathways has not been established. SHPTP1 (PTP1C, HCP, SHP) is expressed primarily in hematopoietic cells (35). The physiological importance of SHPTP1 has been graphically illustrated by the finding that mutations in SHPTP1 result in *motheaten* (*me*/

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me) and *motheaten viable* (me^v/me^v) mice (37, 41). The *me* allele is an early frameshift mutation, such that *me*/*me* mice make no detectable SHPTP1 protein, whereas me^v/me^v mice synthesize SHPTP1 protein products with substantially decreased PTP activity (19, 37). Although *me*/*me* mice survive for only 2 to 3 weeks, me^v/me^v mice live for a few months. Presumably, residual PTP activity accounts for the increased viability of me^v/me^v mice, emphasizing the physiological importance of the enzymatic activity of SHPTP1. However, the specific signal transduction pathways regulated by SHPTP1 are largely unknown.

Recent studies have suggested that SHPTP1 negatively regulates signaling by the IL-3 and EPO receptors (IL3R and EPOR) (18, 51). Following IL-3 addition to a factor-dependent hematopoietic cell line, SHPTP1 was reported to bind directly to the tyrosyl-phosphorylated IL3R beta chain (IL3Rβ), although the precise binding site was not identified (51). Moreover, lowering the level of SHPTP1 protein by the inducible expression of antisense SHPTP1 RNA was reported to lead to IL3Rβ hyperphosphorylation. Similarly, upon addition of EPO, SHPTP1 associates with the tyrosyl-phosphorylated murine EPOR, expressed ectopically in either BaF3 or 32D cells (18). Mutation of the binding site for SHPTP1 on the EPOR results in prolonged phosphorylation of the EPOR-associated Janus kinase Jak2 and correlates with increased sensitivity to EPO-induced mitogenesis. The latter results suggested that SHPTP1 may regulate cytokine receptor signaling by promoting the dephosphorylation of receptor-associated Janus PTKs. However, the relevance of such results, obtained by using factor-dependent cell lines, has not been assessed in vivo by using *me*/*me* or me^v/me^v mice. To address the role of SHPTP1 in IFN-α/β signal transduction, we examined whether it associated with known components required for Stat activation by IFN-α/β. We found that SHPTP1 is basally associated with the IFNα/βR alpha-subunit (IFNα/βR-α) complex but reversibly dissociates upon IFN addition. This association is functionally important in vivo, because bone marrow-derived macrophages (BMDM) from *me*/*me* mice exhibit hyperstimulation of selective components of the IFN-α/β signaling cascade. Upon IFN stimulation, *me*/*me* BMDM show remarkably enhanced Jak1 tyrosyl phosphorylation, with only minimally increased Tyk2 phosphorylation. This correlates with increased tyrosyl phosphorylation of Stat1α but not Stat2 and increased transcription complex formation on a GRR element but not on an ISRE. Our data establish that SHPTP1 negatively regulates IFNα/βR signal transduction in vivo. Moreover, SHPTP1 can selectively regulate specific components within a cytokine receptor signal transduction pathway by dephosphorylating specific Janus PTKs.

MATERIALS AND METHODS

Cells. Mono Mac 6 (MM6) cells (53) were grown in RPMI 1640 supplemented with 10% fetal calf serum. Cultures of BMDM were prepared from 2-week-old C3HeB/FeJLe-a/a-*me*/*me* mice or their unaffected littermates by a modification of previously described procedures (42). Briefly, bone marrow cells extruded from femurs were incubated for 24 h in Dulbecco's modified Eagle's medium with 20% L-cell conditioned medium (L-CM) as a source of CSF-1. On day 1, adherent cells were discarded and nonadherent cells were collected and seeded on 100-mm-diameter plates at 1.5×10^5 cells per ml in Dulbecco's modified Eagle's medium plus 20% L-CM. On day 7, adherent BMDM were deprived of CSF-1 (L-CM) for 24 h and then stimulated with 10^4 U of IFN-α A/D (Hoffmann-LaRoche) per ml.

Whole cell extracts. Cells were diluted with ice-cold phosphate-buffered saline (PBS) and centrifuged at $1,500 \times g$ for 10 min at 4°C. Cells were washed with PBS and resuspended in 200 μl of lysis buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.4], 1 mM vanadate, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride). For some experiments, 1% digitonin was substituted for Triton X-100. Lysate were vortexed, incubated on ice for 10 min, and centrifuged at $18,000 \times g$ for 10 min at 4°C, and the supernatants were transferred to new tubes. Supernatants were incubated with various glutathione *S*-transferase (GST) fusion proteins bound to glutathione-agarose beads. The construction and expression of GST fusions to either the entire coding region of the cytoplasmic domain of IFNα/βR-α (the cDNA clone of the human IFNα/βR described by Uze et al. [44]), its carboxy-terminal 50 amino acids, or the membrane-proximal 50 amino acids of its cytoplasmic tail (lanes 5 and 6) have been described in detail previously (3). Beads were incubated with extracts at 4°C for 12 h, pelleted at $1,500 \times g$ for 2 min, and washed three times with ice-cold lysis buffer (1 ml for each wash). Proteins were separated on sodium dodecyl sulfate (SDS)–4 to 16% gradient polyacrylamide gels and transferred to Immobilon. Membranes were blocked and then incubated with an monoclonal antibody against Tyk2, Jak1, SHPTP1 (all from Transduction Laboratories), or phosphotyrosine (PY20; ICN), using concentrations and conditions recommended by the manufacturer. Immunoblots were developed by using appropriate secondary antibodies and enhanced chemiluminescence (ECL) (16).

EMSA. Electrophoretic mobility shift assays (EMSAs) were performed as previously described, using whole cell extracts prepared with Triton X-100-containing buffers as described above (10, 20, 48). Probes, consisting of the GRR found within the promoter of the FcγRI gene (5′ AGCATGTTTCAAGGATT TGAGATGTATTTCCCAGAAAAG 3′) and the ISRE of IFN-stimulated gene 15 (5′ GATCCATGCCTCGGGAAAGGGAAACCGAAACTGAAGCC 3′) were end labeled by using polynucleotide kinase and [γ-^{32}P]ATP.

Immunoprecipitations. Cell lysates (350 μg) prepared with Triton X-100 (Fig. 1, 2, 4, and 5)- or digitonin (Fig. 3)-containing buffers were incubated sequentially with an anti-Jak1 antiserum (Upstate Biotechnology Inc.) for 2 h, an anti-Tyk2 antibody (Santa Cruz Biotechnology) for 2 h, and a mixture of anti-Stat1α and anti-Stat2 antibodies overnight (20). For the experiments shown in Fig. 2, antisera for Tyk2 and Jak1 were prepared from rabbits immunized with peptides corresponding to the carboxy terminus of Tyk2 or the kinase-like domain of Jak1 (3, 16). Antibodies to IFNα/βRα were generated against the extracellular domain by immunizing mice with CHO cells expressing a high concentration of cell surface IFNα/βR-α. The antibodies were screened with IFNα/βR-α fusion protein and also by cell surface binding using fluorescence-activated cell sorting analysis. Immune complexes were collected after each immunoprecipitation on protein A beads, resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (8% polyacrylamide gel), immunoblotted with monoclonal antiphosphotyrosine antibody 4G10 (Upstate Biotechnology Inc.) and detected by using ECL. Membranes were stripped and reprobed with an anti-Jak1, anti-Tyk2, anti-Stat1α, or anti-SHPTP1 antibody.

RESULTS

To identify specific PTPs involved in IFN-α/β signal transduction, we examined whether SHPTP1 associated with components of this signaling cascade. Tyk2, a PTK required for IFNα/β activation of Stat1α and Stat2, has been shown to interact with GST fusion proteins containing either the entire cytoplasmic domain of IFNα/βR-α or its membrane-proximal 50 amino acids (3). We used these fusion proteins to determine whether other signaling components interact with this receptor subunit. Extracts were prepared from MM6 cells which had been incubated with or without IFN-β for 10 min. These cells are very responsive to IFN-α/β (53) and express SHPTP1 (data not shown). Glutathione-agarose beads containing GST fusion proteins to either the entire 100-amino-acid cytoplasmic domain of IFNα/βR-α (Fig. 1, lanes 1 and 2), its membrane-proximal 50 amino acids (lanes 5 and 6), or its carboxy-terminal 50 amino acids (lanes 3 and 4) were incubated with the extracts, and bound material was separated by SDS-PAGE, transferred to nylon membranes, and subjected to immunoblotting with antibodies specific for Tyk2 (Fig. 1A), Jak1 (Fig. 1B), or phosphotyrosine (Fig. 1D). As reported previously (3), Tyk2 associated with the GST fusion protein containing the complete IFNα/βR-α cytoplasmic domain (Fig. 1A, lanes 1 and 2) as well as with its membrane-proximal 50 amino acids (lanes 5 and 6). We also detected interaction of Jak1 with both the full-length and membrane-proximal GST fusion proteins. The carboxy-terminal 50 amino acids (lanes 3 and 4) did not bind to either Tyk2 or Jak1, serving as a control for specificity. In addition, GST alone did not bind to any of the proteins that were analyzed (data not shown). When the same blots were reprobed for SHPTP1, it was found to associate with the mem-

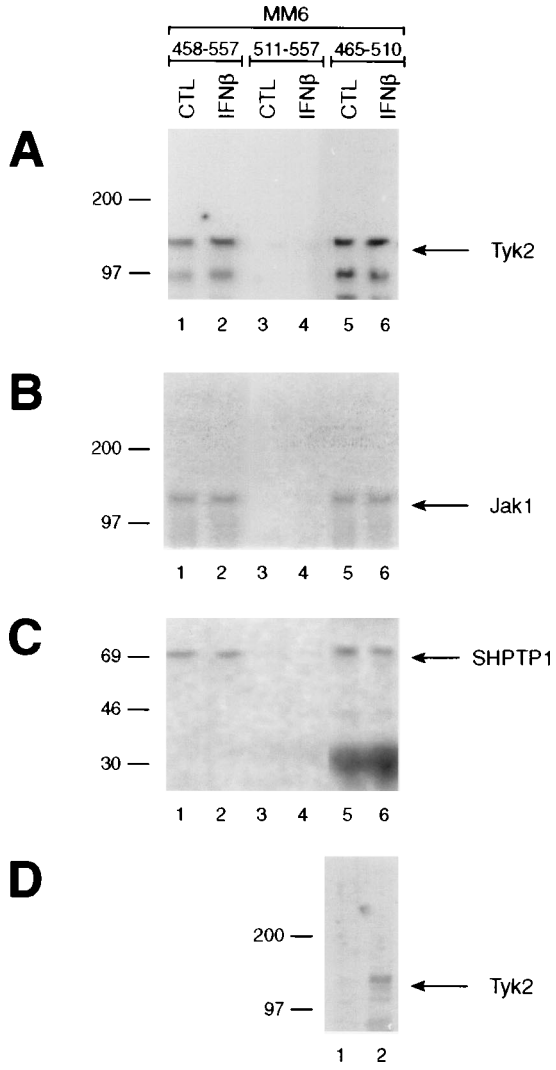

FIG. 1. Interaction of proteins with the cytoplasmic domain of IFNα/βR-α. MM6 cells (1×10^6 to 2×10^6 per sample) were incubated for 10 min at 37°C with (IFNβ) or without (CTL [control] 10^3 U of recombinant human IFN-β per ml, and cell extracts were prepared. The supernatants were incubated with GST fusion proteins bound to glutathione-agarose beads. Either the entire coding region of the cytoplasmic domain of the α subunit (lanes 1 and 2), the carboxy-terminal 50 amino acids of the cytoplasmic tail (lanes 3 and 4), or the membrane-proximal 50 amino acids of the cytoplasmic tail (lanes 5 and 6) were used. Proteins bound to the beads were separated on SDS–4 to 16% gradient polyacrylamide gels, transferred to Immobilon, and immunoblotted with a monoclonal antibody against Tyk2 (A), Jak1 (B), SHPTP1 (PTP1C) (C) (all from Transduction Laboratories), or phosphotyrosine (PY20; ICN) (D). Immunoblots were developed by using ECL. The composition of the faster-migrating product detected with the Tyk2 antibody is not known. The prominent tyrosine-phosphorylated band labeled Tyk2 in panel D aligned with the Tyk2 immunoblot shown in panel A. Sizes are indicated in kilodaltons.

brane-proximal and complete cytoplasmic domains of IFNα/βR-α in MM6 cells (Fig. 1C). Similar interactions were detected in lysates prepared from other cultured hematopoietic cell lines as well as from primary human peripheral blood monocytes (data not shown).

Although SHPTP1 associated with IFNα/βR-α, its interaction initially appeared unaffected by IFN-β treatment (Fig. 1; compare lanes 1 and 2, 5, and 6 [but see below]). In addition, we did not observe any IFN-induced change in SHPTP1 tyrosylphosphorylation. Under the same conditions, IFN-induced Tyk2 tyrosylphosphorylation was readily detected, while IFN-α stimulated tyrosine phosphorylation of Jak1 was variable and often marginally detectable (Fig. 1D). It should be noted that in most experiments, there was low but detectable tyrosine phosphorylation of both Tyk2 and Jak1 in cells incubated without cytokine (see Discussion).

Tyk2 and Jak1 represent two potential substrates for SHPTP1. To determine whether these PTKs are in a complex with SHPTP1 in vivo, Tyk2 and Jak1 immunoprecipitates from MM6 cell extracts were resolved by SDS-PAGE, blotted onto nylon membranes, and probed for the presence of SHPTP1 (Fig. 2A). Both anti-Tyk2 and to a lesser extent anti-Jak1 coimmunoprecipitated SHPTP1 from MM6 cells, whereas a control monoclonal antibody of the same isotype did not cross-react with the protein detected by the SHPTP1 antibody (Fig. 2B). From the data in Fig. 1 and 2, we concluded that SHPTP1 is associated with one or more components of the IFNα/βR-α signaling complex in vivo.

The association of SHPTP1 with the IFNα/βR-α complex suggested that it might regulate IFN-α/β signal transduction. However, we were surprised to find that this association appeared to be independent of IFN addition (for 10 min). This result contrasted with previous reports on the association of SHPTP1 with the IL3R (51) and EPOR (18, 39) and with the receptor tyrosine kinase c-Kit (50), in which SHPTP1-receptor association is ligand inducible. Since we also detected no change in SHPTP1 tyrosyl phosphorylation upon IFN treatment, it was unclear how SHPTP1 regulation of IFN-α/β signaling was effected.

Therefore, we investigated the kinetics of SHPTP1 association with components of the IFN-α/β signal transduction pathway in more detail (Fig. 3). MM6 cells were stimulated with IFN for various periods of time, and extracts were immunoprecipitated with antibodies against IFNα/βR-α (Fig. 3A), Jak1 (Fig. 3B), and Tyk2 (Fig. 3C), resolved by SDS-PAGE, and subjected to anti-SHPTP1 immunoblotting. These studies indicated that SHPTP1 reversibly associates with these three components of the IFNα/βR-α signaling complex. As observed in Fig. 2, prior to stimulation, SHPTP1 can be coimmunoprecipitated with both anti-Jak1 and anti-Tyk2 antibodies, and as predicted by the results of Fig. 1, SHPTP1 also is found in anti-IFNα/βR-α immunoprecipitates. However, within 30 s of IFN stimulation, the amount of SHPTP1 associated with IFNα/βR-α, Jak1, and Tyk2 decreases substantially, and by 1 min, virtually no SHPTP1 is detectable in any of these immunoprecipitates (Fig. 3). By 2 min following stimulation, reassociation of SHPTP1 with the IFNα/βR-α complex was observed, and basal levels of association were restored by 5 min. These blots were reprobed with anti-IFNα/βR-α, anti-Jak1, and anti-Tyk2 antibodies, respectively, to ensure that comparable amounts of these three signaling molecules were immunoprecipitated at each time point following stimulation. IFN-induced dissociation of SHPTP1 from the IFNα/βR-α complex suggested that its removal might be necessary to allow activation of Jak1 and Tyk2. Its return to the receptor complex by 5 min poststimulation suggested that SHPTP1 might help regulate the magnitude and/or duration of IFN-induced Janus PTK activation.

However, since PTPs have been implicated in both positive and negative functions in IFN signaling (see the introduction), the role(s) of SHPTP1 required clarification. The availability of a murine model of SHPTP1 deficiency (*me*/*me* mice) provided the opportunity to define the function of SHPTP1 in IFN signaling in more detail. We compared IFN-α/β signaling in BMDM isolated from *me*/*me* mice or their unaffected littermates. We used *me*/*me* mice, which are SHPTP1 protein null

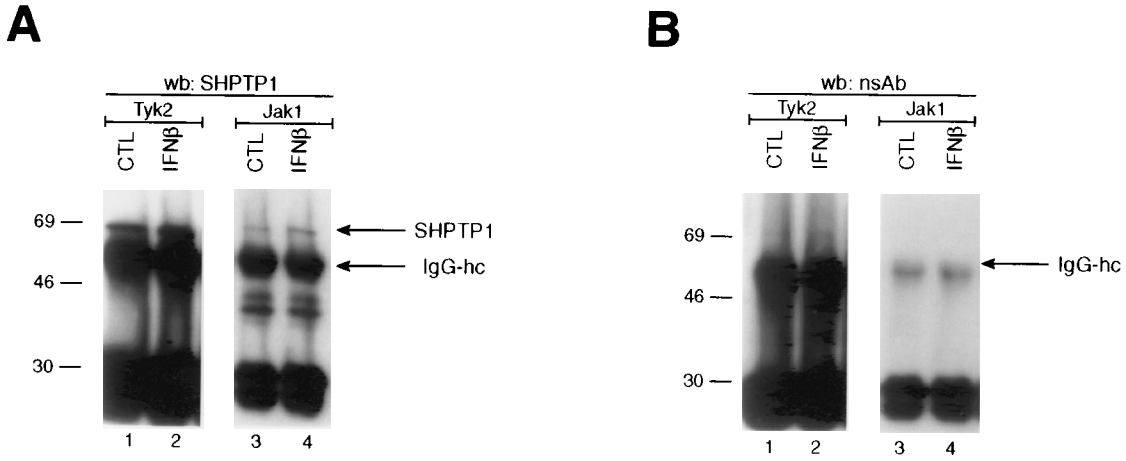

FIG. 2. SHPTP1 is associated with Tyk2 and Jak1 in MM6 cells. MM6 cells were incubated without (CTL [control]) or with (IFNβ) IFNβ, and extracts were prepared as in described in Materials and Methods. Lysates were incubated with an affinity-purified antibody raised against either Tyk2 (lanes 1 and 2) or Jak1 (lanes 3 and 4). Immunoprecipitates were resolved by SDS-PAGE, transferred to Immobilon, and probed for the presence of SHPTP1 (A) or an isotype-specific monoclonal antibody (B) as described in the legend to Fig. 1. wb, Western blot (immunoblot); IgG-hc, immunoglobulin G heavy chain. Sizes are indicated in kilodaltons.

animals, to avoid the potential confounding effects of residual PTP activity in me^v/me^v mice. BMDM were allowed to proliferate for 7 days in CSF-1, which was then removed from the cultures for 24 h prior to incubation of cells with or without IFN-α for 30 min. Whole cell extracts were prepared before and after IFN treatment, and Stat1α and Stat2 functions were assessed by EMSA using GRR and ISRE probes (33, 48). IFN-α-induced complexes from normal and *me/me* BMDM bound to both GRR and ISRE probes (Fig. 4A, lanes 2, 4, 6, and 8). Although there was no difference in the amount of IFN-stimulated gene factor 3 (ISGF3) complex in IFN-α/β-stimulated *me/me* and normal extracts (lanes 6 and 8), there was a substantial increase in the amount of the IFN-α-stimulated factor binding to the GRR in the same extracts (lanes 2 and 4). This factor contained Stat1α by supershift analysis (data not shown). In parallel, the amount of tyrosyl-phosphorylated Stat1α was also significantly increased in *me/me* BMDM, whereas the levels of tyrosine-phosphorylated Stat2 (a component only of ISGF3) were not enhanced (Fig. 4B, lanes 2 and 4). As demonstrated by anti-Stat1 immunoblotting (Fig. 4B), the increase Stat1α tyrosine phosphorylation was not due to an increased level of Stat1α protein in *me/me* BMDM; instead, there is increased tyrosine phosphorylation of comparable amounts of Stat1α in *me/me* cells. An additional IFN-α-stimulated tyrosyl-phosphorylated protein, which did not react on immunoblots with either Stat1α or Stat2, was also observed in Stat1α and/or Stat2 immunoprecipitates. This protein may represent a related Stat family member, since upon tyrosyl phosphorylation, Stats dimerize and can then be coimmunoprecipitated (7, 23, 36). Alternatively, this additional protein could represent a novel, non-Stat tyrosyl phosphoprotein that either shares epitopes or forms a complex with Stat family members. It is not known whether this additional IFN-α-stimulated tyrosyl-phosphorylated protein contributes to the enhanced GRR binding seen in extracts prepared from BMDM from *me/me* mice.

Both Tyk2 and Jak1 are required for IFN-α/β activation of Stat1α and Stat2 (25, 45). These PTKs are tyrosyl phosphory-

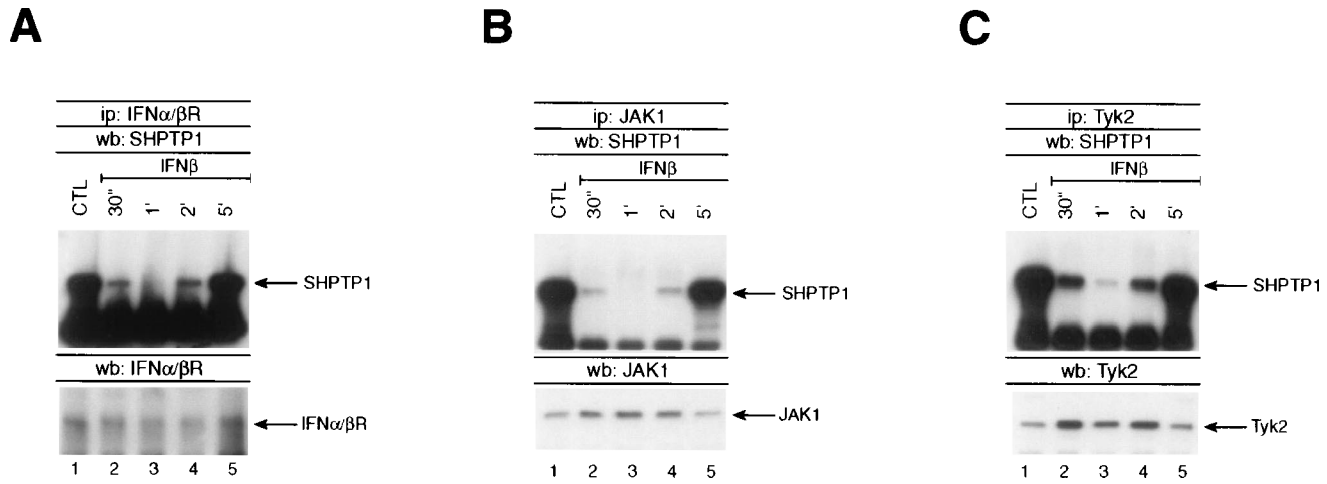

FIG. 3. Reversible association of SHPTP1 with components of the IFNα/β/R-α signaling complex. MM6 cells were incubated without (CTL [control]) or with (IFNβ) IFNβ for 30 s (30″), 1 min (1′), 2 min (2′), or 5 min (5′). Extracts were prepared as for Fig. 1 except that digitonin-containing buffer was used for lysis. Lysates were immunoprecipitated (ip) with antibodies against IFNa/β/R-α (A), Jak1 (B), or Tyk2 (C), resolved by SDS-PAGE, and subjected to anti-SHPTP1 immunoblotting (Western blotting [wb]) (top panels). Blots were then stripped and reprobed for the indicated proteins to ensure comparable loading (bottom panels).

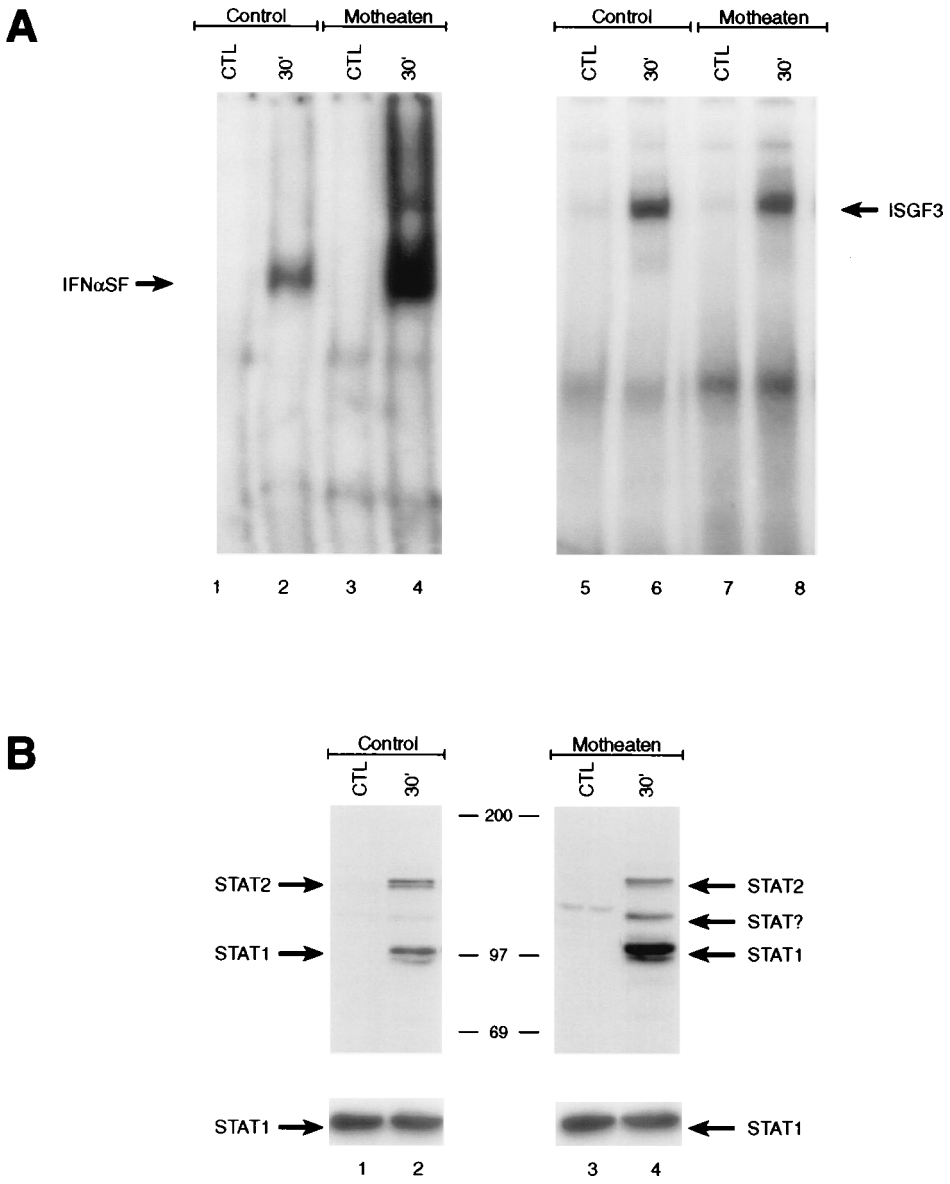

FIG. 4. BMDM from *me*/*me* mice display enhanced IFN-α/β activation of the Jak/Stat pathway. (A) IFN-α/β stimulates enhanced formation in *me*/*me* macrophages of a complex that binds to the GRR in the promoter of the high-affinity (FcγR1 gene but not to the ISRE. Whole cell extracts were prepared from cultures of BMDM from *me*/*me* mice or their unaffected littermates. EMSAs were performed with equal amounts of protein and ^{32}P-labeled oligonucleotide probes corresponding to the GRR in the high-affinity FcγR1 gene (lanes 1 to 4) or the ISRE from IFN-stimulated gene 15 (lanes 5 to 8) (33, 48). CTL, control; 30′, 30 min; IFNαSF, IFN-α-stimulated factor; ISGF3, IFN-stimulated growth factor 3. (B to D). Loss of SHPTP1 in *me*/*me* BMDM leads to selective hyperactivation of Jak1 and Stat1α. Cell lysates (350 μg) were incubated sequentially with an anti-Jak1 antiserum, an anti-Tyk2 antibody, and a mixture of anti-Stat1α and anti-Stat2 antibodies (20). Immune complexes were collected on protein A beads, subjected to SDS-PAGE (8% gel), immunoblotted with monoclonal antiphosphotyrosine antibody 4G10, and detected by ECL. Membranes were stripped and reprobed with the anti-Jak1, anti-Tyk2, or anti-Stat1α antibody. (B) Tyrosine phosphorylation of Stat1α and Stat2 in macrophages from *me*/*me* and normal mice. Extracts prepared from BMDM as for Fig. 3A were immunoprecipitated with a mixture of Stat1α- and Stat2-specific antisera (20). The immunoprecipitates were resolved by SDS-PAGE and immunoblotted with the antiphosphotyrosine antibody (upper panel). The membrane was then reprobed with Stat1α (lower panel). (C) IFN-α/β-stimulated tyrosine phosphorylation of Tyk2 in macrophages from normal and *me*/*me* mice. The antiphosphotyrosine blot was stripped and reprobed for Tyk2 (lower panel). (D) IFN-α/β-stimulated tyrosine phosphorylation of Jak1 in macrophages from normal and *me*/*me* mice. The antiphosphotyrosine blot was stripped and reprobed for Jak1 (lower panel).

lated as a result of treatment of cells with IFN-α, and tyrosine phosphorylation of these proteins correlates with an increase in their kinase activities (25, 45). To examine whether activation of Tyk2 and/or Jak1 is modified in BMDM from *me*/*me* mice, BMDM were incubated with IFN-α, and cellular extracts were prepared and subjected to immunoprecipitation with a Tyk2 or Jak1 antiserum followed by SDS-PAGE and antiphosphotyrosine immunoblotting (Fig. 4C and D). Interestingly, Jak1 displayed drastically increased tyrosine phosphorylation in IFN-α-treated macrophages from *me*/*me* mice compared with cells derived from the control group (Fig. 4D), whereas there was, at most, modestly enhanced tyrosine phosphorylation of the Tyk2 kinase under the same conditions (Fig. 4C). Analysis of the kinetics of tyrosyl phosphorylation of Jak1, Tyk2, Stat1, and Stat2 following IFN stimulation revealed that tyrosine phosphorylation of Stat1α and Jak1 was enhanced

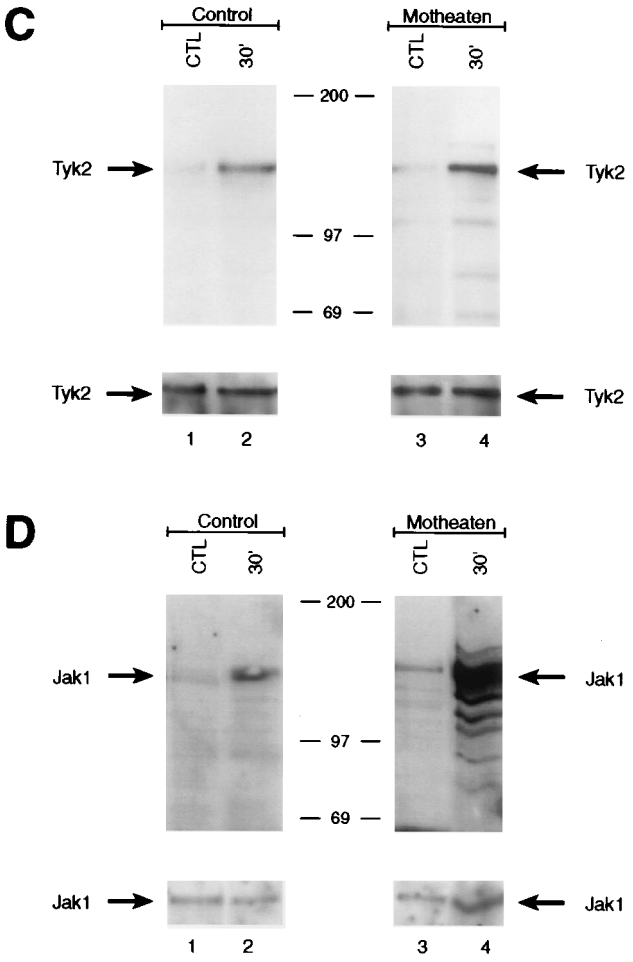

FIG. 4—*Continued.*

after 5, 15, and 30 min of incubation with IFN-α (data not shown). Likewise, selectively enhanced Jak1 and Stat1 tyrosyl phosphorylation was observed with several different IFN concentrations (data not shown). Thus, concentrations of IFN that yield weak responses (as monitored by Jak and Stat phosphorylation) in normal BMDM yield strong responses in *me/me* cells. Our data establish SHPTP1 as an extremely selective negative regulator of IFN-α/β signal transduction, such that loss of SHPTP1 results in hyperactivation of only some components of the pathway.

DISCUSSION

Previous studies suggested that PTPs have both positive and negative functions in IFN-α/β signal transduction, but the specific molecules involved were unclear. We have established that SHPTP1 is a physiologically relevant negative regulator of the IFN-α/β pathway. Both before and after IFN stimulation, SHPTP1 is associated with the IFNα/βR signaling complex (Fig. 1 to 3). This association is reversible, as following IFN addition, and concomitant with activation of Jak1 and Tyk2, SHPTP1 transiently dissociates from the complex, only to return at later time points. Most importantly, comparison of IFN signaling in normal and *me/me* BMDM reveals that negative regulation by SHPTP1 is specific, controlling the tyrosine phosphorylation (and presumably the PTK activity) of Jak1 but not Tyk2, as well as Stat1 but not Stat 2 (Fig. 4 and 5).

The presence of SHPTP1 in the IFNα/βR-α complex does not appear to be necessary to prevent gratuitous Jak1 activation, as basal Jak1 activity in *me/me* BMDM is not significantly elevated. Therefore, SHPTP1 alone cannot account for the PTP activity required to prevent spontaneous Jak and Stat activation, as revealed by PTP inhibitor studies (see the introduction). Conceivably, SHPTP1 could function primarily to limit the extent and duration of Jak and Stat activation, while another PTP prevents basal activation. However, we suspect that SHPTP1 is associated with the basal IFNα/βR-α complex because it also helps to keep basal Jak1 activity low. One or more additional PTPs may contribute to such regulation, providing a fail-safe mechanism; perhaps an as yet unidentified PTP responsible for Tyk2 inactivation subserves this function. In any case, SHPTP1 probably is not the only negative regulatory PTP in IFN-α/β signaling. Mesenchymally derived cells, which fail to express SHPTP1 (22, 35, 51), presumably have an alternative mechanism for terminating IFN-α/β activation. In addition, the effects of SHPTP1 expression on IFN-α signaling may not be limited to Jak1 activity in that another possible Stat protein may be activated in macrophages from *me/me* mice (Fig. 4B).

Although our results suggest a general model for SHPTP1 regulation of IFN-α/β signaling (Fig. 5), several questions remain. The component(s) (IFNα/βR-α, Jak1, and/or Tyk2?) of the IFNα/βR complex that directly mediate association with SHPTP1, as well as the molecular determinants of association (SH2 domain or non-SH2 domain mediated), remains to be defined. We regularly detect low levels of tyrosine phosphorylation on Tyk2 in unstimulated cells which potentially could mediate SHPTP1 binding to the complex. Alternatively, the C terminus of SHPTP1, which has been implicated in its association with the insulin receptor (43), could be responsible. Studies using purified recombinant proteins produced in nonmammalian cell systems should resolve this issue. Likewise, the mechanism by which SHPTP1 is displaced from the IFNα/βR complex requires clarification. It is clear that association-dissociation of SHPTP1 from the signaling complex does not strictly correlate with tyrosine phosphorylation of Jak1, Tyk2, or the α subunit of the receptor. These components (with the exception of Tyk2) are not tyrosine phosphorylated in untreated cells, and they all remain tyrosine phosphorylated longer than 5 min after exposure of cells to IFN-α (21) (data not shown). One obvious and attractive possibility is that in the basal IFNα/βR complex, SHPTP1 associates with Jak1 and/or Tyk2 on a surface that, following receptor engagement, serves as the PTK dimerization interface. Likewise, the precise mechanism by which SHPTP1 is recruited back to the receptor complex is unclear. Although SHPTP1 appears to dephosphorylate and presumably inactivate Jak1 but not Tyk2, it is not clear whether specificity derives from restricted access of SHPTP1 to Tyk2 within the IFNα/βR complex or instead reflects substrate preference of the SHPTP1 catalytic domain. Most likely, SHPTP1 undergoes one or more IFN-α/β-induced posttranslational modifications (e.g., serine phosphorylation). In fact, recent evidence indicates that IFN-α/β activates ERK2 and stimulates serine phosphorylation of Stat1 and Stat3 (5). However, in contrast to other signaling pathways, tyrosine phosphorylation of SHPTP1 does not appear to increase in response to IFN-α/β. Interestingly, the enhanced IFN-α/β-induced activation of Jak1 in *me/me* BMDM correlates with selectively enhanced Stat1α tyrosine phosphorylation. The much more modest increase in tyrosine phosphorylation of Tyk2 compared with Jak1, and of Stat2 compared with Stat1α, is consistent with the notion that Tyk2 promotes tyrosine phosphorylation of Stat2 in response to IFN-α/β (45).

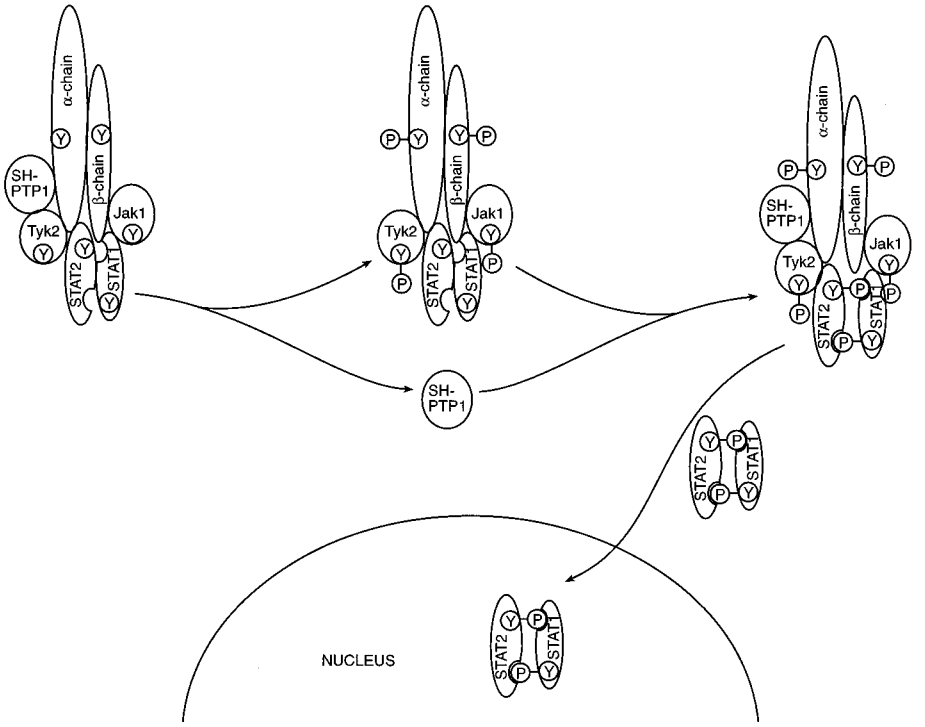

FIG. 5. Model for selective regulation of IFNα/βR signal transduction by SHPTP1. (A) SHPTP1 is basally associated with the IFNα/βR signaling complex. The specific components mediating this association remain to be established. (B) Following addition of IFN, the IFNα/βR is activated, leading to extrusion of SHPTP1, Jak1 and Tyk2 phosphorylation, and tyrosine phosphorylation of the α and β subunits of the receptor (34) and Stat1 and Stat2. (C) SHPTP1 is recruited back to the complex and selectively dephosphorylates Jak1 but not Tyk2. The mechanism of recruitment of SHPTP1 back to the complex remains to be defined and is indicated by the question marks, as is the PTP responsible for Tyk2 dephosphorylation, which remains unidentified. See text for details. P, phosphate.

Previously, we reported that SHPTP1 controls EPOR signal transduction by regulating EPOR-associated Jak2 (18). Our finding that SHPTP1 controls Jak1 tyrosyl phosphorylation in response to IFN-α/β supports our earlier suggestion that inactivation of receptor-associated Janus PTKs may be a general mechanism by which SHPTP1 regulates multiple cytokine receptor signaling pathways. In this regard, the hyperphosphorylation of the IL3Rβ chain observed in DA3 myeloid cells expressing antisense SHPTP1 constructs may be due to aberrant regulation of Jak2 in response to IL-3 stimulation of these cells, rather than to the action of SHPTP1 on IL3Rβ itself as suggested previously (51). Consistent with this notion, we have found that Jak2 is hyperactivated in granulocyte-macrophage CSF-stimulated *me*/*me* BMDM (2a).

However, the current study goes beyond previous work to reveal new complexities in PTP regulation of PTKs. First, although mutation of the binding site for SHPTP1 on EPORs expressed in heterologous myeloid cells correlated with sustained Jak2 activation (18), it remained possible that this was due to another regulatory molecule binding at this site or was a peculiarity of ectopic EPOR expression. Analysis of BMDM from otherwise isogenic normal and *me*/*me* mice provides the distinct advantage of investigating the role of SHPTP1 in primary cells expressing normal levels of cytokine receptors and downstream signaling components. Our studies of IFN-α/β signaling attribute the observed hyperactivation of Jak1 and Stat1 in *me*/*me* to the absence of SHPTP1, but it is formally possible that this may be a secondary effect due to the lack of expression of the protein. Second, the mechanistic details of SHPTP1's interaction with the EPOR and the IFNα/βR differ. Whereas SHPTP1 is basally associated with the IFNα/βR-α complex (Fig. 1 to 3), no such complex with the EPOR could be detected in the absence of EPO stimulation (18). In addition, although loss of SHPTP1 association led to prolonged (18) Jak2 activation in response to EPO stimulation, the extent (i.e., peak magnitude) of activation was not altered, as opposed to our observations of IFN-α/β-stimulated *me*/*me* BMDM (Fig. 4). Furthermore, the downstream signaling consequences of aberrant Jak2 activation were not assessed in our earlier study. We have now shown that hyperactivation of a specific Janus PTK in *me*/*me* BMDM results in selective dysregulation of a particular Stat family member. This finding establishes that PTPs can be highly specific regulatory molecules which can distinguish between closely related target molecules, even when such molecules are in the same signaling complex. In the few previous studies in which discrete substrates for PTPs have been proposed, e.g., Src family PTKs for CD45 (40) and HPTPa (52) for Jak2 and the EPOR (18), such specificity has not been observed.

The finding of selective dysregulation of Stat1 in response to IFN in *me*/*me* BMDM may have interesting consequences for IFN-induced gene expression in these mice. The increase in IFN-induced GRR-binding complexes suggests that genes bearing such elements would be overexpressed in these cells. Indeed, preliminary analysis of IFN regulatory factor 1 expression by Northern (RNA) blotting reveals a severalfold increase in expression in response to IFN (unpublished observations). Typically, however, IFN-α/β results in the generation of both ISRE- and GRR-binding complexes. Lowering the SHPTP1 activity clearly alters the ratio of these two transcription complexes (Fig. 4) as a result of SHPTP1's selective regulation of Jak1 and Stat1. It will be interesting to monitor the expression of a battery of IFN-responsive genes. Furthermore, our findings in this pathological model system raise the possibility that

physiological regulation of SHPTP1 expression and/or activity can modulate cytokine-induced gene expression in similar ways. Therefore, it will be also interesting to examine whether, by regulating SHPTP1 activity, different normal cell types can exhibit distinct responses to IFN-α/β.

Finally, the hypersensitive IFN-α/β signaling pathway in *me*/*me* BMDM may help contribute to abnormal inflammation. One gene controlled by a GRR element in monocytic cells is FcγR1. Our results predict that subthreshold levels of IFN-α/β would lead to FcγR1 expression; threshold levels of IFN-α/β might result in even higher FcγR1 expression. The circulating immune complexes found in *me*/*me* mice might be expected to engage FcγR1 on macrophages and neutrophils, leading to their activation and further cytokine production. Furthermore, FcγR1 signaling in neutrophils and/or macrophages may itself be regulated by SHPTP1, a possibility suggested by the recent finding that SHPTP1 is activated by another member of the FcR family, FcγRIIB, in B cells (4). Thus, IFN-α/β signaling may be part of a proinflammatory positive feedback loop which helps generate the complex *me*/*me* phenotype.

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M.D. and H.E.C. contributed equally to the data in this report.

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REFERENCES

1. **Ahmad, S., D. Banville, Z. Zhao, E. H. Fischer, and S.-H. Shen.** 1993. A widely expressed human protein-tyrosine phosphatase containing *src* homology 2 domains. Proc. Natl. Acad. Sci. USA **90:**2197–2201.
2. **Boulton, T. G., N. Stahl, and G. D. Yancopoulos.** 1994. Ciliary neurotrophic factor/leukemia inhibitory factor/interleukin6/oncostatin M family of cytokines induces tyrosine phosphorylation of a common set of proteins overlapping those induced by other cytokines and growth factors. J. Biol. Chem. **269:**11648–11655.

2a. **Chen, H., and B. G. Neel.** Unpublished data.

3. **Colamonici, O., H. Yan, P. Domanski, R. Handa, D. Smalley, J. Mullersman, M. Witte, K. Krishnan, and J. Krolewski.** 1994. Direct binding to and tyrosine phosphorylation of the type I interferon receptor by p135^{tyk2} tyrosine kinase. Mol. Cell. Biol. **14:**8133–8142.
4. **D'Ambrosio, D., K. L. Hippen, S. A. Minskoff, I. Mellman, G. Pani, K. A. Siminovitch, and J. C. Cambier.** 1995. Recruitment and activation fo PTP1C in negative regulation of antigen receptor signaling by FcγRIIB1. Science **268:**293–296.
5. **David, M., E. F. Petricoin III, C. Benjamin, R. Pine, M. J. Weber, and A. C. Larner.** 1995. Requirement for MAP kinase (ERK2) activity for interferonα/β-stimulated gene expression through Stat proteins. Science **269:**1721–1723.
6. **David, M., G. Romero, Z. Zhang, J. E. Dixon, and A. C. Larner.** 1993. In vitro activation of the transcription factor ISGF3 by IFNα involves a membrane associated tyrosine phosphatase and kinase. J. Biol. Chem. **268:**6593–6599.
7. **Feldman, G. M., E. F. I. Petricoin, M. David, A. C. Larner, and D. S. Finbloom.** 1994. Cytokines that associate with the signal transducer gp130 activate the interferon-induced transcription factor p91 by tyrosine phosphorylation. J. Biol. Chem. **269:**10747–10752.
8. **Feng, G.-S., C.-C. Hui, and T. Pawson.** 1993. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science **259:**1607–1611.
9. **Feng, G.-S., and T. Pawson.** 1994. Phosphotyrosine phosphatases with SH2 domains: regulators of signal transduction. Trends Genet. **10:**54–58.
10. **Finbloom, D. S., et al.** 1994. Growth hormone and erythropoietin activate DNA-binding proteins by tyrosine phosphorylation. Mol. Cell. Biol. **14:**1477–1486.
11. **Freeman, R. M., Jr., J. Plutzky, and B. G. Neel.** 1992. Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of *Drosophila* corkskew. Proc. Natl. Acad. Sci. USA **89:**11239–11243.
12. **Fu, X.-Y., D. S. Kessler, S. A. Veals, D. E. Levy, and J. E. Darnell, Jr.** 1990. ISGF-3, the transcriptional activator induced by IFN-α, consists of multiple interacting polypeptide chains. Proc. Natl. Acad. Sci. USA **87:**8555–8559.
13. **Fu, X.-Y., C. Schindler, T. Improta, R. Aebersold, and J. E. J. Darnell.** 1992. The proteins of ISGF-3, the interferon α-induced transcriptional activator, define a gene family involved in signal transduction. Proc. Natl. Acad. Sci. USA **89:**7840–7843.
14. **Igarashi, K., M. David, D. S. Finbloom, and A. C. Larner.** 1993. In vitro activation of the transcription factor gamma interferon activation factor by gamma interferon: evidence for a tyrosine phosphatase/kinase signaling cascade. Mol. Cell. Biol. **13:**1634–1640.
15. **Igarashi, K., M. David, A. C. Larner, and D. S. Finbloom.** 1993. In vitro activation of a transcription factor by gamma interferon requires a membrane-associated tyrosine kinase and is mimicked by vanadate. Mol. Cell. Biol. **13:**3984–3989.
16. **Igarashi, K., G. Garotta, L. Ozmen, A. Ziemiecki, A. F. Wilks, A. G. Harpur, A. C. Larner, and D. S. Finbloom.** 1994. Interferon-γ induces tyrosine phosphorylation of interferon-γ receptor and regulated association of protein tyrosine kinases, Jak1 and Jak2, with its receptor. J. Biol. Chem. **269:**14333–14336.
17. **Ihle, J. N., B. A. Witthuhn, F. W. Quelle, K. Yamamoto, W. E. Thierfelder, B. Kreider, and O. Silvennoinen.** 1994. Signaling by the cytokine receptor superfamily: JAKS and STATS. Trends Biochem. **19:**222–227.
18. **Klingmuller, U., U. Lorenz, L. C. Cantley, B. G. Neel, and H. F. Lodish.** 1995. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. Cell **80:**729–738.
19. **Kozlowski, M., I. Mlinaric-Rascan, G. S. Feng, T. Pawson, and K. A. Siminovitch.** 1993. Expression and catalytic activity of the tyrosine phosphatase PTP1C is severely impaired in motheaten and viable motheaten mice. J. Exp. Med. **178:**2157–2163.
20. **Larner, A. C., M. David, G. M. Feldman, K. Igarashi, R. H. Hackett, D. A. S. Webb, S. M. Sweitzer, E. F. Petricoin III, and D. S. Finbloom.** 1993. Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. Science **261:**1730–1733.
21. **Larner, A. C., and D. S. Finbloom.** 1995. Protein tyrosine phosphorylation as a mechanism which regulates cytokine activation of early response genes. Biochim. Biophys. Acta **1266:**278–287.
22. **Matthews, R. J., D. B. Bowne, E. Flores, and M. L. Thomas.** 1992. Characterization of hematopoietic intracellular tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. Mol. Cell. Biol. **12:**2396–2405.
23. **Meyer, D. J., G. S. Campbell, B. H. Cochran, L. S. Aretsinger, A. C. Larner, D. S. Finbloom, C. Carter-Su, and J. Schwartz.** 1994. Growth hormone induces a DNA binding factor related to the interferon-stimulated 91-kDa transcription factor. J. Biol. Chem. **269:**4701–4704.
24. **Milarski, K. L., and A. R. Saltiel.** 1994. Expression of catalytically inactive Syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. J. Biol. Chem. **269:**21239–21243.
25. **Muller, M., et al.** 1993. The protein tyrosine kinase JAK1 complements defects in the interferon-α/β and -γ signal transduction. Nature (London) **366:**129–135.
26. **Neel, B. G.** 1993. Structure and function of SH2-domain containing tyrosine phosphatase. Semin. Cell Biol. **4:**419–443.
27. **Noguchi, T., T. Matozaki, K. Horita, Y. Fujioka, and M. Kasuga.** 1994. Role of SH-PTP2, a protein tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. Mol. Cell. Biol. **14:**6674–6682.
28. **Novick, D., B. Cohen, and M. Rubinstein.** 1994. The human interferon α/β receptor: characterization and molecular cloning. Cell **77:**391–400.
29. **Pearse, R. N., R. Feinman, and J. V. Ravetch.** 1991. Characterization of the promoter of the human gene encoding the high-affinity IgG receptor: transcriptional induction by γ interferon is mediated through common DNA response elements. Proc. Natl. Acad. Sci. USA **88:**11305–11309.
30. **Pearse, R. N., R. Feinman, K. Shuai, J. E. Darnell, Jr., and J. V. Ravetch.** 1993. Interferon γ-induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of transcription factor ISGF3. Proc. Natl. Acad. USA **90:**4314–4318.
31. **Perkins, L.** Personal communication.
32. **Perkins, L. A., I. Larsen, and N. Perrimon.** 1992. The *Drosophila corkscrew* gene encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase *torso*. Cell **70:**225–236.
33. **Petricoin, E. F., R. H. Hackett, H. Akai, K. Igarashi, D. S. Finbloom, and A. C. Larner.** 1992. Modulation of interferon signaling in human fibroblasts by phorbol esters. Mol. Cell. Biol. **12:**4486–4495.
34. **Platanias, L. C., S. Uddin, and O. R. Colamonici.** 1994. Tyrosine phosphorylation of the α and β subunits of the type I interferon receptor. J. Biol. Chem. **269:**17761–17764.
35. **Plutzky, J., B. G. Neel, and R. D. Rosenberg.** 1992. Isolation of a src homology 2-containing tyrosine phosphatase. Proc. Natl. Acad. Sci. USA **89:**1123–1127.
36. **Shuai, K., C. M. Horvath, L. H. Tsai Huang, S. A. Qureshi, D. Cowburn, and J. E. Darnell, Jr.** 1994. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell

76:821–828.
37. **Shultz, L. D., P. A. Schweitzer, T. V. Rajan, T. Yi, J. N. Ihle, J. Matthews, M. L. Thomas, and D. R. Beier.** 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. Cell **73:**1445–1454.
38. **Tang, T. L., R. M. Freeman, Jr., A. M. O'Reilly, B. G. Neel, and S. Y. Sokol.** 1995. The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development. Cell **80:** 473–483.
39. **Tauchi, T., G.-S. Feng, R. Shen, M. Hoatlin, G. C. Bagby, Jr., D. Kabat, L. Lu, and H. E. Broxmeyer.** 1995. Involvement of SH2-containing phosphotyrosine phosphatase Syp in erythropoietin receptor signal transduction pathways. J. Biol. Chem. **270:**5631–5635.
40. **Trowbridge, I. S.** 1991. CD45, a prototype for transmembrane protein tyrosine phosphatases. J. Biol. Chem. **266:**23517–23520.
41. **Tsui, H. W., K. A. Siminovitch, L. DeSouza, and F. W. L. Tsui.** 1993. Motheaten and viable motheaten mice have mutations in the hematopoietic cell phosphatase gene. Nat. Genet. **4:**124–129.
42. **Tushinski, R. J., I. T. Oliver, L. J. Guilbert, P. W. Tynan, J. R. Warner, and E. R. Stanley.** 1982. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. Cell **28:**71–81.
43. **Uchida, T., T. Matozaki, T. Noguchi, T. Yamao, K. Horita, T. Suzuki, Y. Fujioka, C. Sakamoto, and M. Kasuga.** 1994. Insulin stimulates the phosphorylation of Tyr^{538} and the catalytic activity of PTP1C, a protein tyrosine phosphatase with Src homology-2 domains. J. Biol. Chem. **269:**12220–12228.
44. **Uze, G., G. Lutfalla, and I. Gresser.** 1990. Genetic transfer of a functional interferon a receptor into mouse cells: cloning and expression of its cDNA. Cell **60:**225–234.
45. **Velazquez, L., M. Fellous, G. R. Stark, and S. Pellegrini.** 1992. A protein tyrosine kinase in the interferon α/β signaling pathway. Cell **70:**313–322.
46. **Vogel, W., R. Lammers, J. Huang, and A. Ullrich.** 1993. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. Science **259:** 1611–1614.
47. **Welham, M. J., U. Dechert, K. B. Leslie, F. Jirik, and J. W. Schrader.** 1994. Interleukin (IL)-3 and granulocyte/macrophage colony-stimulating factor, but not IL-4, induce tyrosine phosphorylation, activation, and association of SHPTP2 with Grb2 and phosphatidylinositol 3′-kinase. J. Biol. Chem. **269:** 23764–23768.
48. **Wilson, K. C., and D. S. Finbloom.** 1992. Interferon γ rapidly induces in human monocytes a DNA-binding factor that recognizes the γ response region within the promoter of the gene for the high-affinity Fcγ receptor. Proc. Natl. Acad. Sci. USA **89:**11964–11968.
49. **Xiao, S., D. W. Rose, T. Sasaoka, H. Maegawa, T. R. Burke, Jr., P. P. Roller, S. E. Shoelson, and J. M. Olefsky.** 1994. Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. J. Biol. Chem. **269:**21244–21248.
50. **Yi, T., and J. N. Ihle.** 1993. Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. Mol. Cell. Biol. **13:**3350–3358.
51. **Yi, T., A. L.-F. Mui, G. Krystal, and J. N. Ihle.** 1993. Hematopoietic cell phosphatase associates with the interleukin-3 (IL-3) receptor β chain and down-regulates IL-3-induced tyrosine phosphorylation and mitogenesis. Mol. Cell. Biol. **13:**7577–7586.
52. **Zheng, X. M., Y. Wang, and C. J. Pallen.** 1992. Cell transformation and activation of $pp60^{c\text{-}src}$ by overexpression of a protein tyrosine phosphatase. Nature (London) **359:**336–339.
53. **Ziegler-Heitbrock, H. W. L., E. Thiel, A. Futterer, V. Herzog, A. Wirtz, and G. Reithmuller.** 1988. Establishment of a human cell line (Mono Mac6) with characteristics of mature monocytes. Int. J. Cancer **41:**456–461.