Signal Transduction of IL-6, Leukemia-Inhibitory Factor, and Oncostatin M: Structural Receptor Requirements for Signal Attenuation

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*J Immunol* 2000; 165:2535-2543; doi: 10.4049/jimmunol.165.5.2535

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Signal Transduction of IL-6, Leukemia-Inhibitory Factor, and Oncostatin M: Structural Receptor Requirements for Signal Attenuation

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Stimulation of the IL-6R complex leads to Src homology domain containing tyrosine phosphatase 2 (SHP2) recruitment to the receptor subunit gp130 and its subsequent tyrosine phosphorylation. SHP2 is a two-SH2 domain-containing protein tyrosine phosphatase that is activated by many cytokines and growth factors. SHP2 counteracts the activation of transcription factors of the STAT family and the induction of IL-6-responsive genes. Tyrosine 759 of gp130, the signal transducing subunit of the IL-6R complex, is essential for the phosphorylation of SHP2. Mutation of tyrosine 759 to phenylalanine leads to an enhanced inducibility of IL-6-dependent genes. Here we demonstrate that no further tyrosines in the cytoplasmic part of gp130 are required for the phosphorylation of SHP2. We also tested whether the tyrosine 759 motifs in both subunits of the gp130 dimer are required for SHP2 association and tyrosine phosphorylation. Interestingly, one SHP2-recruiting phosphotyrosine motif in a single chain of the gp130 dimer is sufficient to mediate SHP2 association to the gp130 receptor subunit and its tyrosine phosphorylation as well as to attenuate IL-6-dependent gene induction. Furthermore, we show that repression of gene induction via Y759 does not require the presence of the SHP2 and STAT recruitment sites within the same receptor subunit, but within the same receptor complex. The Y759 motif in gp130 also attenuates gene induction mediated by the oncostatin M and leukemia inhibitory factor receptor complexes, which both contain gp130 as the shared subunit. The Journal of Immunology, 2000, 165: 2535–2543.
by the solution of the three-dimensional structure of SHP2 (26). The structure implicates an inhibitory function of the N-terminal SH2 domain on the phosphatase activity that can be overcome by the binding of phosphotyrosine peptides to the SH2 domain(s) of SHP2.

In this study we analyzed the requirements for the tyrosine phosphorylation of SHP2 at the gp130 signal transducing subunit of the IL-6R complex. We found that apart from Y759 of gp130, no further cytoplasmic tyrosine residues are involved in SHP2 phosphorylation. Since SHP2 contains two SH2 domains, we investigated whether both Y759 motifs in the gp130 homodimer are needed for SHP2 to exert its action on IL-6 signal transduction and acute phase protein promoter activation. We extended our studies to the heterodimeric gp130/LIF-R and gp130/OSM-R complexes. Finally, it has been studied whether the SHP2 and the STAT recruitment sites on gp130 are required to be present on the same chain of a receptor complex and whether there is cross-talk between different receptor complexes.

Materials and Methods

**Materials**

Restriction enzymes were purchased from Roche (Mannheim, Germany). Oligonucleotides were synthesized by MWG-Biotech (Ebersberg, Germany). Vent polymerase was obtained from New England BioLabs (Schwalbach/Ts, Germany), and RL-5 was purchased from Cell Concepts (Unnikirch, Germany). Abs to the extracellular domain of the IL-5R α-chain were gifts from Jan Tavernier (Gent, Belgium). Abs to the extracellular domains of the IL-5R β-chain and to SHP2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphotyrosine Abs (4G10) were purchased from Upstate Laboratories (Lake Placid, NY). Abs to gp130 were gifts from Dr. J. Wijdenes (Besançon, France). Recombinant IL-6 and -LIF were prepared as described previously (27, 28). The sp. act. of IL-6 was 2 × 10^5 B cell stimulatory factor-2 U/mg of protein.

**Construction of expression vectors**

Plasmid constructions were conducted by standard procedures (29). pGL3α-M-215Luc contains the promoter region −215 to +8 of the rat α2-macroglobulin (α2M) gene fused to the luciferase-encoding sequence and was described previously (15). pSVL-gp130 (YYYYYY) is an expression vector for wild-type gp130 (gp130(YYYYYY)) and was described previously (17). This expression vector was modified by PCR mutagenesis to encode a gp130 mutant with all cytoplasmic tyrosine residues of gp130 replaced by phenylalanine (pSVL-gp130(FFFFF)) (mutated residues are bold) (30). The vector coding for the add-back mutant gp130(FYYYY) is identical with pSVL-gp130(FFFFF), but contains a tyrosine codon at position 759. All these vectors encode a C-terminal Myc tag. These vectors were used for stable transfection of murine pre-B cells (Ba/F3 cells).

Vectors encoding IL-5Rα/gp130 fusion proteins of the extracellular IL-5Rα or IL-5Rβ region and the transmembrane and intracellular parts of gp130 (IL-5Rα/gp130(YYYYYY) and IL-5Rβ/gp130(YYYYYY)) were previously described (pSVL-α-130; pSVL-β(31)). pSVL-based expression vectors for IL-5Rα/gp130(YYYYYY) and IL-5Rβ/gp130(YYYYYY) were generated by exchange of the sequence encoding the transmembrane and intracellular parts of wild-type gp130 in pSVL-α-130 and pSVL-β(31). The corresponding sequence of pSVL-gp130(YYYYYY). These vectors were used for transient transfection of COS-7 cells. IL-5Rα/gp130 chimeric receptor constructs were generated by fusing the extracellular domain of the IL-5Rα or the IL-5Rβ with the appropriate mutated cytoplasmic part of chimeric EpoR/gp130 receptors (30). To allow expression encoding the human hepatoma cell line HepG2, these constructs were inserted into the pRC-CMV expression vector (Invitrogen, Groningen, The Netherlands) to give pRC-CMV-IL-5Rα/gp130(YYYYYY), pRC-CMV-IL-5Rβ/gp130(YYYYYY), pRC-CMV-IL-5Rβ/gp130(YYYYYY), pRC-CMV-IL-5Rβ/gp130(FFFFF) and pRC-CMV-IL-5Rβ/gp130(YYYYYY). The expression vectors for EpoR/gp130 chimeric receptor constructs and were described previously (30). Expression vectors for fusion proteins of the extracellular domain of the IL-5Rβ and the transmembrane and cytoplasmic parts of the LIF-R or the OSM-R were used previously by Hermanns et al. (pRC-CMV-IL-5Rβ/OSM-R and pRC-CMV-IL-5Rβ/LIF-R) (32). The sequences of all constructs were verified by fluorescence sequencing.

**Immunoprecipitation and immunoblot analysis**

For immunoprecipitation 2 × 10^7 cells were lysed in 500 μl of lysis buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EDTA; 1% Brij 96 (Sigma, Deisenhofen, Germany); 0.5% Brij 96 in coimmunoprecipitation assays); and 10 μM of each aprotinin, pepstatin, leupeptin. Equal amounts of cellular protein were incubated with the appropriate Abs at 4°C overnight and immunoprecipitated with 2.5 μg of protein A-Sepharose (Pharmacia, Uppsala, Sweden). Immune complexes were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Eschborn, Germany). Abs were detected by incubation with the appropriate primary Ab (4G10, 1/1000 dilution; anti-SHP2 and anti-IL-5Rα, 1/1000 dilution) and HRP-coupled secondary Abs (1/2000; Dako, Hamburg, Germany).

**Transfection and reporter gene analysis**

Human hepatoma cells HepG2 were grown and transiently transfected using the calcium phosphate coprecipitation method as described previously (33). Transfections were adjusted with control vectors to equal amounts of DNA. Cell lysis and luciferase assays were conducted using the luciferase kit (Promega, Madison, WI) according to the manufacturer. All transient expression experiments were performed at least in triplicate. Luciferase activity values were normalized to transfection efficiency monitored by the cotransfected β-galactosidase expression vector (pBRET3a/pCR2; Pharmacia; 1.5 μg). COS-7 cells were grown in DMEM with 10% FCS, 100 mg/l streptomycin, and 60 mg/l penicillin. Approximately 1.5 × 10^7 COS-7 cells were transiently transfected with 25 μg of DNA using the DEAE-dextran method. Briefly, cells were incubated in medium containing DNA, 80 μM chloroquine, and 0.4 mg/ml DEAE-dextran for 80 min, avoiding gas exchange. Afterward, cells were incubated for 1 min in PBS containing 10% DMSO. After 24 h cells were split 1/2, and after an additional 24 h in culture medium cells were stimulated. Chimeric receptor surface expression in COS-7 cells was verified by FACS analysis with Abs to the extracellular domains of the IL-5R α-chain or IL-5R β-chain. Ba/F3 cells were grown and stably transfected as described previously (34). Surface expression of gp130 was verified by FACS analysis with B-P4 Ab specific for the extracellular domain of gp130.

**Results**

Y759 in gp130 is the only tyrosine residue required for SHP2 activation

Tyrosine 759 in gp130 is essential for IL-6-induced SHP2 activation. Substitution of tyrosine 759 to phenylalanine leads to loss of SHP2 phosphorylation, enhanced and prolonged STAT activation, and acute phase protein gene induction (16–18). We examined whether any other tyrosine residues in the cytoplasmic domain of gp130 apart from Y759 are required for SHP2 activation. Therefore, a gp130 receptor mutant with a Y→F exchange of all cytoplasmic tyrosine residues except Y759 was generated (gp130(FYYYYY)). This mutant as well as the wild-type gp130 receptor (gp130(YYYYYY)) and a mutant with all six tyrosines mutated to phenylalanine (gp130(FFFFF)) were stably expressed in Ba/F3 pro-B cells that lack endogenous gp130. As determined by FACS analysis the surface expressions of these various receptor mutants were similar (Fig. 1B). Stimulation of cells expressing wild-type gp130 (gp130(YYYYYY); lanes 1 and 2) or the gp130(FYYYYY) mutant (lanes 3 and 4) with IL-6/IL-6R complexes led to a similar extent of SHP2 tyrosine phosphorylation (Fig. 1A). In contrast, elimination of all cytoplasmic tyrosine residues of gp130 abolished SHP2 phosphorylation (lanes 5 and 6). Thus, the presence of Y759 is sufficient for SHP2 phosphorylation, and none of the other five cytoplasmic tyrosines in gp130 is required for SHP2 activation.
A single Y759 in one receptor chain of the gp130 dimer is sufficient for SHP2 phosphorylation

The phosphorylation of SHP2 after activation of the IL-6R/gp130 complex requires the recruitment of SHP2 to phosphorytose 759 of gp130 (12). To examine whether the tyrosine residues Y759 in both subunits of the activated gp130 dimer are required for SHP2 phosphorylation, we used a heterodimeric chimeric receptor system that enabled us to induce the dimerization of two different cytoplasmic tails (31). These chimeric receptors containing the IL-5Rα or IL-5Rβ extracellular domains fused to the transmembrane and cytoplasmic domains of gp130 were transiently expressed in COS-7 cells. Thus, by stimulation with IL-5 we were able to induce receptor complex formation where only one receptor chain in the dimer contains a Y→F substitution at position Y759 (i.e., IL-5Rα/gp130(YYYYYYY) or IL-5Rβ/gp130(YYYYYY)) or IL-5Rα/gp130(YYYYYY)/IL-5Rβ/gp130(YYYYYYY)). The expression levels of all these chimeric receptor constructs were monitored by FACS analysis and were found to be similar (data not shown). Mutation of Y759 to F in both chimeric receptor chains led to the total loss of SHP2 phosphorylation (Fig. 2, lanes 7 and 8). Restoration of one Y759 in the receptor complex was sufficient to sustain SHP2 phosphorylation regardless of whether the Y759 is present in the IL-5Rα (lanes 3 and 4) or IL-5Rβ chimeric protein (lanes 5 and 6). Thus, for SHP2 phosphorylation, Y759 is required in only one of the chimeric receptor chains in the activated receptor complex.

SHP2 phosphorylation requires its association with the receptor. Therefore, we tried to detect complexes of SHP2 and the chimeric receptors. To date, we have not been able to coprecipitate the chimeric receptors with SHP2 after stimulation with IL-5. Recently, Pratt et al. (35) were able to demonstrate Src/GM-CSF-β-receptor association by expression of Jak2 to simulate receptor activation. Similarly, we simulated receptor activation by overexpression of Jak1 in COS cells (Fig. 3). Immunoprecipitation with Abs to IL-5Rβ led to coprecipitation of SHP2 only when Y759 was present in the IL-5Rβ chimera (IL-5Rβ/gp130(YYYYYYY); lanes 1 and 3), but not in the absence of Y759 in the IL-5Rβ/gp130 chimeric receptor (IL-5Rβ/gp130(YYYYYY); lanes 2 and 4). Thus, Y759 in the IL-5Ra/gp130 chimera is not required for binding of SHP2 to the IL-5Rβ/gp130 chimeric receptor chain.

A single Y759 in the gp130 dimer is sufficient for inhibition of acute phase protein induction

A further downstream effect of SHP2 activation is the attenuation of IL-6-induced acute phase protein expression in liver cells (17, 18). To examine whether a single Y759 in the gp130 dimer is sufficient for the repression of acute phase protein induction we analyzed whether a single Y759→F substitution in the cytoplasmic part of the gp130 dimer receptor complex is sufficient to abolish the negative effect of SHP2 on acute phase protein promoter induction in human hepatoma cells. The IL-5Rα/gp130 chimeric receptors were expressed in HepG2 cells transfected with an αs2M-promoter luciferase construct (Fig. 4). Stimulation of cells expressing the IL-5Ra/gp130(YYYYYYY) and IL-5Rβ/gp130(YYYYYY) chimeric receptors led to a 20-fold induction of the luciferase reporter gene (lanes 1 and 2). Elimination of Y759 in both chimeric receptor chains (IL-5Ra/gp130(YYYYYYY)-IL-5Rβ/gp130(YYYYYY); lanes 7 and 8) further enhanced the reporter activity (>2-fold), whereas only moderate increases in reporter activity were measured in cells expressing the IL-5Ra/gp130(YYYYYYY)-IL-5Rβ/gp130(YYYYYY) (lanes 3 and 4) or IL-5Rα/gp130(YYYYYY)-IL-5Rβ/gp130(YYYYY) (lanes 5 and 6) heterodimeric receptor chimeras, demonstrating that in these cells a single tyrosine 759 is able to down-regulate acute phase gene induction.

Tyrosine 759 in gp130 also represses gp130/LIF-R- and gp130/OSM-R-mediated gene induction

LIF and OSM signal through gp130/LIF-R or gp130/OSM-R heteromeric receptor complexes. In human cells, OSM also uses gp130/LIF-R complexes (36). We analyzed whether tyrosine 759 in the gp130 receptor chain of these receptor complexes also affects gene induction. The transmembrane and cytoplasmic domains of wild-type LIF-R and OSM-R were fused to the extracellular part of IL-5Rβ. These IL-5Rβ/OSM-R and IL-5Rβ/LIF-R chimeric proteins were coexpressed with the chimeric IL-5Ra/gp130 constructs IL-5Ra/gp130(YYYYYY) (Fig. 5, lanes 5 and 6...
and lanes 7 and 8) or IL-5Rα/gp130(YFFFFF) (lanes 9 and 10) or IL-5Rα/gp130(YYYYYY) (lanes 7 and 8 and lanes 11 and 12) in HepG2 cells and analyzed for αM-promoter-luciferase construct gene activation after IL-5 stimulation. Activation of the IL-5Rα/IL-5Rβ chimeric receptor pairs containing the cytoplasmic wild-type receptor domains (IL-5Rα/gp130(YYYYYY)/IL-5Rβ/gp130(YYYYYY) (lanes 1 and 2), IL-5Rα/gp130(YYYYYY)/IL-5Rβ/LIF-R (lanes 5 and 6), or IL-5Rα/gp130(YYYYYY)/IL-5Rβ/OSM-R (lanes 9 and 10)) led to the induction of the luciferase reporter gene. Elimination of Y759 in the cytoplasmic part of gp130 (IL-5Rα/gp130(YFYYYY)/IL-5Rβ/gp130(YFYYYY) (lanes 3 and 4), IL-5Rα/gp130(YFYYYY)/IL-5Rβ/LIF-R (lanes 7 and 8), or IL-5Rα/gp130(YFYYYY)/IL-5Rβ/OSM-R (lanes 11 and 12)) resulted in a further increase in reporter gene activity. These observations demonstrate that LIF and OSM signaling can also be down-regulated by the presence of tyrosine 759 of gp130 within the receptor complex.

The SHP2 recruitment site (Y759) does not have to be located at the same receptor chain as the STAT activation sites to affect receptor-mediated gene induction

One central function of gp130 is the recruitment and activation of STAT factors at the four distal tyrosine motifs. We investigated whether repression of gene induction via the tyrosine 759 motif of gp130 also occurs in receptor complexes in which the SHP2 and STAT recruitment sites are located not on the same but on different receptor chains (Fig. 6A). Therefore, we measured the reporter gene induction mediated by an IL-5Rα/gp130(YFFFFF)/IL-5Rβ/gp130(YFYYYY) receptor complex (lanes 9 and 10) in which SHP2 can only be recruited to the IL-5Rα/gp130 chimera and STATs can only be activated at the IL-5Rβ/gp130 receptor chain. Interestingly, this receptor combination was as potent as the IL-5Rα/gp130(YFFFFF)/IL-5Rβ/gp130(YYYYYY) complex (lanes 5 and 6), where SHP2 and STAT are activated at the same chain. For comparison, the IL-5Rα/gp130(YFFFFF)/IL-5Rβ/gp130(YFFFFF) complex (lanes 7 and 8), in which both Y759 motifs are lacking, was again much more potent to mediate reporter gene activation after stimulation of the cells with IL-5. The receptor combinations with mutated STAT binding sites in the IL-5Rα/gp130 chain show less efficient gene induction than the corresponding unmutated receptor chain, most likely because of the lower number of STAT activation sites in the receptor complex (compare IL-5Rα/gp130(YYYYYY)/IL-5Rβ/gp130(YYYYYY) (lanes 1 and 2) with IL-5Rα/gp130(YFFFFF)/IL-5Rβ/gp130(YYYYYY) (lanes 5 and 6) and IL-5Rα/gp130(YFFFFF)/IL-5Rβ/gp130(YFFFFF) (lanes 7 and 8)).

Thus, to attenuate gp130-mediated gene induction, the SHP2 recruitment site does not have to be located on the same receptor chain as the STAT activation sites.

These results led us to the question whether Y759 in the gp130 receptor chain influences gene induction when the STATs can only be recruited at the OSM-R or LIF-R chain. Therefore, we modified the experiment described in Fig. 5 by additionally mutating the four STAT binding sites in the cytoplasmic part of gp130 (Fig. 6B). Activation of the receptor complexes IL-5Rα/gp130(YFFFFF)/IL-5Rβ/LIF-R (lanes 3 and 4) and IL-5Rα/gp130(YFFFFF)/IL-5Rβ/OSM-R (lanes 7 and 8) led to an enhanced reporter gene induction compared with the complexes containing Y759 (IL-5Rα/gp130(YFFFFF)/IL-5Rβ/LIF-R (lanes 1 and 2) and IL-5Rα/gp130(YFFFFF)/IL-5Rβ/OSM-R (lanes 5 and 6)). Thus, tyrosine 759 in gp130 is able to affect signal transduction even when STAT activation occurs exclusively at the LIF-R or OSM-R.
Tyrosine 759 in gp130 inhibits gp130/LIF-R- and gp130/OSM-R-mediated gene induction. HepG2 cells were transfected with expression vectors for the receptors as indicated. Experiments were performed as described in Fig. 4.

Discussion

IL-6, IL-11, OSM, LIF, cardiotoxpin 1, CNTF, and the recently discovered novel neutrophin 1 (37) are members of the family of IL-6-type cytokines. These cytokines signal through receptor complexes that contain gp130 as homodimer (IL-6 and IL-11) or as part of a heterodimer together with LIF-R or OSM-R (1). The involvement of SHP2 in gp130-mediated signal transduction was first described by Boulton et al. (38). Binding of SHP2 to gp130 was found for cells stimulated with IL-11 (39). Using chimeric receptors, Stahl et al. (12) identified Y759 of gp130 to be crucial for SHP2 phosphorylation. This observation was also confirmed for native gp130 receptor subunits (17).

In the platelet/endothelial cell adhesion molecule-1 two neighboring phosphotyrosine motifs (Y663 and Y686) are required for SHP2/platelet/endothelial cell adhesion molecule-1 interaction (40, 41). Similarly, immunoreceptor tyrosine-based activation motifs comprise two tyrosine modules of a conserved sequence separated by six to eight residues (42). Thus, the presence of two SH2 domains in SHP2 could be a hint that SHP2 binds to two cytoplasmic phosphotyrosine residues in the signal transducer gp130. This idea is supported by in vitro studies revealing a regulatory potential of the SH2 domains for the catalytic activity of SHP2. SHP2 mutants lacking the SH2 domains are constitutively active (24, 25).

In the present study we report that no other tyrosine residue apart from Y759 in the cytoplasmic domain of gp130 is required for SHP2 phosphorylation after receptor activation. Mutation of all cytoplasmic tyrosine residues except Y759 to phenylalanine in.
A gp130 dimer is required for SHP2 phosphorylation and its inhibitory function on acute phase protein gene induction. Furthermore, we were able to demonstrate that for the attenuation of receptor-mediated gene induction the SHP2 recruitment site does not have to be located within the same chain of the receptor complex as the STAT binding sites (Fig. 6A).

The two IL-6-type cytokines LIF and OSM signal through gp130/LIF-R or gp130/OSM-R heteromeric receptor complexes. These receptor complexes contain only a single gp130-SHP2 recruitment site. Schiemann et al. (44) have proposed an additional SHP2 binding site at the LIF-R. Analysis of the SHP2 binding site of gp130 within the gp130/LIF-R and gp130/OSM-R complexes shows that the Y759 motif in gp130 is also functional in attenuating LIF and OSM signaling (Fig. 5). Within these heterodimeric receptor complexes the Y759 of gp130 could act specifically on the gp130 receptor chain or might also affect LIF-R- or OSM-R-originated gene activation. Interestingly, APP promoter activation was also impaired when gp130-mediated STAT activation was prevented by mutation of the STAT recruitment sites in the IL-6R/gp130 receptor chain (Fig. 6B). Thus, similar to the gp130 homodimer, the inhibitory activity of Y759 of gp130 in the gp130/OSM-R and gp130/LIF-R heterodimeric complexes is evident even when the STATs are activated only at the OSM-R or LIF-R chain. The putative SHP2 binding site in the LIF-R might also affect gp130/LIF-R-mediated gene induction. However, to date this site was only examined in homodimeric receptor chimeras, containing the LIF-R cytoplasmic tail, with regard to MAPK activation (44).

After stimulation of cells with a variety of cytokines and growth factors, SHP2 is recruited at the tyrosine-phosphorylated receptors and becomes activated. Thus, it is possible that SHP2 activated at one receptor complex could inhibit the signaling through another receptor. If such receptor cross-talk could occur, the SHP2 activation at one receptor complex should influence signal transduction events elicited by another receptor. From our experiments (Fig. 7) no evidence for “trans-inhibition” between different receptor complexes was obtained. This indicates that the downstream activities that mediate Y759-dependent attenuation of APP gene induction act only on signals derived from the receptor complex from which they originate and therefore are unlikely to be mobile (trans-acting) proteins.

SHP2 becomes activated by binding to the tyrosine 759 motif of gp130 and tyrosine phosphorylated by the Jaks with transient and rapid kinetics similar to those of receptor phosphorylation (17, 18, 39). In turn, the activated receptor-bound phosphatase may dephosphorylate the tyrosine-phosphorylated Janus kinases and becomes activated. Thus, it is possible that SHP2 activated at one receptor complex could inhibit the signaling through another receptor. If such receptor cross-talk could occur, the SHP2 activation at one receptor complex should influence signal transduction events elicited by another receptor. From our experiments (Fig. 7) no evidence for “trans-inhibition” between different receptor complexes was obtained. This indicates that the downstream activities that mediate Y759-dependent attenuation of APP gene induction act only on signals derived from the receptor complex from which they originate and therefore are unlikely to be mobile (trans-acting) proteins.

Another mechanism of feedback inhibition for cytokine signaling has become obvious by the discovery of the cytokine-induced SOCSs (suppressors of cytokine signaling) proteins (45–47). The SOCSs bind to the Janus kinases to inhibit signal transduction. Recently, we found that the IL-6-induced SOCS3 also requires tyrosine 759 of gp130 to exert its negative action on IL-6 signaling. Furthermore, we have shown that SOCS3 interacts with a peptide corresponding to the phosphorylated tyrosine 759 motif of gp130. This interaction did not depend on the presence of SHP2 (48). Thus, SOCS3 has to be recruited to phosphorytose 759 of gp130 to inhibit the receptor-associated Jaks and cannot act in

FIGURE 6. Separation of SHP2 and STAT recruitment sites to different receptor chains does not abolish the effect of Y759 on gene induction. A and B, HepG2 cells were transfected with expression vectors for the receptors as indicated. Experiments were conducted as described in Fig. 4.
FIGURE 7. Tyrosine 759 of one receptor complex does not affect signal transduction of another receptor complex. A and B. HepG2 cells were transfected as described in Fig. 4, but with expression vectors for the receptors indicated in this figure. The cells were stimulated for 16 h with 10 ng/ml IL-5 plus 7 U/ml Epo where indicated, and cellular extracts were prepared for the determination of luciferase activity. Luciferase activity is presented in relation to the activity in cell extracts from stimulated HepG2 cells expressing the wild-type chimeric receptors. Error bars are the SD.
trans on other receptor complexes. This requirement of SOCS3 to bind gp130 is in agreement with the observed lack of trans-inhibition (Fig. 7).

In previous work from our group it was demonstrated that IL-6 signaling was inhibited by TNF-α pretreatment of macrophages. This effect is very likely due to TNF-α-induced SOCS3 gene induction (49). Thus, SOCS3 has been suggested to be a mediator of receptor cross-talk. Nevertheless, the inhibitory function of SOCS3 on IL-6 signal transduction depends on the presence of tyrosine 759 of the activated signal transducer gp130 (48). Therefore, cross-talk with TNF-α signaling has to occur at the level of SOCS3 gene induction (upstream of binding to the Y759 motif) and not at the level of SOCS3 function (downstream of binding to the Y759 motif). In conclusion, the two differently activated receptor complexes do not influence each other directly but, rather, through the induction of proteins that, in turn, affect receptor activities.

The activation of the Ras/Raf/MAPK cascade has been described to inhibit IL-6 signal transduction (50, 51). Tyrosine 759 in gp130 is essential for IL-6-dependent activation of the MAPK cascade (19). Since the activated MAPK is a trans-acting protein, it has been concluded that activation of the Ras/Raf/MAPK cascade is unlikely to be directly responsible for the Y759-dependent re-duction of APP induction. Nevertheless, MAPK could contribute indirectly to the mechanism of attenuation investigated in this work. For example we found that MAPK activation by PMA increases the level of SOCS3 mRNA, which, in turn, results in the tyrosine 759-dependent repression of IL-6 signal transduction (52).

Further efforts are required to elaborate whether SOCS3 and/or SHP2 are the mediators of Y759-dependent attenuation of IL-6-, OSM-, and LIF-induced gene induction. Work on the role and the mode of action of SOCS3 and SHP2 in IL-6-type cytokine-induced inhibition of gene expression is in progress.

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