Interaction and Functional Interference of Glucocorticoid Receptor and SOCS1*

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Cytokine and glucocorticoid (GC) hormone signaling act in an integrated fashion to control inflammation and immune response. Here we establish a new mode of interaction of these two pathways and propose Suppressor of Cytokine Signaling (SOCS)-1 as an essential player in mediating cross-talk. We observed that glucocorticoid receptor (GR) and SOCS1 form an intracellular complex through an interaction, which required the SH2 domain of SOCS1 and the ligand binding domain of GR. Furthermore, GC stimulation was found to increase the nuclear level of SOCS1. SOCS1 binding to the GR did not require ligand binding of the receptor; however, it was abolished after long term GC stimulation, suggesting a functional role of the interaction for the early phase of GC action. The interaction between GR and SOCS1 appeared to negatively influence the transcription of the two GR-regulated genes, FKBP5 and MKP1, because the GC-dependent expression of these genes was inhibited by the SOCS1 inducer IFNγ and enhanced in SOCS1-deficient murine embryonic fibroblasts as compared with IFNγ-treated wild-type cells. Our results suggest a prominent role of SOCS1 in the early phase of cross-talk between GR and cytokine signaling.

Key principles determining the cellular response to cytokine signaling are duration of stimulus, prevalence of inhibitory feedback mechanisms, and operation of cross-talk with other cellular pathways. All of these factors interact to influence the strength and the quality of the physiological response. For cytokines operating via the Janus kinase (JAK)²/signal transducers and activators of transcription (STAT) pathway, both suppressor of cytokine signaling (SOCS) proteins and the glucocorticoid receptor (GR) have been identified as important intracellular regulators in this respect.

SOCS proteins have been shown to serve both as feedback inhibitors as well as mediators of cross-talk with other signaling pathways. They comprise a family of 8 members, initially described as negative feedback regulators of the JAK/STAT pathway. All SOCS proteins have two functional domains: an SH2 domain, which primarily enables binding of phosphorylated tyrosine residues, and a C-terminal SOCS box, which serves as a recruiting site for ubiquitin ligases, thereby combining specific inhibition of JAK catalytic activity with generic mechanisms such as competition over binding sites and targeting of associated proteins to proteasomal degradation (1, 2).

Recent evidence suggests that the negative regulatory function of SOCS is not restricted to the JAK/STAT pathway. Through interaction with other signaling intermediates SOCS proteins can interfere with crucial signaling pathways such as the NF-κB and insulin receptor signaling pathways (3). Furthermore glucocorticoids (GC) can also influence cytokine signaling. They act together with cytokines in an integrated fashion to control inflammation, immune response, and other more diverse physiological functions in mammals (4). The effect of GCs is mediated by the GR, a member of the nuclear receptor superfamily (5), which can serve as a transactivator or repressor gene expression by interfering with other transcription factors (6). In this respect, the GR has been described to interact directly or indirectly with essential transcription factors in cytokine signaling, namely AP-1, NF-κB, and STAT proteins. Interactions of the GR with AP-1 and NF-κB are involved in the negative cross-talk between cytokine and GC signaling (7), whereas the interaction between GR and STATs can be either synergistic or antagonistic, depending on the cell type, duration of stimulus, and STAT factor involved in the interaction (4, 8).

Here we establish a novel mode of interaction between cytokine and GC signaling, which was apparent in the early phase of GC stimulation. We provide evidence that SOCS1 is associated with the GR, and forms an intracellular complex that is disassembled after long term stimulation with GC. Furthermore, SOCS1 nuclear levels were found to increase in GC-treated cells. We tested the hypothesis that the interaction between GR and SOCS1 is influencing GC signaling. Our results led us to propose that SOCS1 is able to inhibit the transactivation activity of the GR and thereby to attenuate the transcriptional activation of GC-regulated genes. The study establishes a new link between cytokine and GC signaling with important implica-
Glucocorticoid Receptor and SOCS1 Interaction

tions for the regulation of inflammation as well as other more diverse physiological processes, where interactions of GC with JAK/STAT signaling are involved.

EXPERIMENTAL PROCEDURES

Cell Culture—COS7 monkey kidney cells, were propagated in MEM medium supplemented with 10% heat-inactivated FCS and 50 μg/ml gentamicin; MEFs and NIH3T3 cells were propagated in Dulbecco’s modified Eagle’s medium with 10% FCS and gentamicin (50 μg/ml); P388D1 murine lymphoma cells were kept in RPMI with 10% FCS and gentamicin (50 μg/ml). Murine BMDM were prepared as described recently (9). All cells were cultivated in 5% CO₂ at 37 °C and subcultured every 3–4 days. To eliminate basal activation of the glucocorticoid receptor by serum glucocorticoids, all cells were washed prior to experiments three times with prewarmed serum-free F-12/DMEM supplemented with 50 μg/ml gentamicin and 200 μg/ml Albumax II (Invitrogen).

Hormones, Cytokines, and Plasmids—Murine IFNγ was purchased from PeproTech (Rocky Hill, NJ) and used at a concentration of 20 ng/ml. Dexamethasone (Sigma) was diluted in 70% ethanol and applied in concentrations ranging from 100 nM to 1 μM. RU486 was kindly provided by Dr. E. Baulieu and used at a final concentration of 1 μM. Myc-tagged wild-type SOCS1 and SOCS1 deletion mutants were described previously (10). Rat GR mutants (11) were kindly provided by Dr. Starr.

Transient Transfections—Transient transfections were carried out using Transfast transfection reagent (Promega, Madison, WI). For a 10-cm cell culture dish, 3 μg of DNA were mixed with 9 μl of Transfast in 1200 μl of serum-free Opti-Mem I (Invitrogen). After 3 h, medium supplemented with 10% FCS was added.

Antibodies—Rabbit polyclonal anti-GR antibody (M-20, Santa Cruz Biotechnologies) was used at a dilution of 1:350 for immunoblotting and 1:50 for immunofluorescence microscopy. Anti-c-Myc mouse monoclonal antibody (9E10, Santa Cruz Biotechnologies) was used at 1:1000 and 1:500 for immunoblotting and immunofluorescence microscopy, respectively. Anti-GAPDH (MAB374, Chemicon Int.) was used at a 1:1500 dilution, anti-SOCS1 (4H1, MBL International, Woburn, MA) at 1:200, and anti-GR (MAI-510, ABR, Golden, CO) at 1:350.

Immunoprecipitation and Immunoblotting—for total cell lysates (TCL), cells were washed three times with ice-cold PBS and then lysed in coIP lysis buffer (50 mM HEPES, pH 7.5, 1 mM EGTA, 2 mM EDTA, 12.5 mM β-glycerophosphate, 3.2 mM MgCl₂, 10% glycerol, 1% Triton X-100, 5 mM dithiothreitol, 1.19 mM Na₃VO₄, 2.5 mM NaF) for 30 min at 4 °C. Lysates were centrifuged (21,000 g) at 4 °C for 30 min to remove insoluble fractions and precleared and 50 μl of G-Plus-agarose (Santa Cruz Biotechnology) for 1 h.

4 °C for 30 min to remove insoluble fractions and precleared and 50 μl of G-Plus-agarose (Santa Cruz Biotechnology) for 1 h. Immune complexes, G-Plus-agarose was added. Agarose beads were collected by centrifugation. Pellets were washed three times with lysis buffer, resuspended in lysis buffer, and boiled in SDS sample buffer before analysis by SDS-PAGE. Nuclear and cytosolic extracts were prepared as described previously (12). Proteins were separated in 10 or 14% gels by SDS-PAGE depending on molecular weight and transferred to Immobilon-FL membranes (Millipore, Marlborough, MA). Membranes were blocked with Rockland blocking reagent for near infra-red (Rockland, Gilbertsville, PA) for 1 h, and primary antibodies were applied at the indicated dilutions overnight in Rockland blocking reagent at 4 °C. Anti-rabbit IR 800 (Rockland) and anti-mouse IR 680 (Molecular Probes, Leiden, Netherlands) were used at 1:5000 dilution. Immunoreactive bands were detected using a LICOR Odyssey Infrared Imager (LI-COR, Biosciences, Lincoln, NB). Quantifications were performed using Odyssey Application Software Version 2.1 provided by LICOR. Further data analysis was carried out using Prism 4.0 for Macintosh (GraphPad Software, San Diego, CA).

Immunofluorescence Microscopy—COS7 cells were grown on glass coverslips and transiently transfected. 36 h after transfection, cells were washed with PBS, fixed with 4% paraformaldehyde in PBS for 10 min on ice and permeabilized by methanol/acetone (1:1) for 5 min. Prior to antibody incubation, cells were blocked with 3% bovine serum albumin (Sigma-Aldrich) in PBS for 1 h. Primary and secondary antibodies were diluted in PBS with 3% bovine serum albumin and incubated for 1 h. Samples were washed three times with 0.1% Tween 20 in PBS after incubation with the primary and Alexa-labeled secondary antibodies. Fixed samples were mounted in Mowiol 4-88 mounting medium containing 2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO, Sigma-Aldrich) and analyzed by confocal microscopy using a Zeiss Axiovert 2000 equipped with a LSM510 META detector (Zeiss, Jena, Germany). Fluorescent spillover was checked with single dye-treated samples.

Quantitative Real-time PCR—The mRNA abundance of SOCS1 and the glucocorticoid-regulated genes MKP1 and FKBP5 was assessed by quantitative RT-PCR using the TaqMan method. Total RNA was extracted from cultured cells using the RNaseasy kit (Qiagen, Hilden, Germany). Integrity of prepared RNA was evaluated by determination of ethidium bromide-stained 18S and 28S ribosomal RNA bands in an agarose gel. 0.5 μg of total RNA was reverse-transcribed with Superscript III (Invitrogen) according to the manufacturer’s instructions. All PCR reactions were carried out on an ABI Prism thermocycler (PerkinElmer) as described previously (13). To control for variations in RNA quality and quantity, expression of the gene of interest was normalized to the expression of the TATA box-binding protein (TBP). Relative expression levels were calculated according to the formula: 2−ΔΔCT, where ΔΔCTsample was defined as CTgene of interest − CTTBP and ΔCT as DDCTsample − DDCT normalization sample. Sequences of primers were designed using Primer3 software (http://frodo.wi.mit.edu) and synthesized by MWG Biotech (Ebersberg, Germany).
RESULTS

SOCS1 Associates with the GR—To test whether SOCS1 associates with the GR in co-immunoprecipitation experiments, we transiently expressed Myc-tagged SOCS1 and GR in COS7 cells. Cell lysates were subjected to immunoprecipitation using antibodies directed against either the Myc tag of SOCS1 or the GR. Co-immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted for SOCS1 and GR (Fig. 1A). SOCS1 was detected in the GR immune complex and conversely, GR was pulled-down with the anti-Myc tag antibody, indicating that both proteins form a complex. Detection of endogenous SOCS1 has been proven to be difficult and has been reported only for the thymus or tissues from IFN-γ-treated animals, and required the combination of immunoprecipitation and immunoblotting (14, 15). To demonstrate the association of endogenous SOCS1 and GR we performed co-immunoprecipitation experiments with extracts prepared from livers of IFN-γ-treated mice (Fig. 1B). As described previously (14), untreated mouse liver showed no detectable SOCS1 protein expression (Fig. 1B, lane 2) and therefore served as a negative control for SOCS1 co-immunoprecipitation. However, SOCS1 could be detected in anti-GR immunoprecipitates in liver lysates from IFN-γ-treated mice (Fig. 1B, lane 1), indicating that SOCS1 and GR are also intracellularly associated in a complex when expressed at physiological levels. Liver lysates from IFN-γ-treated mice (Fig. 1B, lane 4) and thymus lysates (Fig. 1B, lane 5) immunoprecipitated with an anti-SOCS1 antibody served as a control for specificity of the precipitation product.

SOCS1 Binding to the GR Is Abolished after Long Term GC Stimulation—The GR is a ligand-dependent transcription factor and has been shown to interact with various chaperone proteins, co-activators, and co-repressors in a ligand-dependent fashion (16). We investigated which domain of the GR is required for SOCS1 interaction and whether ligand binding has any implications on the interaction of the receptor with SOCS1. COS7 cells were transiently co-transfected with SOCS1 and full-length GR or GR deletion mutant constructs. We used wild-type (wt) full-length GR, which we have shown to bind SOCS1 (Fig. 1), the C-terminal deletion mutant (N525) lacking the ligand binding domain (LBD) and the d107–318 deletion mutant lacking the t1 enhancer region (11). Cell lysates were precipitated with an antibody against the Myc tag of SOCS1 and analyzed by Western blotting. We observed that the mutant N525 lacking the C terminus of GR did not associate with SOCS1, indicating that the interaction of SOCS and GR requires the LBD. Deletion of the t1-containing region resulted in diminished binding, suggesting an additional contribution of this domain. A deletion of the complete DNA binding domain (d428–490) (17) did not interfere with the ability of the GR to bind to SOCS1 (data not shown).

To test whether ligand binding has an effect on the stability of the complex, COS7 cells were co-transfected with SOCS1 and GR constructs. Cell lysates were prepared before and after GC stimulation, and anti-GR immunoprecipitates were analyzed for SOCS1 abundance. Interestingly, the amount of co-immunoprecipitated SOCS1 decreased after stimulation with the GR agonist dexamethasone (Dex, Fig. 2B). After a 24-h treatment interval, SOCS1 binding to the GR was strongly reduced. We further investigated whether this time-dependent decrease in binding of SOCS1 to the GR was the result of transcriptional activation by the agonist Dex or occurs also with the antagonist RU486. As shown in the last two lanes of Fig. 2B, both agonist and antagonist treatment resulted in the same decrease of SOCS1 binding, indicating that the effect does not depend on a
Transcriptionally active GR. The result was further corroborated by experiments with a mutant GR (cs-2 GR) previously described by Lanz and Rusconi (18), that is only able to bind RU486 but not Dex and is devoid of any transcriptional activity. There, RU486 treatment for 24 h resulted in decreased binding of SOCS1 to the mutated GR (Fig. 2C), whereas Dex treatment did not show any effect on SOCS1 GR interaction as expected. We furthermore observed that the SOCS1 mutant dc40, which lacks the C-terminal 40 amino acids including the SOCS box, exhibits similar dissociation kinetics as the wt SOCS1, indicating that proteosomal degradation of other GR-associated proteins initiated by SOCS1 cannot account for the observed decrease in binding after GC stimulation (data not shown). Taken together these results suggest that long term ligand binding abolishes the interaction of GR and SOCS1 (Fig. 2, A and B). These effects apparently do not depend on GR transcriptional activity and SOCS box-mediated proteasomal targeting.

**The SH2 Domain of SOCS1 Is Required for GR Binding**—To map the site on SOCS1 responsible for the interaction with GR, wild-type and mutant constructs of SOCS1 were tested in the COS7 overexpression system (Fig. 3): SOCS1 carrying a point mutation in the SH2 domain (R105E), which disrupts binding to tyrosine-phosphorylated JAK2, and the dc40 mutant, which lacks the SOCS box still bound the GR. Deletion of the first 77 amino acids of the N terminus (dn77) also did not result in a decreased binding ability. However binding was abolished in the case of a deletion mutant lacking the N terminus and the SH2 domain (c-box). Taken together, these results indicate that the SH2 domain is required for GR SOCS1 interaction, but not the ability of the SH2 domain to bind to tyrosine-phosphorylated peptides, which requires Arg-105 of SOCS1. Because the SH2 domain is highly conserved within the SOCS family, we investigated whether other members of this family share the capability to bind to the GR. As shown in supplemental Fig. S1, CIS, SOCS2, and SOCS3 were co-immunoprecipitated with the GR, although with lower efficiency as indicated by the decrease of recovery in immunopre-
Cytosolic R105E mutant migrates slower in SDS gel as described recently (10). Siently transfected with GR and wild-type SOCS1 or SOCS1 mutants (see results). SH2 of SOCS1 is required for binding GR. GC Increases Nuclear Accumulation of SOCS1—The strong association observed in co-immunoprecipitation experiments raised the question whether ligand induced nuclear translocation of the GR can influence the nuclear concentration of SOCS1 in transfected COS7 cells. Therefore, the abundance of GR and SOCS1 in nuclear and cytoplasmic fractions, prepared from co-transfected cells stimulated for 3 h with Dex or from untreated cells, was investigated by Western blotting (Fig. 4A). As expected, Dex stimulation resulted in nuclear translocation of the GR. Dex also induced a significant change in the nuclear to cytosolic SOCS1 ratio (2.5-fold increase), suggesting that SOCS1 is translocated into the nucleus in a GR-dependent mode. However, cells only transfected with SOCS1 expression plasmids did not show any change in nuclear localization upon Dex stimulation. We further investigated the subcellular localization of GR and SOCS1 by confocal microscopy in COS7 cells co-transfected with expression vectors for both proteins or with each one alone. Cells were stimulated with Dex for 3 h or left unstimulated, fixed, stained, and investigated by confocal microscopy. In cells transfected with GR alone, a strong nuclear accumulation of the GR was observed upon Dex stimulation as expected. By contrast, in cells transfected with SOCS1, Dex treatment did not show an effect on the subcellular localization of SOCS1 (supplemental Fig. S2). However, when both proteins were co-expressed, the localization of SOCS1 changed, and both proteins showed a strong colocalization. In unstimulated cells, SOCS1 was retained in the cytoplasm, and Dex treatment resulted in nuclear translocation of SOCS1, indicating that the GR is capable of influencing the subcellular localization of SOCS1 and confirming our results from biochemical fractionation experiments (Fig. 4).

SOCS1 Inhibits GR Transactivation—To investigate whether the interaction of SOCS1 and GR had an impact on the transactivation activity of the GR, the effect of SOCS1 expression on the transcription of GC-regulated genes was determined in P388 lymphoma cells, murine bone marrow-derived macro-phages (BMDM), and NIH3T3 fibroblasts. SOCS1 expression was therefore induced by IFNγ treatment for 2.5 h, followed by activation of the GR with Dex for 3.5 h (Fig. 5A). FKBPs5 and MKP1 were chosen as GC indicator genes, because they have been shown to be strongly induced by GC (19, 20). FKBPs5 encodes for a 51-kDa FK506-binding protein, which forms part of the Hsp90 steroid receptor complex (19). MAP Kinase phos-
Glucocorticoid Receptor and SOCS1 Interaction

**FIGURE 5. Effect of SOCS1 on GR transactivation.** A, P388 murine lymphoid tumor cells, NIH3T3 murine fibroblasts and primary murine BMDM were cultured as described under "Experimental Procedures." Prior to the experiment, cells were washed three times for 30 min with GC-free medium. Cells were then prestimulated with 200 units of IFNγ for 2.5 h followed by 3.5 h stimulation with 1 μM Dex as indicated. Relative mRNA expression of the GC-regulated gene MKP1 and of SOCS1 was determined by quantitative RT-PCR using the Taq-Man method. SOCS1 expression was induced by IFNγ for all cell lines tested (data not shown). B, embryonal fibroblast derived from socs1−/− (socs1−/− MEF) and control mice (control MEF) were grown in serum-free medium. Cells were either prestimulated with 200 units of IFNγ (upper panels) for 2 h or not (lower panels), followed by Dex stimulation for the indicated time points. Fold induction levels of FKBP5 and MKP1 as determined by RT-PCR are shown as a function of time. The induction of SOCS1 expression in control MEFs and the absence of SOCS1 expression in socs1−/− MEFs was verified by RT-PCR (not shown). The data presented are representative for two independent experiments.

**DISCUSSION**

Interactions of different signaling pathways play an important role in the integration and fine-tuning of the cellular response. During acute inflammation, a balance between pro- and anti-inflammatory signaling inputs has to be maintained to ensure optimal host defense and limited tissue damage. Whereas GCs

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GR-interacting protein, STAT5, has also been described to be influenced by the GR (21). Also the treatment of cells with GCs resulted in nuclear accumulation of STAT5, suggesting that STAT5 localization is coupled with GR nuclear translocation. Although we have not determined the actual stoichiometric ratio between endogenous GR and SOCS1, we estimate that under physiological conditions, GR is present in abundance and that a significant proportion of intracellular SOCS1 molecules remains associated and shuttles into the nucleus together with the GR. The notion of a significant GR/SOCS1 association is further supported by the efficient pull-down of SOCS1 with a GR antibody from extracts of IFNγ-stimulated murine livers (Fig. 1B).

Experiments with transfected GR mutants revealed the C-terminal part of the GR adjacent to the DNA binding domain to be required for the interaction with SOCS1 (Fig. 2A). This region of the GR harbors the LBD, chaperone binding sites as well as transcription activation domains (16, 22, 23). All these functional domains are interconnected and depend on the structural integrity of the GR. An intriguing possibility is that an association of SOCS1 with the LBD could interfere with the recruitment of co-activator proteins such as steroid receptor coactivator-1 (SRC-1) and transcriptional intermediary factor 2 (TIF2) (24, 25), which could explain the negative regulatory effect of SOCS1 on GR transactivation observed in our study (Fig. 5). The reduced binding of SOCS1 to the GR after removal of the N-terminal transactivation t1 domain points again to the possible role of co-activator involvement. The association of SOCS1 and GR in the nucleus could thus have far reaching implications on GR function by interfering with the recruitment of transcriptional co-factors and histone deacetylases to target genes, which has to be addressed in further studies.

The GR interaction domain of SOCS1 was mapped to the SH2 domain (Fig. 3). This domain has previously been shown to mediate the binding of a variety of proteins in a phosphotyrosine-dependent and -independent fashion (10, 26–28). Further studies with additional mutants will be required to map the interaction domains of the GR and SOCS1 in more detail. Interestingly, the interaction of the GR was not restricted to SOCS1 but was also observed with the SH2 domain-containing proteins SOCS2, SOCS3, and CIS (supplemental Fig. S1), indicating that the binding to the GR could be a common function of SOCS proteins and proposing a further level of complexity for cross-talk between GR and cytokine signaling. Because the affinity of the other proteins was substantially lower compared with SOCS1, the physiological significance of these interactions remains to be established.

SOCS proteins have been shown to induce ubiquitination and degradation of interacting proteins via their SOCS box (3, 29), suggesting the possibility that the negative effect of SOCS1 on GR action involves SOCS1-mediated GR degradation. However, in experiments with GR- and SOCS1-transfected cells designed to determine GR protein stability in the presence or absence of SOCS1, we could not observe an influence of SOCS1 on GR stability. Furthermore, we did not see a change in the level of GR ubiquitination in these cells in the presence or absence of SOCS1,3 arguing against an important role of SOCS1 in GR ubiquitination. A SOCS1 box-mediated ubiquitination also does not appear to be responsible for the ligand-induced dissociation of GR and SOCS1, because co-expression of either wt SOCS1 or the SOCS box deletion mutant (dc40) resulted in similar dissociation kinetics. These data suggest that the observed effect of SOCS1 on GR transactivation is not a consequence of SOCS1-dependent degradation of the GR.

Implications of the Transient Nature of GR/SOCS1 Interaction—The stability of the intracellular complex of GR and SOCS1 was strongly influenced by GR ligand binding with slow kinetics (Fig. 2). This destabilizing effect of ligand occurred well after the effect of ligand on the early stages of receptor activation, which involves dissociation of chaperones, nuclear translocation, and transactivation. The effect apparently did not require transactivation via the GR, because it was observed with a GR mutant deficient in transcriptional activity (Fig. 2C) as well as after binding of the GR antagonist RU486.

The interaction between SOCS1 and GR adds to the more extensively studied effects of the GR on two other key cytokine signaling molecules namely, AP-1 and NF-κB (6, 7). Other mechanisms for the functional interference of GC signaling with the JAK/STAT pathway have been described, which comprise long term effects of the GR via activation and/or repression of GR-regulated genes (4, 30, 31). Specifically, GR was shown to cooperate with STAT1, a key mediator of inflammation via the IFNγ JAK/STAT pathway (31). In this report GC-mediated enhancement of STAT1 transcriptional activity was observed leading to enhanced transcription of the Fcγ receptor I, and this was attributed to the cycloheximide-sensitive induction of transcriptional co-regulators by the GR. In another report, long term GC treatment negatively regulated the amplitude of IFNγ signaling by down-regulating STAT1 expression at the mRNA level (30). By contrast, the novel mechanisms for negative interference between GC action and the JAK/STAT-induced SOCS1 described by us, was most prominent in the early phase of GC action. As shown in Fig. 5B, the interaction of GR with SOCS1 apparently inhibited GR transactivation, and this effect was only apparent early after GC stimulation and not observed after long term incubation in accordance with the dissociation of the GR SOCS1 complex under these conditions.

Potential Role of GR-SOCS1 Interaction in Disease—An intriguing implication of our findings on the negative effect of SOCS1 on GR function is that constitutively high expression levels of SOCS1 as observed in chronic inflammatory diseases like rheumatoid arthritis (32) could result in diminished sensitivity to GC under chronic inflammatory conditions and contribute to clinical treatment failure. Until now, attenuation of GR function in inflammation has been mechanistically linked to the action of proinflammatory cytokines, which can induce the accumulation of the dominant negative β-isofrom of GR and thereby reduce GC sensitivity (33), or to a negative effect of NF-κB on GR transactivation (33, 34).

Both the GR (35) and SOCS1 (1) have been recognized for a long time as potent intracellular regulators of the anti-inflamm-

3 M. C. Haffner, unpublished data.
Glucocorticoid Receptor and SOCS1 Interaction

matory response. However, so far they have been considered to act completely independently from each other. Our findings provide a novel molecular link by which the two proteins can interact with each other. One intriguing possibility is that the negative cross-talk between GR and SOCS1 represents a means to mitigate a potentially harmful effect of a simultaneous action of these two potent inhibitors of cytokine signaling. To test this hypothesis and to further assess the relative importance of the interaction in the fine-tuning of the cellular response, it will be important to define mutants of the GR or SOCS1 that are defective in negative cross-talk.

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