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Suppressors of cytokine signaling (SOCS) are cytokine-inducible proteins that modulate receptor signaling via tyrosine kinase pathways. We investigate the role of SOCS in renal disease, analyzing whether SOCS regulate IgG receptor (FcγR) signal pathways. In experimental models of immune complex (IC) glomerulonephritis, the renal expression of SOCS family genes, mainly SOCS-3, significantly increased, in parallel with proteinuria and renal lesions, and the proteins were localized in glomeruli and tubulointerstitium. Induction of nephritis in mice with a deficiency in the FcγR γ-chain (γ−/− mice) resulted in a decrease in the renal expression of SOCS-3 and SOCS-1. Moreover, blockade of FcγR by Fc fragment administration in rats with ongoing nephritis selectively inhibited SOCS-3 and SOCS-1, without affecting cytokine-inducible Src homology 2-containing protein and SOCS-2. In cultured human mesangial cells (MC) and monocytes, IC caused a rapid and transient induction of SOCS-3 expression. Similar kinetics was observed for SOCS-1, whereas SOCS-2 expression was very low. MC from γ−/− mice failed to respond to IC activation, confirming the participation of FcγR. Interestingly, IC induced tyrosine phosphorylation of SOCS-3 and Tec tyrosine kinase, and both proteins coprecipitated in lysates from IC-stimulated MC, suggesting intracellular association. IC also activated STAT pathway in MC, which was suppressed by SOCS overexpression, mainly SOCS-3. In SOCS-3 knockdown studies, specific antisense oligonucleotides inhibited mesangial SOCS-3 expression, leading to an increase in the IC-induced STAT activation. Our results indicate that SOCS may play a regulatory role in FcγR signaling, and implicate SOCS as important modulators of cell activation during renal inflammation.


The recently identified suppressors of cytokine signaling (SOCS) family is constituted by cytokine-inducible proteins that modulate receptor signal transduction via tyrosine kinases, mainly the Janus kinase (JAK)/STAT pathway (1–3). Until now, eight members of this family have been identified, i.e., cytokine-inducible Src homology 2-containing protein (CIS) and SOCS-1 to SOCS-7, and their biological functions are currently being examined (4, 5).

SOCS proteins inhibit cytokine signal transduction through several mechanisms. CIS and SOCS-2 bind to receptor sites blocking the recruitment and activation of STAT5, whereas both SOCS-1 and SOCS-3 can inhibit JAK tyrosine kinase activity (4–6). SOCS-1 binds to the kinase domain of JAK1, JAK2, and JAK3, then suppressing the JAK catalytic activity (1, 5). In contrast, SOCS-3 binds to the cytokine receptor, but it can also interact with some target sequences present within JAKs and STATs, revealing the complexity of the SOCS-3 regulatory mechanism (7). Little is known about the functions of the other SOCS family members.

In addition to cytokine receptors, SOCS interact with insulin, insulin-like growth factor I, leptin, growth hormone, and chemokine receptors (6, 8–11). Moreover, SOCS-3 induced by cytokines and growth factors is also rapidly tyrosine phosphorylated (9, 10, 12, 13), although the importance of this phosphorylation remains unclear. SOCS proteins also modulate the Grb/Ras pathway by binding and suppression of nonreceptor tyrosine kinases (Tec and Syk) (14–16). Both kinases are implicated in signaling via T cell and B cell Ag receptors and IgG receptors (FcγR) (17, 18), thus suggesting that SOCS may have a broader range of action than originally thought. Recent papers described that SOCS proteins regulate the TCR signaling through the inhibition of the calcineurin/NFAT pathway (16, 19). Although there are some parallels in signaling between cytokine receptors, TCR, and FcγR, the role of SOCS in the regulation of FcγR-mediated signaling has not previously been reported.

Evidence is emerging for the involvement of SOCS proteins in diseases of the immune system, such as rheumatoid arthritis, colitis, and Crohn’s disease (5, 7, 20–22). In this sense, inhibition of SOCS-1 expression resulted in a more severe colitis and arthritis in mice (20, 21), whereas SOCS-3 gene therapy reduced the progression of the disease (22). In the kidney, differential SOCS expression was observed in renal cells stimulated with cytokines (23), but the involvement of SOCS in the pathogenesis of immune renal diseases has not been reported.

There is compelling evidence for the important role of immune complexes (IC) as the pathogenic factor triggering inflammation in many immunological diseases, including glomerulonephritis (24).
Studies in animal models of immune glomerular injury and in IC-stimulated renal cells revealed that the presence of FcyR in resident and infiltrating cells is critical for the initiation and progression of renal damage (25–29). Similar to other immunoreceptor tyrosine-based activation motif-containing receptors, such as the TCR, FcγRI and FcγRIII signaling pathways involve the immunoreceptor tyrosine-based activation motif tyrosine phosphorylation in the associated γ-chain by Src (17), and the subsequent activation of Syk, Lyn, Tec, phosphatidylinositol 3-kinase, phospholipase Cγ, and mitogen-activated protein kinase (MAPK) in many cell types, including glomerular mesangial cells (MC) (17, 30, 31).

In this study we examined the relation of SOCS, especially SOCS-3, with FcγR-mediated signaling and the potential implication of SOCS in IC-mediated renal diseases. We show that SOCS are induced in resident and infiltrating cells after FcγR stimulation, both in vivo (models of IC-mediated glomerulonephritis) and in vitro (cultured MC and monocytes incubated with IC). Moreover, SOCS-3 modulates cell signaling by interaction with Tec tyrosine kinase and inhibition of FcγR-mediated STAT activation.

Materials and Methods

Reagents

IC containing IgG were obtained by heat aggregation (63°C, 30 min) of monomeric human IgG (Sigma-Aldrich, St. Louis, MO) and mouse IgG (Cappel/ICN, Aurora, OH), as previously described (31). IgG Fc fragments were obtained by digestion with activated papain (Sigma-Aldrich) and purification by chromatography (29). The presence of endotoxin in all preparations was excluded using the Limulus amebocyte assay (Ingelheim Diagnostica, Barcelona, Spain). Genistein, erbstatin, PD98059, and SB203580 were from Calbiochem (La Jolla, CA), and cycloheximide from Sigma-Aldrich. Polyclonal Abs against SOCS-3, SOCS-1, and SOCS-2 (sc-7009), SOCS-1 (sc-7005), and SOCS-2 (sc-7007), Tec (sc-1109), STAT1 (sc-346), and JAK2 (sc-294), and mAbs against STAT3 (sc-8019) and JAK1 (sc-1677) were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-SOCS-1, mAb anti-tubulin, and mAb anti-phosphotyrosine (P-Tyr, PY20, and RC20 biotinylated) were from Immunogenex (Los Angeles, CA). Phosphorothioate-modified oligodeoxynucleotides (ODN) were from BD Biosciences. The reporter vector containing the 0.7-kb human renin promoter and luciferase gene; Promega, Madison, WI) at a 10:1 ratio, by using the FuGENE 6 reagent (Roche, Barcelona, Spain) (34). The expression vectors for SOCS-3 and SOCS-1 cloned in the psiHA vector were a gift from Dr. S. Lamas (Centro de Investigaciones Biológicas, Madrid, Spain) (34). The expression vectors for SOCS-3 and SOCS-1 cloned in the psI3HA plasmid were a gift from Dr. H. Boef (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France) (35). MC (5 × 10⁵) on 24-well plates were transfected during 24 h with luciferase plasmids and pRL-TK vector (containing the Renilla luciferase gene; Promega, Madison, WI) at a 10:1 ratio, by using the FuGENE 6 reagent (Roche, Barcelona, Spain). In some cases, cotransfection with SOCS-3 and SOCS-1 expression vectors or with the control empty vector (psi3HA) was made. After 24 h transfection, cells were stimulated for 24 h and luciferase activity in cleared lysates was assayed using a luminometer. Firefly luciferase activity was normalized for total protein content and for variations in transfection efficiency (Renilla activity). In knockdown studies, cells were pretreated during 24 h with SOCS-3 ODN (antisense and sense, 1 μM) before transfection.

Statistical analysis

The results are given as mean ± SD or representative experiments, when indicated. Values were analyzed by ANOVA and Tukey-Kramer tests using Instat, Graphpad software (San Diego, CA). A value of p < 0.05 was considered significant.
Results

Expression of SOCS proteins in experimental models of immune glomerulonephritis

We first investigated the expression levels of SOCS and identified the cells producing these proteins in different models of immune-mediated renal inflammation: chronic IC-mediated glomerulonephritis in rats (by repeated injection of OVA) and acute mesangio proliferative glomerulonephritis in mice (by single injection of anti-murine mesangial cell Ab).

The chronic glomerulonephritis model was characterized by glomerular immune deposits, cell proliferation, matrix accumulation, inflammatory cell infiltration, and intense proteinuria (29). Then we evaluated whether SOCS expression is implicated in the pathogenesis of immune glomerulonephritis. As indicated in Fig. 1A, the renal expression of SOCS genes in healthy control rats was very weak, with the exception of SOCS-2. Induction of IC-mediated glomerulonephritis in these animals markedly increased SOCS expression (n-fold vs control: CIS, 3.1 ± 0.2; SOCS-1, 1.8 ± 0.1; SOCS-2, 1.7 ± 0.2; SOCS-3, 5.2 ± 0.4). By immunohistochemistry, few glomerular and tubular cells positive for SOCS-3 and SOCs-1 were observed in healthy control rats (glomerular score: SOCS-3, 0.3 ± 0.1; SOCS-1, 0.5 ± 0.3; Fig. 1, B and C). In diseased kidney, SOCS proteins increased, mainly in glomeruli, (glomerular score: SOCS-3, 2.8 ± 0.2; SOCS-1, 2.1 ± 0.1; p < 0.001 vs control) and in some tubular epithelial cells and infiltrating cells (Fig. 1, D and E).

The acute glomerulonephritis model was characterized by severe proteinuria and hematuria, with maximal glomerular lesions (cell lysis, cell proliferation, and inflammatory infiltrate) at days 5–7 (32). In this model, SOCS-3 gene expression was maximal at day 5 (Fig. 2A) and the protein was principally localized in glomeruli and proximal tubules (glomerular score at day 7: control, 0 ± 0; nephritis, 2.2 ± 0.1, p < 0.01, n = 4; Fig. 2, B and C). Similarly, the number of SOCS-1-positive cells increased in nephritic mice (Fig. 2D), with a wide distribution in glomeruli (mesangial and endothelial cells) and tubules (glomerular score at day 7: control, 0.5 ± 0.1; nephritis, 3.0 ± 0.5, p < 0.01, n = 4).

We evaluated whether the SOCS production by renal cells was a process mediated by FcγR. Then, mesangio proliferative glomerulonephritis was studied in mice lacking functional FcγRI and FcγRIII (γ−/− mice) in parallel to their wild-type littermates. Induction of nephritis in γ−/− mice resulted in a reduction in proteinuria levels and glomerular and tubulointerstitial lesions (66 ± 11%, 71 ± 4%, and 73 ± 11% decrease vs nephritis in wild-type at day 5, respectively; p < 0.05). Moreover, SOCS-3 mRNA expression significantly decreased in γ−/− mice (Fig. 2A). By immunohistochemistry, the production of both SOCS-3 (Fig. 2F) and SOCS-1 (Fig. 2G) appeared significantly diminished in renal tissues from γ−/− mice (65 ± 16% and 42 ± 8% decrease vs nephritis in wild-type, respectively; p < 0.05). Additionally, in vivo FcγR blockade was studied by injection of IgG Fc fragments into rats with ongoing immune nephritis. We have previously described that Fc fragment administration prevents renal injury in immune glomerulonephritis and decreases the generation of inflammatory mediators (29, 31). Consistent with this, the renal mRNA expression of SOCS-3 and SOCS-1 was significantly reduced in rats treated with Fc fragments (71 ± 3% and 60 ± 5% inhibition vs untreated nephritis, respectively; p < 0.01, n = 5). Interestingly, the expression levels of other SOCS family members were not significantly affected by Fc fragment administration (CIS, 18 ± 5%; SOCS-2, 15 ± 6% inhibition vs untreated nephritis; p > 0.05), suggesting that the renal expression of SOCS-3 and SOCS-1 could be selectively linked to FcγR stimulation.

**FIGURE 1.** Expression of SOCS genes in IC-mediated glomerulonephritis in rats. A. The renal mRNA expression of SOCS family genes was analyzed in control (C) and nephritic (N) rats by RT-PCR (28–35 cycles). Quantification of mRNA expression was performed by densitometry and values were corrected by the GAPDH expression. Each bar represents the mean ± SD of five animals analyzed in quadruplicate; *, p < 0.01 vs control rats. Detection of SOCS proteins in kidneys from control (B and C) and nephritic (D and E) rats was performed by immunoperoxidase technique using specific anti-SOCS-3 (B and D) and anti-SOCS-1 (C and E) Abs. An increase in the positive staining for SOCS-3 (D) and SOCS-1 (E) was observed in glomerular and tubular cells of nephritic rats. Original magnification, ×200.
IC rapidly induce SOCS-3 expression in cultured MC and monocytes

To assess in vitro the potential role of SOCS family in stimulated renal cells, we examined its expression in cultured glomerular MC and monocytes. Exposure of human MC to 200 μg/ml IC induced a time-response expression of SOCS-3 with a maximum at 1–2 h, as determined by RT-PCR (Fig. 3A) and confirmed by Northern blot (1.6, 2.8, 2.1, 1.9, and 1.6 n-fold increase at 1, 2, 4, 6, and 8 h of incubation; data not shown). Preincubation with 50 μM cycloheximide did not affect the SOCS-3 mRNA expression induced by IC (2.1 ± 0.2 vs 2.3 ± 0.2, n-fold increase vs basal at 2 h, p > 0.05, n = 4), indicating that de novo protein synthesis is not required for SOCS gene induction.

In monocytes (THP-1 cell line), IC dose dependently induced SOCS-3 mRNA expression (1.2 ± 0.1, 2.4 ± 0.2, and 2.7 ± 0.2 n-fold increase with 50, 100, and 200 μg/ml at 2 h), and the temporal course was very similar to that observed in MC (peak at 1 h, 3.3 ± 0.2 n-fold increase, n = 3).

As positive control, we used the cytokines IL-6 and IFN-γ (100 U/ml), which induced a high SOCS-3 mRNA expression in MC (4.5 ± 0.6 and 4.7 ± 0.7 n-fold increase at 2 h, respectively, n = 4). In these conditions, preincubation with IC for 1 h significantly reduced the SOCS-3 expression induced by both IL-6 and IFN-γ (76 ± 11% and 67 ± 14% inhibition, respectively; p < 0.05). This suggests that FcγR could negatively regulate the cytokine receptor signaling in these cells.

The synthesis of SOCS proteins was determined by Western blot. In THP-1 monocytes (Fig. 3B) and human MC (data not shown), SOCS-3 protein was detected within 30 min of IC stimulation, peaked at 1 to 2 h, and returned to near basal levels within 7 h. Similar kinetics was observed for SOCS-1 protein expression (Fig. 3C), whereas SOCS-2 production was very low (data not shown).

The involvement of FcγR in the IC-induced SOCS expression was assessed in cultured MC from γ−/− mice. As shown in Fig. 4, MC from wild-type mice expressed high levels of SOCS-3 and SOCS-1 mRNA after incubation with murine IC, which remained increased even after 6 h. By contrast, SOCS expression was significantly attenuated when MC from γ−/− mice were stimulated.

SOCS-3 is tyrosine phosphorylated in response to IC

Lysates from IC-stimulated human MC were immunoprecipitated with anti-SOCS-3 or P-Tyr Abs and then immunoblotted for tyrosine phosphorylation of SOCS-3 protein, respectively. As indicated in Fig. 5A, IC induced a rapid (15–30 min) and transient tyrosine phosphorylation of SOCS-3 in MC. By contrast, no tyrosine phosphorylation of SOCS-1 protein was observed under the same experimental conditions (data not shown).

We next analyzed the signaling pathways leading to SOCS-3 protein induction by IC. Pretreatment of cells with the general tyrosine kinase inhibitors genistein (Fig. 5, B and C) or erbstatin (data not shown) diminished the tyrosine phosphorylation and mRNA expression levels of SOCS-3 (82 ± 10% and 59 ± 7% inhibition at 1 h, respectively, n = 2 to 4). This suggests that a tyrosine kinase may be involved not only in SOCS-3 phosphorylation, but also in its induction by IC. To investigate the involvement of MAPK pathway in the induction of SOCS-3 we used PD98059 (inhibitor of MAPK kinase) and SB203580 (inhibitor of p38). Both compounds had partial inhibitory effects on the SOCS-3 mRNA expression elicited by IC at 2 h (percentage of inhibition: PD98059, 31 ± 3%; SB203580, 40 ± 8%, n = 2) without affecting the SOCS-3 phosphorylation levels. These data
Tec kinase was detected in Blotted for Tec. As shown in Fig. 6 were precipitated with anti-SOCS-3 and subsequently immuno-

Tec (64 kDa). In additional experiments, lysates from human MC

-m-fold increase vs basal) and transient tyrosine phosphorylation of

indicated in Fig. 6, IC induced a rapid (peak at 15 min, 3.7

A

analyzed the activation of Tec kinase in IC-stimulated cells. As

position of molecular markers is indicated. Data of densitometric analysis

are expressed respect to basal and are mean ± SD of three to six different

experiments; *, p < 0.01 vs basal.

suggest that IC uses tyrosine kinase and MAPK pathways for

SOCS-3 induction in MC.

Interaction of SOCS-3 with Tec kinase in IC-stimulated cells

The rapid tyrosine phosphorylation of SOCS-3 suggests that it could be a substrate for a FcγR-activated tyrosine kinase. Previous reports have described that SOCS can inhibit the activity of several nonreceptor tyrosine kinases, including Tec, a kinase that is also implicated in the FcγR signaling in monocytes (17). Therefore, we analyzed the activation of Tec kinase in IC-stimulated cells. As indicated in Fig. 6A, IC induced a rapid (peak at 15 min, 3.7 ± 0.5 n-fold increase vs basal) and transient tyrosine phosphorylation of Tec (64 kDa). In additional experiments, lysates from human MC were precipitated with anti-SOCS-3 and subsequently immuno-blotted for Tec. As shown in Fig. 6B, Tec kinase was detected in SOCS-3 immunoprecipitates of IC-stimulated cells, suggesting a possible interaction between SOCS-3 and Tec after FcγR ligation.

IC induce activation of STAT transcription factors in MC

Because there are similarities between cytokine receptor and FcγR signaling, we next investigated the involvement of SOCS in different FcγR-mediated signals, focusing on JAK/STAT, the classical pathway modulated by SOCS (6). As indicated in Fig. 6C, IC elicited the tyrosine phosphorylation of STAT1 (84–91 kDa) and STAT3 (92 kDa) in MC, reaching peak levels after 60 and 30 min of stimulation, respectively. In contrast, no tyrosine phosphorylation of JAK1 and JAK2 was detected upon FcγR stimulation (data not shown). This is consistent with previous works describing that, in addition to JAK, other tyrosine kinases may regulate STAT pathway (36).

To analyze whether FcγR ligation induce STAT transcriptional activity, MC were transiently transfected with the pGAS-Luc, pISRE-Luc, or pSTAT3-Luc plasmids, which contain STAT1/STAT1, STAT1/STAT2, and STAT3 binding sites, respectively, and luciferase activity was measured after 24 h of stimulation with IC or the positive control (IFN-γ plus IL-6). As shown in Fig. 6D, IC elicited the expression of the three STAT-driven reporter vectors, with the maximal luciferase activity corresponding to the STAT1/STAT2 combination. No increase in luciferase activity was seen with the control plasmid pGL2-Luc.

**SOCS expression inhibits the STAT activation in response to IC**

The regulatory role of SOCS in IC-induced transcription was analyzed in cells cotransfected with STAT-responsive luciferase reporter plasmid and the SOCS expression vectors, or with the control empty vector (p513HA). As shown in Fig. 7A, the IC-induced STAT reporter activity was impaired by SOCS-3 and SOCS-1 overexpression, although maximal decrease was achieved with SOCS-3. By contrast, both SOCS-3 and SOCS-1 expression vectors inhibited in a similar manner the STAT activity induced by the positive control (IFN-γ plus IL-6; Fig. 7A). In parallel experiments using murine MC, the IC-stimulated luciferase activity was significantly inhibited by overexpression of SOCS, mainly SOCS-3 (Fig. 7B). In these experiments, STAT-mediated transcription was attenuated in MC from γ−/− mice (Fig. 7B), corroborating the implication of FcγR.

We also evaluated whether SOCS proteins were able to inhibit IC-induced transcriptional activity of a STAT-regulated gene. Therefore, we transfected MC with pNiSOS-Luc, a plasmid containing the DNA binding sites for the transcription factors Oct-1, NF-IL-6, NF-kB, GAS/ISRE, and AP-1 (34). Similar to the STAT reporter assay, the iNOS promoter transcriptional activity stimulated by IC (n-fold increase vs basal, 3.8 ± 0.9, n = 4) was partially decreased when cells were cotransfected with SOCS expression vectors, with the highest inhibition corresponding to SOCS-3 (percentage of inhibition; SOCS-3, 51 ± 3%; SOCS-1, 33 ± 3%; n = 3).
To determine which components of the JAK/STAT pathway could be modulated by SOCS-3 protein, MC transfected with SOCS-3 expression vector or control empty plasmid (p513HA) were stimulated with IC, and lysates were analyzed by immunoprecipitation and Western blot. In MC transfected with the control empty plasmid, IC caused tyrosine phosphorylation of SOCS-3, Tec, STAT1, and STAT3 (Fig. 7C), but not JAK1 and JAK2 (data not shown). However, SOCS-3 overexpression inhibited the IC-induced STAT1 and STAT3 activation, without affecting Tec tyrosine phosphorylation. Consistent with data in intact cells, SOCS-3 coimmunoprecipitated with Tec in SOCS-3 overexpressing cells (Fig. 7D). Association with Tec was detectable in the absence of IC, but the stimulus increased the interaction.

As an alternative approach to examine the role of SOCS-3 in IC-stimulated STAT activation, knockdown studies were done using SOCS-3 antisense ODN, which was designed to hybridize to human SOCS-3 mRNA at the translation start site, blocking translation and leading to decreased protein expression (37). As shown in Fig. 8A, preincubation of cells with SOCS-3 antisense ODN dose dependently decreased the SOCS-3 production in response to IC (total inhibition at 1 μM). By contrast, no effect was observed in the presence of sense ODN (Fig. 8B). In the reporter gene assay,
pretreatment of cells with antisense ODN led to a significant increase in the IC-stimulated STAT luciferase activity compared with cells treated with sense ODN (Fig. 8C). In other experiments, cells were preincubated with antisense and sense ODN, then co-transfected with STAT-reporter and SOCS-3 expression vectors and stimulated with IC. As shown in Fig. 8D, SOCS-3 overexpression significantly diminished the positive effect of antisense ODN on IC-induced STAT activation, indicating that the antisense ODN had a specific effect on the reduction of SOCS-3 protein expression.

Discussion
The SOCS family of cytokine-induced proteins constitutes a negative feedback loop of JAK/STAT pathway (1–3, 5). However, SOCS can also associate with different receptors, such as growth hormones, T cell and B cell Ag receptors (6–11, 16, 19), suggesting that SOCS have more diverse functions that simply acting as negative regulators of cytokine receptor signaling. In this work we evaluate the potential role of SOCS in regulating inflammatory and immune responses in the kidney by analyzing two models of IC glomerulonephritis. Induction of chronic immune glomerulonephritis in rats markedly increased renal expression of SOCS genes (SOCS-3 > CIS > SOCS-1 > SOCS-2). The more selective expression of SOCS-3 compared with other SOCS proteins suggests its important role in the diseased kidney. SOCS proteins were also detected in glomeruli and tubules of mice with mesangiotrophilic glomerulonephritis. Expression of SOCS-3 was previously seen in several inflammatory diseases, such as human and experimental colitis and experimental arthritis (20–22). The fact that SOCS are expressed by hemopoietic and nonhemopoietic cell types in vivo (5) suggests that this family importantly participates in inflammation during immune disease. Because SOCS proteins are expressed in the damaged kidney by both resident and infiltrating cells, we next analyzed their expression in cultured MC and monocytes. Both SOCS-3 and SOCS-1, but not SOCS-2, were rapidly and transiently expressed in IC-stimulated MC and monocytes. Moreover, SOCS-3 expression was resistant to the protein synthesis inhibitor cycloheximide, indicating that it is an immediate-early gene induced by IC.

In these studies we used knockout mice for γ-chain, the common activation subunit required for surface assembly and signaling of some Fc receptors (FcεRI, FcγRI, FcγRIII, and FcεRI) (17). Previous studies with these mice have documented the importance of FcγR in promoting IC-mediated inflammation in several tissues, including the kidney (17, 24–26, 29). In this study we show that the increase in SOCS expression observed in wild-type mice with glomerulonephritis was attenuated in the γ−/− mice. In vitro, MC from γ−/− mice failed to respond to IC activation, as shown by impaired SOCS expression, and to STAT transcriptional activity. These data confirm that FcγRI and/or FcγRIII may be involved in IC-mediated responses in renal cells, and reinforce the key role of FcγR in the initiation/progression of inflammation during immune glomerular injury. Moreover, in the rat glomerulonephritis model, treatment with Fc fragments (inhibitors of IC binding to FcγR) selectively prevented the renal expression of SOCS-3 and SOCS-1, without affecting CIS and SOCS-2 expression levels, thus suggesting that SOCS isoforms may selectively regulate FcγR signaling in renal cells.

The further mechanism involved in IC-induced SOCS expression was investigated by using several kinase inhibitors. The mesangial SOCS-3 mRNA expression was abolished by tyrosine kinase inhibitors. Furthermore, although both SOCS-3 and SOCS-1 proteins are induced by IC, only SOCS-3 is tyrosine phosphorylated, thus suggesting that phosphorylation may be important in SOCS-3 function, as previously reported in other systems (9, 10, 12, 13). In this sense, in cytokine-stimulated T cells, whereas
SOCS-3 inhibits STAT pathway, phosphorylated SOCS-3 sustains MAPK activation through the interaction with Ras (13), indicating that SOCS may act as molecular switch of different signaling pathways. The rapid tyrosine phosphorylation of SOCS-3 in response to IC indicates that SOCS-3 is a substrate for an IC-activated tyrosine kinase. By immunoprecipitation assays, we demonstrated that SOCS-3 coprecipitated with Tec tyrosine kinase, indicating association between SOCS-3 and Tec after FcγR stimulation. Moreover, SOCS-3 is not required for Tec activation because Tec is phosphorylated soon after ligand stimulation, when endogenous SOCS-3 is not yet expressed.

The mechanism by which synthesized SOCS inhibit signaling differs among the various isoforms (1–3, 4, 6). It has been described that SOCS-3 indirectly inhibits STAT phosphorylation through association with the cytokine receptor (6), although it can also inhibit JAK1 and suppress STAT and MAPK pathways (38). SOCS proteins also inhibit Tec tyrosine kinase, suggesting that their spectrum of activity may extend beyond the JAK family (17). Tec kinases associate with multiple cell surface receptors, including Ag receptor and FceR (17, 18), and are able to interact with and induce STAT tyrosine phosphorylation and transcriptional activity, without JAK activation (36, 39). In MC we observed Tec kinase activation by FcγR cross-linking, and SOCS-3 association with Tec, but not with JAK1 and JAK2 in IC-stimulated cells. The inability of SOCS-3 to interact with JAK, even in the presence of IC stimulation, confirms that SOCS-3 does not act by directly inhibiting the JAK activity. Other authors also described that SOCS-1 inhibits Kit receptor tyrosine kinase signaling through the interaction with Grb-2 and Vav (14), and that the association SOCS-1/Syk/ε-chain (16) and SOCS-3/calcineurin (19) mediates suppression of NFAT activation by TCR.

In this report we also described for the first time the activation of STAT pathway after FcγR stimulation. Indeed, STAT1/STAT3 tyrosine phosphorylation and STAT transcriptional activity were suppressed by overexpression of SOCS-3 and, to a lesser extent, by SOCS-1. SOCS-3 also inhibited the IC-mediated induction of the iNOS gene promoter, a STAT-regulated gene, similarly to previous data in LPS-stimulated macrophages (40). To further test the role of SOCS-3 in the negative regulation of IC-induced STAT activation, we examined the effect of knocking down SOCS-3 using a specific antisense ODN (37). The results indicate that reduced expression of SOCS-3 protein leads to an increase in the magnitude of IC response, implying that SOCS-3 may play a negative regulatory role in FcγR signaling.

Mesangial SOCS-3 expression was partially decreased by inhibitors of MAPK kinase and p38 MAPK, indicating that IC use multiple pathways for maximal SOCS-3 induction. Previous studies have described the involvement of MAPK in FcγR signaling in several cell types (17, 31), and the involvement of SOCS in the STAT inhibition by MAPK (39, 41, 42). Accordingly, we conclude that SOCS proteins, mainly SOCS-3, appear to be responsible for the termination of FcγR-mediated STAT activation in renal cells. The mechanism of this regulation is currently unknown, but several possibilities exist. We postulate that SOCS-3 may down-regulate the activity of Tec by direct interaction. However, we cannot discard the association of SOCS family members with other kinases or phosphatases of the FcγR signaling pathways.

We also noted that IC significantly inhibited mesangial SOCS-3 expression in response to IL-6 and IFN-γ. Similarly, FcγR ligation inhibits IFN-γ and IL-10 signaling in monocytes (43, 44), suggesting a negative cross-talk between cytokine receptors and FcγR. It is also possible that, through differential activation of SOCS, IC may influence STAT activation and modulate cytokine signaling. In this sense, IFN-γ-induced STAT1 is inhibited by SOCS-3 and SOCS-1, but only SOCS-3 affects IL-6-induced STAT3 activation (45). Then, we postulate that the diminished responsiveness to these cytokines, and thus inadequate suppression of cell activation, may contribute to chronicity and severity of inflammation in IC-mediated diseases.

Our work describes the importance of SOCS proteins, especially SOCS-3, in inflammatory renal processes and raises the possibility that therapeutic strategies based on the manipulation of renal SOCS might be of clinical benefit. It is difficult at this point to
directly test the role of SOCS-3 in inflammation in vivo without viable SOCS-3 knockout or transgenic mice (5, 6). The evidence is that SOCS proteins inhibit FcγR-mediated activation of STAT pathway in renal cells, suggesting a potential protective role during renal inflammation. However, additional studies are required to define the role of SOCS in renal pathologic processes or even in repairing after inflammation-induced renal damage.

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