Suppressor of Cytokine Signaling-1 Regulates the Sensitivity of Pancreatic β Cells to Tumor Necrosis Factor*

Mark M. W. Chong, Helen E. Thomas, and Thomas W. H. Kay‡

From the Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

Suppressor of cytokine signaling-1 (SOCS-1) is a negative regulator of the Jak-STAT (signal transducer and activator of transcription cytokine) signaling pathway but may also regulate other pathways. At least in vitro, SOCS-1 inhibits the action of multiple cytokines. By studying the effects of SOCS-1 deficiency, we investigated whether SOCS-1 is involved in preventing cytokine-induced death of pancreatic islet cells, a potential mechanism of insulin deficiency in autoimmune diabetes. Tumor necrosis factor (TNF) + interferon-γ (IFNγ) was more potent at inducing cell death in SOCS-1−/− islets than in wild type. Individually, these cytokines did not induce cell death. The titration of the two cytokines suggested that this increased cell death was because of hypersensitivity to TNF. Interleukin-1 + IFNγ induced the same level of cell death in SOCS-1−/− and wild-type islets, suggesting that the sensitivity of islets to IFNγ or interleukin-1-mediated cytotoxicity is not affected by SOCS-1 deficiency. Additionally, SOCS-1−/− β cells were responsive to lower concentrations of TNF measured by class I major histocompatibility complex up-regulation. The TNF + IFNγ damage of islets was mediated by inducible nitric-oxide synthase (iNOS), and increased iNOS expression and nitric oxide production were found in SOCS-1−/− islets following cytokine treatment. A further analysis revealed that SOCS-1 deficiency results in augmented TNF signaling via the p38 mitogen-activated protein kinase pathway but not NFκB or c-Jun N-terminal kinase pathways. Increased p38 signaling may be responsible for the increased iNOS expression in SOCS-1−/− islets. Therefore, these findings provide evidence that physiological levels of SOCS-1 negatively regulate TNF signaling.

Multiple immune mediators are implicated in the destruction of pancreatic β cells in autoimmune diabetes including pro-inflammatory cytokines, perforin, and Fas (reviewed in Ref. 1). Pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interferon-γ (IFNγ) are secreted by autoreactive T cells and activated macrophages that infiltrate the islets during the pathogenesis of diabetes (2–4). In vitro, combinations of these cytokines cause β cell damage (5–9). IL-1 together with IFNγ induces the expression of iNOS in mouse and human islets, and the subsequent nitric oxide (NO) production is largely responsible for islet dysfunction (5, 10–12). TNF in combination with IFNγ also damages primary islets and β cell lines in vitro (5, 6, 9). We have previously shown that β cells express TNFRI, the receptor for TNF (9). Therefore, β cell damage could arise from the direct action of TNF, and this is the case in β cell lines. TNF-induced cell death in combination with lipopolysaccharide and IFNγ in primary β cells appears to occur indirectly by stimulating intra-islet macrophages to secrete IL-1, which leads to NO-dependent β cell cytotoxicity (5, 13).

Suppressor of cytokine signaling-1 (SOCS-1) was identified as a negative regulator of the Jak-STAT signaling pathway of cytokines. The overexpression of SOCS-1 inhibits signaling by a variety of cytokines that activate this pathway including interferons (14–16) and members of the IL-6 family (17–19). In hepatocytes, SOCS-1 deficiency has been shown to prolong STAT1 activation by IFNγ (20). SOCS-1 is thought to act on this pathway by binding to and inhibiting activated Jak kinases (18, 19, 21, 22). In overexpression systems, SOCS-1 has also been shown to bind to adaptor proteins such as Grb2 and Nck (23), nucleotide exchange factors such as vav (23, 24), and cytoplasmic tyrosine kinases such as Tec and Syk (25, 26), suggesting that it may act on other signaling pathways in addition to the Jak-STAT pathway. Because overexpression systems have been used to demonstrate the binding of SOCS-1 to these proteins, it is still unclear whether these interactions are physiologically relevant.

We have previously demonstrated that SOCS-1 is expressed in β cells (16). Because SOCS-1 was identified as a negative regulator of Jak-STAT signaling and in particular signaling by IFNγ, we expected that it would also be a negative regulator of IFNγ signaling in β cells. However, we found that the levels of SOCS-1 expressed in β cells after cytokine treatment were insufficient to terminate IFNγ signaling at the level of STAT1 activation and transcription of IFNγ-regulated genes including class I MHC. This finding suggested that IFNγ signaling in β cells may not be negatively regulated by SOCS-1. Consistent with this observation, SOCS-1 deficiency did not affect the intensity or kinetics of IFNγ signaling in β cells nor the sensitivity of β cells to the cytokine.

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‡ To whom correspondence should be addressed: The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Parkville, Victoria 3050, Australia. Tel.: 61-3-9345-2457; Fax: 61-3-9347-0852; E-mail: kay@wehi.edu.au.

1 The abbreviations used are: TNF, tumor necrosis factor; IL-1, interleukin-1; IFNγ, interferon-γ; NO, nitric oxide; TNFRI, tumor necrosis factor receptor; SOCS-1, suppressor of cytokine signaling-1; MHC, major histocompatibility complex; STAT, signal transducer and activator of transcription; iNOS, inducible nitric-oxide synthase; IRAP, IL-1 receptor antagonist protein; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; CHX, cycloheximide; FACs, fluorescence-activated cell sorting; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.

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The aim of this study is to investigate whether SOCS-1 in β cells has a role in regulating signaling by other cytokines. In particular, we have examined the effects of SOCS-1 deficiency on the sensitivity of β cells to damage by IL-1 and TNF. Unlike IFNγ, IL-1 and TNF do not activate Jak-STAT pathway. By analyzing the effects of SOCS-1 deficiency rather than using overexpression systems, we have also addressed the issue of whether the action of SOCS-1 on signaling pathways other than the Jak-STAT pathway is physiologically relevant.

EXPERIMENTAL PROCEDURES

Isolation of Murine Islets—SOCS-1−/−/IFNγ−/− and IFNγ−/− mice maintained on mixed C57Bl/6–129SvV backgrounds are described elsewhere (27). Islets were isolated according to the method of Liu and Shapiro (28) by intraductal digestion of the pancreas with collagenase P (Roche Diagnostics) and the separation on a Histopaque-1077 (Sigma) density gradient. The islets were handpicked to purity and cultured in CMRL-1066 (Invitrogen) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum.

Reagents—The cytokines used were recombinant murine TNF (Genentech, South San Francisco, CA), recombinant murine IFNγ (Genentech), and recombinant human IL-1 (Genzyme Corp., Cambridge, MA). The iNOS inhibitor aminoguanidine (Sigma) was used at 1 mM, the caspase inhibitor zVAD-fmk (Enzyme Systems Products, Livermore, CA) was used at 100 μM, and IL-1 receptor antagonist protein (IRAP) (Amen Inc., Thousand Lakes, CA) was used at 5 μg/ml. Sodium nitroprusside (Sigma) was used as a NO donor compound. The protein synthesis inhibitor cycloheximide (CHX, Sigma) was used at 7.5 μg/ml.

Cell Death Assays—The quantification of cell death was performed according to the method described by Nicoletti et al. (29), which measures the fragmentation of nuclei. After cytokine treatment, the islets were dispersed into single cells with 0.2% trypsin (Calbiochem), 10 mM EDTA in Hanks' solution and then were allowed to recover in complete medium at 37 °C for 30 min. Nuclear DNA was stained by incubating the cells overnight in a hypotonic fluorochrome solution (50 μM propidium iodide, 0.1% sodium citrate, 0.1% Triton X-100) at 4 °C. The cells were then analyzed on a FACScan (BD Pharmingen). Apoptotic bodies were identified by their reduced DNA content. Greater than one fragment can be derived from each apoptotic cell. Alternatively, cell viability was quantitated by trypan blue (Sigma) exclusion. After cytokine treatment, the islets were dispersed into single cells and then resuspended in 0.1% trypsin blue in phosphate-buffered saline. Viable cells, which are not stained by trypan blue, were counted on a hemocytometer.

Flow Cytometric Analysis of β Cells—Islets were dispersed into single cells and allowed to recover in complete medium at 37 °C for 30 min. The measurement of class 1 MHC expression by flow cytometry using an anti-H-2Dm monoclonal antibody (28-14-8, BD Pharmingen) is described elsewhere (16). Analyses were performed on a FACScan. The β cell population was identified by their high flavin adenine dinucleotide autofluorescence (30). Cell sorting was performed on a FACStar (BD Pharmingen).

Western Blot Analysis—For the detection of iNOS, 100 islets were lysed in protein sample buffer (4% SDS, 20% 2-mercaptoethanol, 0.25 M Tris-HCl, pH 6.6, bromphenol blue) and heated to 95 °C for 5 min. Half of the protein extract (~15 μg) was then subjected to SDS-PAGE, and iNOS protein was detected by Western blotting as described previously (9) using an anti-iNOS polyclonal antibody (NOS2, Santa Cruz Biotechnology Inc, Santa Cruz, CA) followed by horseradish peroxidase-conjugated mouse anti-rabbit immunoglobulin (Dako, Carpinteria, CA). iNOS protein was visualized with Lumi-Light (Roche Diagnostics). The blots were stripped with stripping buffer (2% SDS, 0.75% 2-mercaptoethanol, 50 mM Tris-HCl, pH 6.6) at 65 °C for 45 min. As a loading control, they were then reprobed with an anti-hsp 70 antibody (obtained from Dr. Hansa Puthalakakk, the Walter and Eliza Hall Institute of Medical Research).

For the analysis of JNK and p38 MAPK phosphorylation, whole cell lysate from 200 islets/sample were prepared as described by Larsen et al. (31). The protein extract (~30 μg) was divided and run separately on two SDS-polyacrylamide gels. One filter was stained with Coomassie blue and blotted with a rabbit polyclonal antibody specific for phospho-JNK or phospho-p38. As a loading control, the other filter was probed with a rabbit polyclonal antibody to detect total JNK or p38. All of the anti-MAPK antibodies were purchased from Cell Signaling Technology (Beverly, MA).

Nitrite Assays—NO production by islets was assayed by measuring the culture medium for nitrite using the Griess assay (32).

Caspase 3 Assays—Caspase 3 activity was measured according to the method of Silke et al. (33). 300 islets were lysed in 30 μl of lysis buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, protease inhibitors). The exoextract was added to 50 μl of 2X caspase buffer (0.2 mM HEPES, pH 7.5, 20% glycerol, 0.2% CHAPS, 5 μl of 1 mM DEVD-AMC (fluorogenic substrate) (Bachem, Bubendorf, Switzerland), and 1 μl of 1 mM dithiothreitol to a final volume of 100 μl. The fluorescence was monitored in a fluorometric instrument (TECAN) at 37 °C for 120 min with individual readings made every 5 min (excitation filter 360 nm; emission filter 465 nm). DEVD-AMC cleavage rates are determined by the change in fluorescence over time.

Statistical Analysis—Two-way analyses of variance were used for statistical comparisons between treatment groups and genotypes. The calculations were performed using Prism version 2.0a (GraphPad Software Inc., San Diego, CA).

RESULTS

SOCS-1 Deficiency Increases TNF + IFNγ-induced Islet Cytotoxicity—We have previously shown that SOCS-1 does not regulate the sensitivity of β cells to IFNγnor the kinetics of IFNγ signaling (16). Therefore, we were interested in investigating whether SOCS-1 regulates the sensitivity of β cells to other cytokines. This was done by studying the effects of SOCS-1 deficiency on cytokine-induced cell death of pancreatic islets. SOCS-1 deficiency is neonatal lethal in mice (34, 35). However, SOCS-1−/− mice that are also deficient in IFNγ remain healthy and survive to adulthood (27, 36), which allows for the isolation and analysis of SOCS-1−/− islets. Islets were isolated from SOCS-1−/−IFNγ−/− mice and from IFNγ−/− and wild-type mice as controls and were treated with 1000 units/ml TNF, 100 units/ml IFNγ, or both. After 6 days, cell death was quantitated in a flow cytometric analysis for nuclear fragmentation (Fig. 1 A and B). Neither TNF nor IFNγ alone induced cell death in SOCS-1−/− or wild-type islets. However, nuclear fragmentation was induced by a combination of both cytokines. This effect was more pronounced in SOCS-1−/− islets. Cell viability was also assessed by trypan blue exclusion (Fig. 1C). The viability of SOCS-1−/− islet cells following treatment with TNF and IFNγ was significantly reduced compared with wild type. These results suggest that SOCS-1 has a role in regulating the sensitivity of pancreatic islets to TNF and/or IFNγ-mediated cytotoxicity.

Increased TNF + IFNγ-induced Cell Death in SOCS-1−/− Islets Is the Result of Hypersensitivity to TNF—The relative contributions of IFNγ and TNF to the increased death of SOCS-1−/− islet cells were assessed by varying the concentrations of the two cytokines. When IFNγ was maintained at 100 units/ml and TNF was added at increasing concentrations, cell death in both SOCS-1−/− and control islets was induced in a dose-depended manner (Fig. 2 A). TNF at 300 units/ml or greater was required to induce nuclear fragmentation in control islets. However, in SOCS-1−/− islets, 30 units/ml of TNF was sufficient to induce nuclear fragmentation, suggesting that SOCS-1 deficiency increases the sensitivity of islets to TNF-mediated cytotoxicity. Furthermore, at all of the TNF concentrations, a higher level of cell death was induced in SOCS-1−/− islets compared with wild type. In the converse experiment when TNF was maintained at 1000 units/ml and IFNγ was gradually increased, cell death was again induced in a dose-dependent manner (Fig. 2B). SOCS-1−/− islets were not responsive to lower concentrations of IFNγ compared with wild type. Only at 50 units/ml or greater was IFNγ able to induce nuclear fragmentation in either SOCS-1−/− or wild-type islets. This result is consistent with our previous findings, which show that the sensitivity of islets to IFNγ alone measured by STAT1 activation and the up-regulation of class I MHC, is not affected by SOCS-1 deficiency (16).

The interpretation of these experiments remains arguable,
because at each increasing concentration of IFNγ with fixed TNF, there was more DNA fragmentation in SOCS-1−/− islets than in wild type. Therefore, we undertook additional experiments to clarify whether the increased cell death was because of hypersensitivity to TNF, IFNγ, or both. To confirm that this increased cell death was not the result of increased sensitivity to IFNγ, we examined the sensitivity of SOCS-1−/− islets to cell death induced by IFNγ in combination with another cytokine, IL-1 (Fig. 2C). IL-1 activates common signaling pathways (reviewed in Refs. 37 and 38) and has similar effects on islet cells as TNF (11, 39). 10 units/ml IL-1 in combination with 100 units/ml IFNγ induced the same level of nuclear fragmentation in SOCS-1−/− and wild-type islets. Neither 10 units/ml IL-1 alone nor 1 unit/ml in combination with IFNγ damaged SOCS-1−/− or wild-type islets. This finding suggests that SOCS-1 deficiency does not increase the sensitivity of islets to either IFNγ or IL-1-mediated cytotoxicity.

**SOCS-1-deficient β Cells Are More Sensitive to TNF-induced Class I MHC Expression**—To investigate whether TNF signaling in the absence of IFNγ as well as in combination is also
Regulated by SOCS-1, we examined the effect of SOCS-1 deficiency on TNF-stimulated class I MHC expression. Class I MHC is a well characterized TNF-inducible gene in β cells (40). The islets were treated with 0–2500 units/ml TNF for 48 h, and then the β-cell population was analyzed for class I MHC expression by flow cytometry. The class I MHC staining is expressed as the mean fluorescence intensity. The results represent the means ± S.D. of three experiments. Statistical significance: *, p < 0.05 versus wild type or IFN-γ−/−. B, the β-cell population from SOCS-1−/− IFN-γ−/− and IFN-γ−/− islets were analyzed for TNF receptor expression by flow cytometry.

To ensure that the increased sensitivity of SOCS-1−/− β cells to TNF in vitro was not because of prior responsiveness to cytokines in vivo, freshly isolated β cells (without culturing) were analyzed for basal class I MHC expression. Basal class I MHC levels on SOCS-1−/− IFN-γ−/− β cells were found to be the same as on the controls (data not shown). Moreover, we did not find any evidence for constitutive activation of IFN-γ (STAT1) and TNF (NFκB and MAPK) signaling molecules in SOCS-1−/− IFN-γ−/− islets (data not shown). These data suggest that the increased sensitivity of SOCS-1−/− β cells to TNF is unlikely to be a result of priming in vivo.

We have previously shown that β cells express TNFR1 at the mRNA and protein levels but have been unable to detect TNFR2 expression (9). Increased expression of one or both TNF receptors could be responsible for the increased responses of SOCS-1−/− β cells to the cytokine. However, SOCS-1−/− β cells were found to express the same levels of TNFR1 as controls, whereas TNFR2 expression was undetectable on both SOCS-1−/− and wild-type β cells (Fig. 3B).

SOCS-1 Regulates the Sensitivity of β Cells to Direct Damage by TNF. A, islets from SOCS-1−/− IFN-γ−/− and IFN-γ−/− mice were treated with 1000 units/ml TNF + 100 units/ml IFN-γ in the presence or absence of IRAP. After 6 days, nuclear fragmentation was measured by flow cytometry. Islets treated with 10 units/ml IL-1 + 100 units/ml IFN-γ in the presence of IRAP were used a control for IRAP activity. The results represent the means ± S.D. of three experiments. B, FACS-purified β cells from SOCS-1−/− IFN-γ−/− and IFN-γ−/− islets were treated with 1000 units/ml TNF + 100 units/ml IFN-γ for 6 days, and then cell death was analyzed by measuring nuclear fragmentation. The means ± S.D. of two experiments are shown. Statistical significance: *, p < 0.001 versus untreated and IFN-γ−/−.
Enhanced TNF-induced cell death in SOCS-1−/− islets is a result of increased iNOS expression and NO production. A, islets from SOCS-1−/−, IFNγ−/−, IFNγ−/−, and wild-type mice were treated with 1000 units/ml TNF + 100 units/ml IFNγ in the presence of the caspase inhibitor zVAD-fmk or the iNOS inhibitor aminoguanidine (AG). After 6 days, cell death was analyzed by measuring nuclear fragmentation. The results represent the means ± S.D. of four experiments. B, measurement of the intrinsic sensitivity of islets to NO-mediated damage. Islets were treated with 0–0.8 mM sodium nitroprusside, a NO donor compound. After 16 h, nuclear fragmentation was measured. The means ± S.D. of three experiments are shown. C, islets were treated with 1000 units/ml TNF + 100 units/ml IFNγ for 48 h and then analyzed for iNOS expression by Western blotting. D, islets were treated with 1000 units/ml TNF + 100 units/ml IFNγ for 4 days, and nitrite released into culture supernatants was measured by Griess assay. The values are the means ± S.D. of four experiments. Statistical significance: *p < 0.005 versus wild type and IFNγ−/−. E, FACS-purified β cells from SOCS-1−/−, IFNγ−/−, and IFNγ−/− islets were treated with 1000 units/ml TNF + 100 units/ml IFNγ for 48 h and then analyzed for iNOS expression by Western blotting.

Not affected by SOCS-1 deficiency—The increased TNF-induced cell death in SOCS-1−/− islets may be attributed to increased sensitivity to NO and/or increased iNOS expression. To investigate whether the intrinsic sensitivity of islets to NO is affected by SOCS-1 deficiency, cell death was induced with the NO donor compound, sodium nitroprusside (Fig. 5B). Sodium nitroprusside was found to be cytotoxic to all islets at a threshold concentration of 0.4 mM. This finding suggests that the intrinsic sensitivity of islets to NO is not regulated by SOCS-1.

TNF-induced iNOS expression and NO production is elevated in SOCS-1−/− islets. To further investigate the role of iNOS in the cytotoxicity of SOCS-1−/− islets, iNOS protein expression was analyzed by Western blotting (Fig. 5C). SOCS-1−/− islets were found to express higher levels of iNOS compared with wild-type islets following treatment with TNF + IFNγ. Therefore, the increased TNF-induced cell death in SOCS-1−/− islets may be a result of increased iNOS expression. Consistent with this observation, higher levels of nitrite, the degradation product of NO, were found in the cultures of SOCS-1−/− islets compared with control islets following TNF + IFNγ treatment (Fig. 5D). We also examined IL-1 + IFNγ-induced iNOS expression. SOCS-1−/− and wild-type islets were found to express similar levels of iNOS following cytokine treatment (data not shown).

The lack of protection from TNF + IFNγ-induced cytotoxicity seen when IRAP is present (Fig. 4A) suggests that iNOS expression may be directly induced in SOCS-1−/− β cells by TNF.
SOCS-1 Regulates the Sensitivity of β Cells to TNF

**Fig. 6. SOCS-1 deficiency augments TNF signaling via the p38 MAPK pathway.** SOCS-1−/− IFNγ−/− and SOCS-1−/− islets were stimulated with 1000 units/ml TNF for 1 h, and protein lysates were analyzed for JNK and p38 MAPK activation by Western blotting. Duplicate blots were probed with a polyclonal antibody specific for phospho-p38 or, as a loading control, total p38. Alternatively, blots were probed for phospho-JNK or total JNK. One of two experiments is shown.

+ IFNγ. To verify this observation, iNOS expression was analyzed in FACS-purified β cells by Western blotting (Fig. 5E). SOCS-1−/− but not control β cells expressed iNOS following cytokine treatment, thus confirming that TNF is capable of acting directly on SOCS-1−/− β cells to induce iNOS expression.

**TNF Signaling via the p38 MAPK Pathway Is Dysregulated in SOCS-1−/− Islets**—The increased activation of a known TNF signaling pathway may be responsible for the increased iNOS expression in SOCS-1−/− islets. Alternatively, SOCS-1 deficiency may result in the aberrant activation of a pathway not normally activated by TNF. The NFκB, JNK MAPK, and p38 MAPK pathways are activated by TNF (41) and are involved in up-regulating iNOS expression in β cells (10, 31, 39, 42, 43). Therefore, we investigated whether these pathways are affected by SOCS-1 deficiency. The TNF activation of NFκB measured by gel shift was unaffected by SOCS-1 deficiency (data not shown). However, we found that MAPK signaling was augmented in SOCS-1−/− islets (Fig. 6). More specifically, increased phosphorylation of p38 but not JNK MAPK was detected in SOCS-1−/− islets upon treatment with TNF alone. The increased iNOS expression and NO production in TNF + IFNγ-treated SOCS-1−/− islets therefore may be due to dysregulated TNF signaling via the p38 MAPK pathway.

iNOS expression in β cells also requires the activation of STAT1 (10, 11, 39, 42). TNF signaling does not normally involve the Jak-STAT pathway, but this pathway might be aberrantly activated in SOCS-1−/− islets. SOCS-1 is a recognized negative regulator of the Jak-STAT pathway (17–19). However, we were unable to find any evidence for TNF activation of STAT1 in SOCS-1−/− islets (data not shown).

**Increased TNF-mediated Caspase Activation in the Presence of Cycloheximide in SOCS-1−/− Islets**—Caspase activation did not contribute to the islet cell death induced by TNF + IFNγ (Fig. 5A). However, we found that TNF could induce cell death in islets via the caspase pathway in the presence of CHX, because this cell death was inhibited by the caspase inhibitor zVAD-fmk (Fig. 7A). Furthermore, TNF + CHX was more potent at inducing cell death in SOCS-1−/− islets than in control islets. This appeared to be associated with increased caspase 3 activation by TNF in SOCS-1−/− islets (Fig. 7B).

**DISCUSSION**

Pancreatic β cells from SOCS-1−/− IFNγ−/− mice display increased cell death in response to the combination of TNF + IFNγ. The titration of the concentration of TNF and IFNγ suggested that the hypersensitivity to TNF is probably responsible for the increased cell death in SOCS-1−/− islets. Moreover, there was no difference in the cell death induced by IFNγ + IL-1. SOCS-1−/− islets were also more sensitive to TNF-induced cell death in the presence of cycloheximide. This hypersensitivity to TNF was supported by an increased induction of class I MHC expression by TNF on its own. These data indicate that physiological levels of SOCS-1 expression partly block TNF effects on β cells and suggest that SOCS-1 is one of a number of mechanisms that protect cells from the harmful effects of TNF. It is conceivable that increased sensitivity to TNF may contribute to the lethal inflammatory syndrome in SOCS-1−/− mice that are also known to have increased circu-
lating TNF levels (35). We have previously found that IFNγ signaling is unchanged in SOCS-1−/− β cells (16), even though IFNγ deficiency protects SOCS-1−/− mice from neonatal lethality (27, 36). We showed that the levels of SOCS-1 reached in primary β cells after IFNγ treatment are lower than those found in SOCS-1-transfected β-cell lines in which IFNγ signaling is blocked. Low levels of SOCS-1 were found in β cells treated with TNF; however, these appear sufficient to reduce TNF-induced class I MHC expression. The levels of SOCS-1 expression and effects on cytokine signaling may vary in other cell types, for example, in hepatocytes in which IFNγ signaling is prolonged with SOCS-1 deficiency (20).

IL-1 has overlapping effects and signaling pathways to TNF (37–39, 42), and some effects of TNF on β cells are mediated by IL-1 (5, 13). However, there was no evidence in this study that SOCS-1−/− β cells were more sensitive to IL-1. Increased gene expression and cell death in SOCS-1−/− β cells were only seen with TNF or TNF in combination with IFNγ. Both IL-1 and TNF on their own induced class I expression on β cells, and in combination with IFNγ, both induced iNOS-mediated cell death. Although increased class I MHC expression mediated by TNF occurs in β cells deficient in the IL-1 receptor,2 the death of wild-type β cells mediated by TNF + IFNγ was dependent on intra-islet production of IL-1, most probably from resident macrophages. TNF + IFNγ-induced death of SOCS-1−/− islets while mediated by iNOS was not dependent on IL-1, because it was only partially blocked by the presence of IRAP. Additionally, TNF + IFNγ was toxic to FACS-purified β cells from SOCS-1−/− islets, whereas, as expected, purified β cells from wild-type islets were unaffected because IL-1-producing cells such as macrophages were absent. Therefore, SOCS-1−/− β cells themselves in the absence of other cell types appear to be more sensitive to TNF. The absence of SOCS-1 allows TNF + IFNγ to induce iNOS in β cells directly as IL-1 + IFNγ do in normal β cells. TNF alone did not induce iNOS expression or cell death even in SOCS-1−/− β cells, indicating that pathways stimulated by IFNγ are still necessary for iNOS induction in SOCS-1−/− β cells.

The loss of SOCS-1 increases the sensitivity of β cells to TNF. In contrast, we have previously found that the overexpression of SOCS-1 does not block the responsiveness of β cell lines to TNF (16). NIT-1 insulinoma cells, which overexpress SOCS-1, were protected from IFNγ but not from TNF-mediated cytotoxicity. This result clearly needs to be confirmed in primary islets that overexpress SOCS-1, particularly because NIT-1 cells respond to TNF differently from primary islets (9).

Whereas SOCS-1 does not appear to have a role in regulating β cell sensitivity to IL-1, SOCS-3, a related protein, may have a role. There is a recent report indicating that the overexpression of SOCS-3 can block the IL-1 induction of iNOS and cytotoxicity in rat insulinoma cells (44). However, because this is an overexpression study, it is not known whether physiological levels of SOCS-3 are involved in the negative regulation of IL-1 signaling. It has not yet been possible to study SOCS-3−/− islets to address this issue, because SOCS-3 deficiency is embryonic lethal resulting from placental abnormalities (45). Tissue-specific deletion approaches will be required to investigate the effect of SOCS-3 deficiency on pancreatic islets.

We have examined a number of possible mechanisms for increased β cell responses to TNF. We did not find evidence of previous exposure to cytokines in vivo, because class I MHC levels on freshly isolated β cells from SOCS-1−/− IFNγ−/− mice were the same as from control mice, and we were unable to detect the constitutive activation of either IFNγ or TNF signaling pathways. Furthermore, unlike in mice deficient in SOCS-1 alone, circulating TNF levels are not increased in SOCS-1−/− IFNγ−/− mice.3 Therefore, we investigated whether intracellular signaling pathways are affected by SOCS-1 deficiency. TNF signaling via the p38 MAPK pathway but not JNK MAPK or NFκB pathways was found to be increased in SOCS-1−/− islets. MAPK signaling previously has been shown to be important in IL-1 induction of iNOS (31, 43). Therefore, the increased TNF-induced iNOS expression and NO-dependent cell death in SOCS-1−/− islets are probably the result of increased p38 MAPK signaling. Hypersensitivity to TNF has also been reported in SOCS-1−/− murine embryonic fibroblasts in the presence of cycloheximide (46). However, unlike in SOCS-1−/− β cells, this was associated with decreased p38 MAPK activation. The authors postulated that the loss of p38 MAPK activity leads to a loss of cell survival signals. It is unclear why the loss of SOCS-1 in two different cell types has opposing effects on the same pathway and yet results in increased TNF sensitivity in both cell types.

SOCS-1 was discovered as a factor capable of blocking IL-6 effects (17–19), and it has subsequently been shown especially in overexpression studies to inhibit many cytokines that use the Jak-STAT pathway. This study adds to others that show that its inhibitory effects extend beyond cytokines that directly use this pathway. A variety of mechanisms probably mediate β-cell destruction in human diabetes. To overcome this, it will be desirable to find molecules that can block several pathways which lead to β cell death, and SOCS-1 is a candidate for such a role.

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