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Suppressor of Cytokine Signaling-1 Overexpression Protects Pancreatic β Cells from CD8⁺ T Cell-Mediated Autoimmune Destruction

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In type 1 diabetes, cytokine action on β cells potentially contributes to β cell destruction by direct cytotoxicity, inducing Fas expression, and up-regulating class I MHC and chemokine expression to increase immune recognition. To simultaneously block β cell responsiveness to multiple cytokines, we overexpressed suppressor of cytokine signaling-1 (SOCS-1). This completely prevented progression to diabetes in CD8⁺ TCR transgenic nonobese diabetic (NOD) 8.3 mice without affecting pancreas infiltration and partially prevented diabetes in nontransgenic NOD mice. SOCS-1 appeared to protect at least in part by inhibiting TNF- and IFN-γ-induced Fas expression on β cells. Fas expression was up-regulated on β cells in vivo in prediabetic NOD8.3 mice, and this was inhibited by SOCS-1. Additionally, IFN-γ-induced class I MHC up-regulation and TNF- and IFN-γ-induced IL-15 expression by β cells were inhibited by SOCS-1, which correlated with suppressed 8.3 T cell proliferation in vitro. Despite this, 8.3 T cell priming in vivo appeared unaffected. Therefore, blocking β cell responses to cytokines impairs recognition by CD8⁺ T cells and blocks multiple mechanisms of β cell destruction, but does not prevent T cell priming and recruitment to the islets. Our findings suggest that increasing SOCS-1 expression may be useful as a strategy to block CD8⁺ T cell-mediated type 1 diabetes as well as to more generally prevent cytokine-dependent tissue destruction in inflammatory diseases. The Journal of Immunology, 2004, 172: 5714–5721.

Cytokines acting on β cells may contribute to the pathogenesis of type 1 diabetes in several ways (reviewed in Ref. 1). Many studies have demonstrated the cytotoxicity of the proinflammatory cytokines IFN-γ, TNF, and IL-1 for pancreatic islets in vitro. These cytokines are secreted by activated T cells and macrophages that infiltrate the islets (referred to as the insulitis lesion) during the autoimmune reaction (2). In vitro, combinations of IFN-γ, TNF, and IL-1 induce islet expression of inducible NO synthase (iNOS) (3–6). It is the production of NO by iNOS that is responsible for most of the β cell cytotoxicity. In addition to direct cytotoxicity, cytokines stimulate Fas expression on β cells. The expression of Fas is undetectable on β cells, but is up-regulated upon exposure to combinations of IL-1, TNF, and IFN-γ in vitro (7, 8). Fas expression is also up-regulated on β cells in vivo in some accelerated models of autoimmune diabetes (9).

Cytokines may also enhance recognition of β cells by the immune system. Proinflammatory cytokines, particularly IFN-γ, up-regulate class I MHC expression on β cells (10, 11) and may be important for CD8⁺ T cell recognition of β cells. Transgenic expression of the adenosine E3 region in β cells decreases diabetes in nonobese diabetic (NOD) mice (12). This may be due to the inhibition of class I MHC expression and Ag presentation, although genes in this region also inhibit TNF and Fas action. More recently, cytokines have been shown to induce chemokine expression by β cells that may participate in the development of insulitis. In particular, CXC ligands 9 and 10 are produced by β cells in response to cytokines and attract T cells to islets via the CXCR3 (13). Moreover, CXCR3 deficiency delays diabetes in the lymphocytic choriomeningitis virus-induced model of diabetes. Another molecule produced by β cells in response to proinflammatory cytokines is the γ common cytokine IL-15 (14), a key growth and differentiation factor for T cells in the peripheral immune system.

Despite the numerous effects of cytokines on β cells, there has been surprisingly little effect of deficiency of individual cytokines, such as IFN-γ, on diabetes in NOD mice. Genetic deficiency of IFN-γ or its receptor or overexpression of a dominant negative IFN-γ receptor on β cells appears to have little effect on spontaneously diabetes (10, 15–17). However, IFN-γ-deficient NOD mice are resistant to diabetes after the transfer of splenocytes from spontaneously diabetic NOD donors (18). Additionally, IFN-γ action on β cells appears to be essential in the lymphocytic choriomeningitis virus model (19).

One potential reason for the minimal effect of IFN-γ deficiency on diabetes in NOD mice is redundancy of effects between IFN-γ and closely related (such as IFN-α) or unrelated (such as IL-1 or TNF) cytokines, and also the existence of cytokine-independent mechanisms of β cell killing, such as perforin. For this reason we have investigated ways of blocking several cytokine pathways.
with a single inhibitor. Overexpression of suppressor of cytokine signaling-1 (SOCS-1) inhibits IFN-γ signaling in β cell lines (11), and SOCS-1 deficiency causes β cell hypersensitivity to TNF (5). Studies in other cell types have shown that SOCS-1 overexpression also inhibits responses to many other cytokines that signal via the Janus kinase (JAK)-STAT pathway (reviewed in Ref. 20), including other IFNs (21). Therefore, SOCS-1 overexpression may be a useful strategy for rendering β cells unresponsive to multiple cytokines. The present study investigates the ability of this approach to prevent CD8+ T cell-mediated autoimmune diabetes in TCR transgenic NOD mice.

Materials and Methods

Generation of transgenic mice

The rat insulin promoter (RIP)-SOCS-1 transgene was constructed by subcloning the SOCS-1 cDNA from the REP9/SOCS-1 expression plasmid (11) into the ClaI restriction site of the RIP7 expression vector. NOD embryos were microinjected with the transgene, then transferred in pseudopregnant (CBA × C57BL/6j)F1 fosters. Offspring were genotyped by Southern blot analysis of genomic DNA purified from tail biopsies, using RIP as a probe. Subsequent genotyping was performed by PCR for the RIP-SOCS-1 transgene, using the primers 5'-GGCAAGTGTGTTGAAACTGCA-3' and 5'-CTCAAGGGGGTCCCCAATAAAGAG-3'. NOD.8 mice expressing the TCRαβ rearrangements of the H-2Kk-restricted, β cell-reactive, CD8+ T cell clone NY8.3 have been previously described (22).

Isolation of mouse islets

Islets were isolated according to the method of Liu and Shapiro (23) by intraductal digestion of the pancreas with collagenase P (Roche, Basel, Switzerland) and separation on a Histopaque (Sigma-Aldrich, St. Louis, Missouri) density gradient. Islets were handpicked to purity and cultured in CMRL-1066 (Life Technologies, Gaithersburg, MD), 10 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS.

Adaptive transfer of diabetogenic splenocytes and monitoring of diabetes

Splenocytes from diabetic female NOD mouse donors were prepared as single-cell suspensions. RBC were removed by lysis. Splenocytes (2 × 10^6/mouse) were injected i.v. into 6- to 7-wk-old male recipient mice irradiated at 900 rad.

Mice were monitored for diabetes by measurement of urinary glucose levels. Monitoring was performed by(^2)Easytest (Bayer Diagnostics, Bridgend, U.K.). Those suspected of hyperglycemia were confirmed by measurement of blood glucose levels using Advantage II Glucose Strips (Roche). Mice with blood glucose >18 mM or with two readings of 13–18 mM on consecutive days were considered diabetic.

Real-time RT-PCR analyses

Total RNA was purified from pancreatic islets using RNA-Bee (Tel-Test, Friendswood, TX) and treated with DNaseRQ1 (Promega, Madison, WI). First-strand cDNA synthesis from 500 ng of total RNA was performed in a 20-μl reaction using Moloney leukemia virus reverse transcriptase (Promega). cDNAs of interest were analyzed by real-time PCR on the LightCycler apparatus (Roche). PCR products were quantified based on SYBR Green 1 fluorescence using the LightCycler Data Analysis program (version 3.5.28; Idaho Technology, Idaho Falls, ID). The primers 5'-ATGTTAGCAAGGCGACCCAG-3' and 5'-AAAGAGGAGGAGGAGGAGG-3' amplified a 91-bp SOCS-1 product, 5'-CATCCATCTCTGTTGACTTGTGGTT-3' and 5'-CATCTTACCATGGCTTCGTTT-3' amplified a 126-bp IL-15 product, 5'-TGGGAATGGTCCAGGAC-3' and 5'-CCCCAGTGGTGAACAATGCGCA-3' amplified a 115-bp β-actin product, and 5'-CCACATCGAAGGAGGGATT-3' and 5'-CCAGCTCCAGTGCTTGGCACCATTGTG-3' amplified a 250-bp proinsulin product. The primers for IL-15 and proinsulin span introns and therefore only detect cDNAs. PCRs were performed for 50 cycles, with fluorescence acquisitions made after every cycle. After the 50 cycles, melting curves were measured between 64 and 95°C to determine PCR product purity.

Flow cytometry

Islets were dispersed into single cells with 0.2% trypsin (Calbiochem, La Jolla, CA) and 10 mM EDTA in HBSS, then allowed to recover in CMRL-1066 and 10% FCS at 37°C for 30 min. Lymph nodes were also prepared as single-cell suspensions. Staining was performed using mAbs recognizing the following Ags (clone names in parentheses): CD4 (H129.19), CD8 (53-6.7), CD25 (7D4), CD44 (IM7), CD45 (30-F11), CD69 (H1.2F3), class I MHC/H-2D<sup>+</sup> (28-14-8), and Fas (Jo2). Abs were either biotinylated or directly conjugated to FITC, PE, PerCP/Cy5.5, or allophycocyanin. All Abs were purchased from BD PharMingen (San Diego, CA). Analyses were performed on a FACScan (three color) or LSR (four color; BD Biosciences, Mountain View, CA).

Histology and immunohistochemistry

Pancreata were fixed in Bouin’s fixative for 6 h, then paraffin-embedded. Sections were prepared by standard techniques and stained with H&E. The detection of islet cell hormones was performed by immunohistochemistry. Sections were stained with a guinea pig anti-insulin polyclonal Ab (A0564), rabbit anti-glucagon polyclonal Ab (A0565), or rabbit anti-somatostatin polyclonal Ab (A0566), followed by an appropriate HRP-conjugated secondary Ab (all Abs were purchased from DAKO, Santa Barbara, CA). Stains were developed with 3,3′-diaminobenzidine tetrohydrochloride (Sigma-Aldrich) and were counterstained with hematoxylin.

5-Bromo-2′-deoxyuridine (BrdU) incorporation assay

Mice were initially injected i.p. with 200 μl of 10 μg/ml BrdU, then continuously given BrdU (1 mg/ml) in their drinking water. Two days later, islets and lymph nodes were harvested, and single-cell suspensions were prepared. The cells were fixed in 0.5% paraformaldehyde in PBS for 30 min at room temperature. They were then washed with PBS (twice) and resuspended in 3 M HCl/0.5% Tween 20 for 20 min at room temperature to denature nuclear DNA. After thorough washing with HBSS, the cells were stained FITC-conjugated anti-BrdU mAb (BD Biosciences) for 30 min on ice. Abs recognizing cell surface markers were also added at this stage. The cells were washed with PBS (twice), then resuspended in PBS 2% FCS for analysis.

In vitro stimulation of T cells with islets

Pancreatic islets were isolated 1 day before setting up the T cell cultures and were cultured overnight in CMRL-1066 and 10% FCS. The islets were dispersed into single-cell suspension with 0.2% trypsin and 10 mM EDTA and were allowed to recover in CMRL-1066 and 10% FCS for 30 min, then irradiated at 3000 rad. T cells were purified from spleen and lymph nodes of mice using nylon wool, then cultured with the irradiated islet cells in 96-well, round-bottom plates. The cells were pulsed with 2.5 μCi of [3H]thymidine for the last 24 h of culture.

IFN-γ was measured in supernatants of the T cell/islet cultures using a standard sandwich ELISA. The purified mAb R4-62A and the biotinylated mAb XMG1.2 (BD PharMingen) were used as the capture and detection Abs, respectively.

Results

RIP-SOCS-1 transgene drives high level SOCS-1 expression within pancreatic islets

To investigate the ability of SOCS-1 overexpression to protect pancreatic β cells from autoimmune damage, the RIP was used to drive constitutive SOCS-1 expression in the β cells of NOD mice. Nine transgenic founder mice were obtained, of which four were expanded for functional analyses. The islets from each of these lines (no. 24, 32, 45, and 71) were analyzed for SOCS-1 mRNA expression by real-time RT-PCR, and all were found to express high levels of SOCS-1 mRNA (Fig. 1A).

SOCS-1 overexpression inhibits β cell responsiveness to IFNs

Pancreatic β cells are highly autofluorescent due to flavin adenine dinucleotide accumulation compared with other islet cells (24). We have previously shown that the high autofluorescence islet cell population is indeed highly enriched for insulin-producing β cells, whereas all other islet cells fall within the low autofluorescence population (9). Separating islet cells based on autofluorescence allows for quantitative analysis of pure β cells by flow cytometry.

SOCS-1 overexpression inhibits IFN-γ responses, such as class I MHC up-regulation (11). Hence, functional SOCS-1 expression in RIP-SOCS-1 islets was examined by analyzing the responsiveness of islet cells to IFN-γ-induced class I MHC up-regulation. In
islet cells. Lines 24 and 32 were selected for further studies. Furthermore, IFN-γ-induced STAT1 activation was inhibited (not shown). This suggests that the RIP-SOCS-1 transgene drives functional SOCS-1 expression specifically within β cells.

A

FIGURE 1. The RIP-SOCS-1 transgene drives high level functional SOCS-1 expression specifically within β cells. A, The islets from four independent lines of RIP-SOCS-1 transgenic mice at 30 days of age were analyzed for SOCS-1 mRNA expression by real-time RT-PCR. mRNA levels are expressed relative to the positive control (nontransgenic islets treated with 100 U/ml IFN-γ for 4 h), normalized against β-actin expression. For each transgenic line, the data represent the mean ± SD of two individual mice. The numbers above each bar indicate the approximate number of transgene copies integrated into each line. Transgene copy number was determined by real-time PCR of genomic DNA using the endogenous Socs1 gene (two copies) as an internal reference. B, RIP-SOCS-1 inhibits IFN-γ-induced class I MHC up-regulation specifically in β cells. Purified islets from 35-day-old mice were treated with 100 U/ml IFN-γ for 48 h, then class I MHC expression was measured by flow cytometry. Shown are islets from RIP-SOCS-1 line 24. β cells were identified based on their high flavin adenine dinucleotide autofluorescence compared with other islet cells.

three of the four transgenic lines analyzed (no. 24, 32, and 71), 90–95% of β cells did not up-regulate class I MHC in response to 100 U/ml IFN-γ, whereas all other islets cells were responsive, as measured by flow cytometry (Fig. 1B, data from line 24 are shown). RIP-SOCS-1 β cells were unresponsive to IFN-γ even at the extremely high concentration of 2500 U/ml (data not shown). Furthermore, IFN-γ-induced STAT1 activation was inhibited (not shown). This suggests that the RIP-SOCS-1 transgene drives functional SOCS-1 expression specifically within β cells and not other islet cells. Lines 24 and 32 were selected for further studies.

SOCS-1 overexpression in β cells prevents progression to diabetes in CD8+ TCR transgenic NOD8.3 mice

We next examined the ability of β cell-specific SOCS-1 overexpression to prevent diabetes. TCR transgenic NOD mice are simplified forms of the NOD model because more limited sets of mechanisms may be responsible for diabetes. This therefore allows for the investigation of strategies to inhibit specific mechanisms of β cell destruction. NOD8.3 mice transgenically express a Vβ8 TCR from the diabetogenic CD8+ T cell clone NY8.3 (22). This clone was originally isolated from the pancreas of a diabetic NOD mouse and uses a TCRα rearrangement frequently expressed by islet-infiltrating CD8+ T cells in NOD mice (25). The autoantigen recognized by 8.3 T cells has recently been identified as the islet-specific glucose-6-phosphatase catalytic subunit-related protein (26). NOD8.3 mice were crossed with RIP-SOCS-1 transgenic mice, and the incidence of diabetes was followed in the progeny. As previously described (22, 27), NOD8.3 mice developed hyperglycemia from 44 days of age, and by 160 days, 95% of mice had become diabetic (Fig. 2A). In contrast, all NOD8.3 mice that were also transgenic for RIP-SOCS-1 were still normoglycemic at 160 days of age. And even after 250 days, no RIP-SOCS-1/NOD8.3 mouse had become diabetic (data not shown).

RIP-SOCS-1/NOD8.3 mice develop massive insulitis

Histological examination of pancreas sections from NOD8.3 mice at 60 days of age revealed, as expected, the presence of destructive insulitis with the loss of islet cells (Fig. 2, C and H). In RIP-SOCS-1/NOD8.3 mice of the same age, there was still the accumulation of infiltrating cells around islets, but there was no significant loss of islet architecture or cell mass (Fig. 2, E and H). At 130 days of age, the insulitis in RIP-SOCS-1/NOD8.3 mice had expanded considerably to appear as large nodular structures surrounding islet cells (Fig. 2, F–H). The architecture of islets was severely disrupted, but there was still the presence of substantial islet cell mass. This was confirmed by immunohistochemical staining for insulin, glucagon, and somatostatin (Fig. 2, I–K). It appeared as though the islets cells had been dispersed as infiltrating cells accumulated, but were protected from destruction.

SOCS-1 overexpression prevents the induction of Fas expression on β cells in NOD8.3 mice and in response to TNF and IFN-γ

β cell destruction in NOD8.3 mice is thought to be dependent on Fas, because 8.3 T cells are unable to kill Fas-deficient NODllpr islets in vitro (27). Islet-infiltrating CD8+ T cells in NOD8.3 mice have been shown to express IL-2, IL-10, TNF, and IFN-γ (22). Therefore, the TNF and IFN-γ produced by infiltrating 8.3 T cells may act on β cells to up-regulate Fas expression. The engagement of Fas on β cells by FasL on 8.3 T cells may then induce cell death.

The inhibition of cytokine-induced Fas expression on β cells could be the mechanism by which SOCS-1 overexpression prevents diabetes. Therefore, β cells were analyzed for the expression of Fas during insulitis. We have previously shown that β cells within infiltrated islets can be analyzed by flow cytometry by first gating out infiltrating CD45+ hemopoietic cells and then identifying the high flavin adenine dinucleotide autofluorescence population (9). We found that Fas expression was induced on the β cells of prediabetic NOD8.3 mice at 42 days of age, but not on the β cells of littermate RIP-SOCS-1/NOD8.3 mice (Fig. 3A).

The ability of SOCS-1 overexpression to inhibit cytokine-induced Fas expression on β cells was then confirmed in vitro. Purified islets were cultured with TNF or TNF/IFN-γ, and the β cell population was analyzed for Fas expression (Fig. 3C). TNF up-regulated Fas on nontransgenic β cells, and this was enhanced with the addition of IFN-γ. However, TNF- and TNF/IFN-γ-induced Fas expression were completely inhibited by SOCS-1 overexpression. IFN-γ alone did not induce Fas expression on β cells of either genotype (data not shown).

RIP-SOCS-1 β cells have a reduced ability to stimulate 8.3 T cell proliferation and IFN-γ production

It is possible that SOCS-1 protects β cells through other mechanisms in addition to inhibiting Fas expression. Class I MHC expression was also up-regulated on β cells from prediabetic
NOD8.3 mice, but was prevented in mice that were also RIP-SOCS-1 transgenic (Fig. 3B). Blocking class I MHC up-regulation could affect 8.3 T cell-recognition of /H9252 cells. To investigate whether the lack of class I MHC up-regulation on RIP-SOCS-1 /H9252 cells could affect recognition, CD8 /H11001 T cells were isolated from the

FIGURE 2. Progression to diabetes in CD8 TCR transgenic NOD8.3 mice is prevented by SOCS-1 overexpression in β cells. A, Incidence of diabetes in NOD8.3 transgenic mice and RIP-SOCS-1/NOD8.3 double-transgenic mice. The pooled data from RIP-SOCS-1 lines 24 and 32 are shown. Shown in B–G are the typical insulitis histopathologies that develop in NOD8.3 and RIP-SOCS-1/NOD8.3 mice. B, NOD8.3 mice at 25 days of age. C, Destructive insulitis with the loss of islet tissue in NOD8.3 mice at 60 days of age. D, RIP-SOCS-1/NOD8.3 mice at 25 days of age. E, RIP-SOCS-1/NOD8.3 mice at 60 days of age develop only peri-islet infiltration. F and G, A massive florid infiltrate is evident in RIP-SOCS-1/NOD8.3 mice at 130 days of age. Note the loss of normal islet architecture without significant loss of islet tissue mass. Magnification: B–F, ×200; G, ×40. H, Insulitis scores at 30, 70, and 110 days of age: 0 = no infiltrate, 1 = peri-islet infiltrate, 2 = intraislet infiltrate, and 3 = massive florid infiltrate. Twenty islets from each mouse were scored. Insulin (I), glucagon (J), and somatostatin (K) stainings (brown) of pancreas sections from a RIP-SOCS-1/NOD8.3 mouse at 130 days of age are shown. Magnification: I–K, ×100.

FIGURE 3. Transgenic SOCS-1 overexpression prevents the up-regulation of Fas and class I MHC expression on β cells in NOD8.3 mice. The islets of prediabetic NOD8.3 and age-matched RIP-SOCS-1/NOD8.3 mice were analyzed for Fas (A) and class I MHC (B) expression by flow cytometry. Shown are only β cells (high autofluorescence) and other islet cells (low autofluorescence), with infiltrating CD45 hemopoietic cells excluded. The data from one of four pairs of mice are shown. C, SOCS-1 overexpression inhibits TNF-induced and TNF- plus IFN-γ-induced Fas expression on β cells. Islets from 40-day-old mice were treated with 1000 U/ml TNF with or without 100 U/ml IFN-γ for 24 h. The β cells (gated on high autofluorescence) were then analyzed for Fas expression by flow cytometry.
islets of prediabetic NOD8.3 mice and age-matched RIP-SOCS-1/NOD8.3 mice to assess activation in vivo. Perhaps surprisingly, no difference was observed in the frequency of CD44^{high}, CD25^{+}, CD69^{+}, or proliferating (measured by BrdU incorporation) CD8^{+} T cells in the islets between NOD8.3 and RIP-SOCS-1/NOD8.3 mice (Fig. 4). However, T cell activation was already evident in the draining pancreatic lymph nodes of both mice, with similar frequencies of CD44^{high}, CD69^{+}, and BrdU^{+} CD8^{+} T cells found (Fig. 4). There was also no difference in the frequency of IFN-γ-expressing CD8^{+} T cells, as measured by intracellular cytokine staining (not shown). Therefore, the activated phenotype of islet CD8^{+} T cells may at least in part be a reflection of priming in the draining lymph node.

To address the direct interaction between β and T cells, the ability of purified RIP-SOCS-1 islets to stimulate purified 8.3 T cells was examined in vitro. The proliferation of 8.3 T cells stimulated by RIP-SOCS-1 islets was substantially reduced compared with that of cells stimulated with nontransgenic islets (Fig. 5A). Additionally, the amount of IFN-γ produced by 8.3 T cells stimulated with RIP-SOCS-1 islets was diminished compared with that of cells stimulated with nontransgenic islets (Fig. 5B). Therefore, SOCS-1 overexpression, through inhibiting class I MHC up-regulation, also impedes the recognition of β cells by 8.3 T cells, which could contribute to the protection from diabetes.

**SOCS-1 overexpression suppresses islet expression of IL-15 in response to TNF and IFN-γ**

Microarray studies have shown that in response to IL-1 and IFN-γ, islets express genes that may be important in T cell activation and homing, including chemokines and the cytokine IL-15 (14). The TNF and IFN-γ produced by 8.3 T cells may also induce IL-15 expression in islets. To examine this, purified islets were mixed with T cells from diabetic NOD8.3 mice. After 48 h, the T cell/islet cultures were analyzed for IL-15 mRNA expression by real-time RT-PCR (Fig. 5C). Because IL-15 is not expressed in lymphoid cells (28), the contribution of T cell mRNA was excluded by normalizing IL-15 expression against insulin. IL-15 was up-regulated by 20- to 25-fold in nontransgenic islet cells after incubation with 8.3 T cells. However, this was substantially reduced in RIP-SOCS-1 islet cells.

The reduced IFN-γ production in 8.3 T cell/RIP-SOCS-1 islet cultures could alone account for the diminished IL-15 expression. To directly address whether SOCS-1 overexpression suppresses proinflammatory cytokine-induced IL-15 expression, purified islets were treated with TNF and IFN-γ in vitro, then IL-15 mRNA expression was measured (Fig. 5D). IL-15 mRNA expression was induced by 15- to 20-fold in nontransgenic islets, but only by 2-fold in RIP-SOCS-1 islets.

**β cell up-regulation of Fas and class I MHC expression in mice adoptively transferred with diabetogenic splenocytes is also prevented by SOCS-1 overexpression**

We have found that both Fas and class I MHC expression are up-regulated on β cells in mice adoptively transferred with splenocytes from diabetic NOD donors (9). We therefore examined whether Fas and class I MHC up-regulation on β cells could be inhibited by SOCS-1 overexpression after adoptive transfer. Splenocytes from diabetic NOD mice were transferred into irradiated RIP-SOCS-1 recipients or nontransgenic littermate controls, then the islets of recipient mice were analyzed for Fas and class I MHC expression 14 days later (Fig. 6, A and B). In nontransgenic recipients, all islet cells, including β cells, expressed high levels of both Fas and class I MHC after adoptive transfer. However, in RIP-SOCS-1 recipients, neither Fas nor class I MHC expression was up-regulated on β cells, whereas expression was up-regulated on other islet cells.

Although SOCS-1 overexpression inhibited Fas and class I MHC up-regulation on β cells in both the NOD8.3 and adoptive transfer models, RIP-SOCS-1 mice developed diabetes at the same rate as their nontransgenic counterparts after adoptive transfer (Fig. 6C). This indicates that Fas and class I MHC up-regulation on β cells is not required for transfer-accelerated diabetes.

**β cell SOCS-1 overexpression partially suppresses spontaneous diabetes in NOD mice**

RIP-SOCS-1 mice also developed spontaneous diabetes, but at a significantly reduced incidence compared with nontransgenic littermate controls. In both transgenic lines examined (no. 24 and 32), the percentage of RIP-SOCS-1 mice that had become diabetic by 300 days was half that of nontransgenic mice (Fig. 6D, data...
from line 32 are shown). This suggests that SOCS-1 is capable of partially blocking the effector mechanisms of β cell destruction involved in NOD spontaneous diabetes.

Discussion
How CD8+ T cells and β cells interact can be effectively addressed using TCR transgenic models such as NOD8.3 mice. In the current study we showed that overexpression of SOCS-1 in β cells completely prevented diabetes in NOD8.3 TCR transgenic mice. It has been reported that β cell destruction in NOD8.3 mice is primarily mediated through the engagement of Fas on β cells (27). Initiation and development of insulitis in NOD8.3 mice carrying the RIP-SOCS-1 transgene was normal, but there was a failure to progress to β cell destruction, indicating that protection from diabetes was most likely due to blockade of the effector pathways used by the 8.3 T cells to kill β cells. Consistent with this, Fas expression was detectable on β cells isolated from prediabetic NOD8.3 mice, but not in double-transgenic mice, suggesting that cytokine-dependent Fas up-regulation was blocked by SOCS-1 overexpression.

In contrast, the RIP-SOCS-1 transgene only partially prevented spontaneous NOD diabetes and had no apparent effect on accelerated diabetes caused by the adoptive transfer of splenocytes from...
diabetic NOD donors. The level of protection observed in spontaneous diabetes in NOD mice was comparable to that in another study of NOD RIP-SOCS-1 mice (29). It was also similar to the protection observed in NOD mice that express a dominant negative Fas transgene in β cells (30), suggesting that inhibition of Fas expression may be the mechanism of this partial protection in NOD RIP-SOCS-1 mice. Despite this, we have not been able to detect Fas expression on β cells from unmanipulated NOD mice undergoing spontaneous diabetes (8, 9). Fas expression was detected, however, on β cells of nontransgenic NOD recipients adoptively transferred with splenocytes from diabetic NOD donors. RIP-SOCS-1 blocked Fas up-regulation, but not progression to diabetes after adoptive transfer. This indicates that Fas-independent effector pathways must be able to fully compensate for the loss of Fas expression under these circumstances. Only partial protection from spontaneous NOD diabetes by RIP-SOCS-1 may also be explained by the fact that both CD4+ and CD8+ T cells contribute to β cell destruction. Diabetogenic CD4+ T cell clones can cause β cell destruction independently of CD8+ T cells (31, 32). These results appear consistent with β cell destruction in NOD8.3 mice being caused by Fas on β cells interacting with FasL on CD8+ T cells, and β cell destruction in NOD spontaneous diabetes and that in transfer-accelerated diabetes being more diverse processes, involving CD4+ and CD8+ T cells as well as other molecular mechanisms, including the release of perforin and granzymes (33).

It is possible that effector pathways of β cell destruction other than Fas are also inhibited by SOCS-1. We have found that the direct cytotoxicity of proinflammatory cytokines, such as IFN-γ acting together with TNF or IL-1, for β cells in vitro is also blocked in RIP-SOCS-1 islets (unpublished observations). Furthermore, RIP-SOCS-1 islets did not stimulate NOD8.3 T cells in vitro as well as nontransgenic islets. This may be due to the inability of RIP-SOCS1 β cells to up-regulate class I MHC molecules in response to cytokines. Direct recognition of β cells by CD8+ T cells is essential for diabetes in NOD mice. We have previously shown that diabetes proceeds much more slowly when diabetogenic T cells are transferred into mice not expressing class I MHC on β cells than when class I MHC is expressed (34).

Additionally, reduced expression of IL-15 may contribute to the poor stimulatory capacity of RIP-SOCS-1 islets for 8.3 T cells. IL-15 is a member of the γ common family of cytokines, which have many critical actions on T cells, such as in promoting activation, proliferation, and survival. There is evidence suggesting the importance of a positive feedback loop between T cell production of proinflammatory cytokines, and IL-15 and IL-7 (another γ common cytokine) production by target tissues. IL-7 and IL-15 expression are induced in many tissues by TNF and/or IFN-γ (14, 35), and they can stimulate T cells to produce IFN-γ and TNF (36, 37). These interactions may exist between β cells and CD8+ T cells and could contribute to the activation of T cells that infiltrate the islets.

It is striking that normal migration to the islets and T cell activation occurred in vivo, given the blunted response of 8.3 T cells to RIP-SOCS1 islets in vitro and the reduced IL-15 expression by RIP-SOCS1 β cells. There was no difference in the frequency of activated or proliferating 8.3 T cells in the islets of NOD8.3 mice compared with RIP-SOCS-1/NOD8.3 mice. The fact that the islets of RIP-SOCS-1/NOD8.3 mice were still infiltrated with activated T cells implies that either the mechanisms recruiting 8.3 T cells to the islets are unaffected by SOCS-1 overexpression or that remaining mechanisms are sufficient for recruitment. Islet-reactive CD8+ T cells first encounter β cell Ags in the pancreatic lymph node (38) by CD8+ dendritic cell cross-presentation (39). Subsequent to priming, T cells then home to the islets. Therefore, our data suggest that the impaired ability of RIP-SOCS-1 β cells to act as APCs does not affect this process, but may suppress the reactivation of CD8+ T cells upon infiltration into the islets.

Although IFN-γ effects on RIP-SOCS1 β cells are blocked in these mice, IFN-γ is still likely to act on endothelial and other cells, which is thought to promote T cell recruitment (40). The ability of 8.3 T cells to correctly home to the islets of RIP-SOCS-1/NOD8.3 mice can be explained by the fact that pancreatic endothelial cells in these mice are still able to cross-present β cell Ags and recruiting T cells (41). IFN-γ deficiency perturbs homing of diabetogenic T cells to the islet, perhaps due to the loss of IFN-γ-induced chemokine expression, and Ag cross-presentation by endothelial cells (18). This suggests that SOCS-1 overexpression may also be useful for inhibiting pancreatic endothelial cell responses to IFN-γ and the recruitment of diabetogenic T cells to the islet.

SOCS-1 is one member in a family of structurally related proteins, many of which have also been shown to have inhibitory actions on signaling pathways (20). SOCS-3, in addition to its ability to inhibit JAK-STAT signaling, has been shown to inhibit IL-1- and IFN-γ-induced iNOS expression and cytotoxicity in β cell lines (42). Combined overexpression of SOCS-1 and SOCS-3 could be a useful strategy for blocking a broader range of cytokine-dependent mechanisms of β cell destruction than SOCS-1 alone. Complete protection of β cells from autoimmune destruction is likely to require a combination of therapeutic approaches, but we have shown that inhibition of cytokine action is a potent means of inhibiting Fas expression, class I MHC expression, and production of cytokines by β cells under immune attack. This has been effective in blocking CD8+ T cell-mediated autoimmune diabetes. Finally, cytokines also contribute to tissue destruction in other inflammatory diseases. Our findings suggest that using SOCS-1 to block the responsiveness of tissues to proinflammatory cytokines may be valuable in preventing tissue destruction in inflammatory diseases in general.

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References