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Severe Pancreatitis with Exocrine Destruction and Increased Islet Neogenesis in Mice with Suppressor of Cytokine Signaling-1 Deficiency

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Mice with suppressor of cytokine signaling-1 (SOCS-1) deficiency die within 3 weeks of birth from a multiorgan inflammatory disease. Increased systemic levels and sensitivity of cells to the inflammatory cytokines interferon-γ and tumor necrosis factor may contribute to the disease. Hepatitis and liver failure are thought to be the cause of the neonatal lethality in these mice. Here, we show that the pancreata of SOCS-1$^{-/-}$ mice are also severely affected by inflammation, displaying extensive edema and infiltration by T cells and macrophages. Acinar cells in particular were atrophied and reduced in their zymogen content. The expression of inflammatory markers, including class I major histocompatibility complex and inducible nitric oxide synthase, were increased in the SOCS-1$^{-/-}$ pancreas. Although there was generalized up-regulation of class I major histocompatibility complex, inducible nitric oxide synthase expression was more prominent on exocrine tissues. There appeared to be preferential damage and apoptosis of exocrine over endocrine components. Unexpectedly, increased islet neogenesis, possibly from proliferating ductal cells, was observed in the pancreas of SOCS-1$^{-/-}$ mice. This is reminiscent of the pancreatitis and islet neogenesis that occur in mice that transgenically overexpress interferon-γ and/or tumor necrosis factor. This study suggests that in addition to liver failure, the pancreatitis may also be an important contributor to the neonatal lethality in SOCS-1$^{-/-}$ mice. ***(Am J Pathol 2004, 165:913–921)***

Pancreatitis is characterized by infiltration and inflammatory damage to both exocrine and endocrine components of the pancreas. The proinflammatory cytokines tumor necrosis factor (TNF) and interferon (IFN)-γ, released by infiltrating macrophages[1] and T cells,[2] are thought to be key mediators of damage in pancreatitis.

In animal models of pancreatitis, such as cerulein-induced pancreatitis, increased pancreatic and serum levels of TNF correlate with the severity of pancreatic necrosis and cellular infiltration.[1] Cerulein-induced pancreatitis is attenuated in mice deficient in TNF receptor 1,[3] and in mice administered with TNF-neutralizing antibodies.[4,5] In this model, TNF-induced apoptosis of exocrine cells is thought to be dependent on the nuclear factor-κB pathway.[6] *In vitro*, combinations of TNF, IFN-γ, and interleukin (IL)-1 induce β-cell death through inducible nitric oxide synthase (iNOS) and nitric oxide (NO).[7–9] TNF may also induce caspase-dependent β-cell apoptosis.[10]

Mice expressing IFN-γ under the insulin promoter (Ins-IFN-γ) develop a severe pancreatitis that affects both exocrine and endocrine components despite IFN-γ expression being restricted to the islets.[11] In these mice, IFN-γ is thought to facilitate cellular damage by up-regulating the expression of class I major histocompatibility complex (MHC) on parenchymal cells. In WBN/Kob rats, which develop chronic pancreatitis after ductal obstruction, the progression of pancreatic destruction and fibrosis correlate with IFN-γ mRNA expression levels within the pancreas.[12]

Suppressor of cytokine signaling-1 (SOCS-1) is an intracellular-negative regulator of cytokine signaling. Overexpression of SOCS-1 inhibits signaling by multiple cytokines that activate the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. Because these same cytokines, which include IFNs and the IL-6 family, induce the expression of SOCS-1, SOCS-1 is thought to function in a negative feedback loop to terminate cytokine signaling.[13] SOCS-1 may also suppress cellular responses to cytokines and factors that do not signal through the JAK-STAT pathway such as TNF[8,14] and lipopolysaccharide.[15,16]

Mice deficient in SOCS-1 die within 3 weeks of birth from a disease characterized by inflammation and infiltration of the liver, heart, and pancreas.[17,18] Liver failure is thought to be the cause of the neonatal lethality.[18] T cells and NKT cells are thought to be key cellular mediators of the SOCS-1 disease. T cells appear to be aberrantly activated in these mice,[19] and NKT cells may be responsible for the hepatic damage.[20] The SOCS-1 disease may also be because of increased sensitivity of

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tissues to IFN-γ, in particular the liver.[21] Neonatal mice injected with IFN-γ develop an inflammatory disease similar to that in SOCS-1̃ mice,[22] while IFN-γ-induced STAT1 activation is prolonged in SOCS-1̃ hepatocytes.[23] Moreover, IFN-γ deficiency prevents the neonatal inflammatory disease in SOCS-1̃ mice.[19,21] SOCS-1 deficiency also causes TNF hypersensitivity in fibroblasts and pancreatic β cells.[8,14]

Studies into the pathology of SOCS-1̃ mice have focused primarily on the hepatitis that occurs. This is mainly because liver failure is thought to be the cause of the neonatal lethality. However, the pancreas is also affected by inflammation in these mice. The aim of the present study is to characterize the pancreatic pathology in SOCS-1̃ mice and to investigate the consequence of this inflammation.

Materials and Methods

Mice

SOCS-1̃ and SOCS-1̃ IFN-γ̃ mice have been previously described.[18,21] These were maintained on a mixed C57BL/6–129Sv genetic background, and housed in clean conditions at The Walter Eliza Hall Institute of Medical Research.

Organ Preparation

Tissues were fixed in neutral-buffered formalin or Bouin's fixative before embedding in paraffin. Sections were prepared by standard techniques and stained with hematoxylin and eosin. Tissues for immunohistochemistry and X-gal staining were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C for 2 hours, and subsequently infused with 30% sucrose (in PBS) overnight, before being embedded and frozen in OCT compound (Sakura Finetechnical, Tokyo, Japan).

Immunohistochemistry

Sections were stained with monoclonal antibodies (clone name in parentheses) recognizing murine CD4 (H129.19), CD8 (53-6.7), B220 (RA3-6B2), F4/80 (Cl:A3-1), GR-1, and class I MHC (M1/42) and polyclonal antibodies recognizing porcine insulin (A0564), human glucagon (A0565), human somatostatin (A0566), and murine iNOS (NOS2). The anti-insulin, glucagon, and somatostatin antibodies were purchased from DAKO (Carpinteria, CA), the anti-iNOS antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other antibodies were purchased from BD Pharmingen (San Diego, CA). Primary antibody incubation was followed by an avidin-conjugated peroxidase system (Vectorstain Elite ABC, Vector Laboratories, Burlingame, CA) or horseradish peroxidase-conjugated secondary antibodies. Sections were counterstained with hematoxylin. For immunofluorescence, sections were incubated with fluorescein isothiocyanate- or Texas Red-conjugated antibodies.

X-Gal Staining

Staining for β-galactosidase activity was performed as previously described.[24] Briefly, frozen sections were rinsed in PBS and then in wash buffer (PBS containing 2 mmol/L $MgCl_2$, 5 mmol/L EGTA, 0.02% Nonidet P-40, 0.01% sodium deoxycholate). This is followed by incubation overnight at 37°C in X-gal staining solution [100 mmol/L Na_3PO_4, 5 mmol/L $K_4Fe(CN)_6.3H_2O$, 5 mmol/L $K_3Fe(CN)_6$, 2 mmol/L $MgCl_2$, 5 mmol/L EGTA, 0.02% Nonidet P-40, 0.01% sodium deoxycholate, 0.6 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal)]. Sections were counterstained with nuclear fast red.

TdT-Mediated dUTP Nick-End Labeling (TUNEL) Assay

TUNEL detection of apoptotic cells was performed as previously described with minor modifications on paraffin-embedded sections of paraformaldehyde-fixed organs.[25] Sections were dewaxed and rehydrated before incubation in proteinase K solution (20 μg/ml in 20 mmol/L Tris-HCl, 100 mmol/L ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate, pH 8.0) at 37°C for 30 minutes. After washing with PBS and then TE (20 mmol/L Tris-HCl, 100 mmol/L ethylenediaminetetraacetic acid, pH 8.0), sections were incubated in precooled permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) for 3 minutes. After further washing with PBS, the sections were incubated in terminal deoxynucleotidyl transferase (TdT) buffer (Promega, Madison, WI) for 5 minutes before treatment with TdT-reaction mix containing 25 mmol of $CaCl_2$, 1.6 μl TdT (19U/μl; Promega) and 1 μl biotin-conjugated dUTP (Boehringer-Mannheim, Mannheim, Germany) per 100 μl of TdT buffer, for 60 minutes at 37°C. Sections were then incubated in streptavidin-Texas Red (Amersham) for 30 minutes to detect dUTP-positive nuclei. TUNEL-positive nuclei showing pyknosis (nuclear condensation), fragmentation, and/or lobulation, as demonstrated by 4′,6-diamidino-2-phenylindole staining of nuclear DNA, were considered to be true apoptotic cells.

BrdU Staining

Mice were injected intraperitoneally with 0.1 mg/g of 5′-bromodeoxyuridine (BrdU) in PBS. Eighteen hours later, the pancreas was harvested and fixed in 4% paraformaldehyde before paraffin embedding. For visualization of BrdU-labeled cells, sections were first treated with proteinase K solution (20 μg/ml in 20 mmol/L Tris-HCl, 100 mmol/L ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate, pH 8.0) at 37°C for 30 minutes. They were washed and denatured in 2 mol/L HCl for 30 minutes in a 37°C oven and incubated in precooled permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) for 3 minutes. The sections were then incubated with mouse anti-BrdU IgG (Amersham Biosciences, Uppsala, Sweden), followed by incubation with anti-mouse IgG biotin (DAKO) and streptavidin-Texas Red. Co-localization was detected by confocal laser scanning microscopy using a

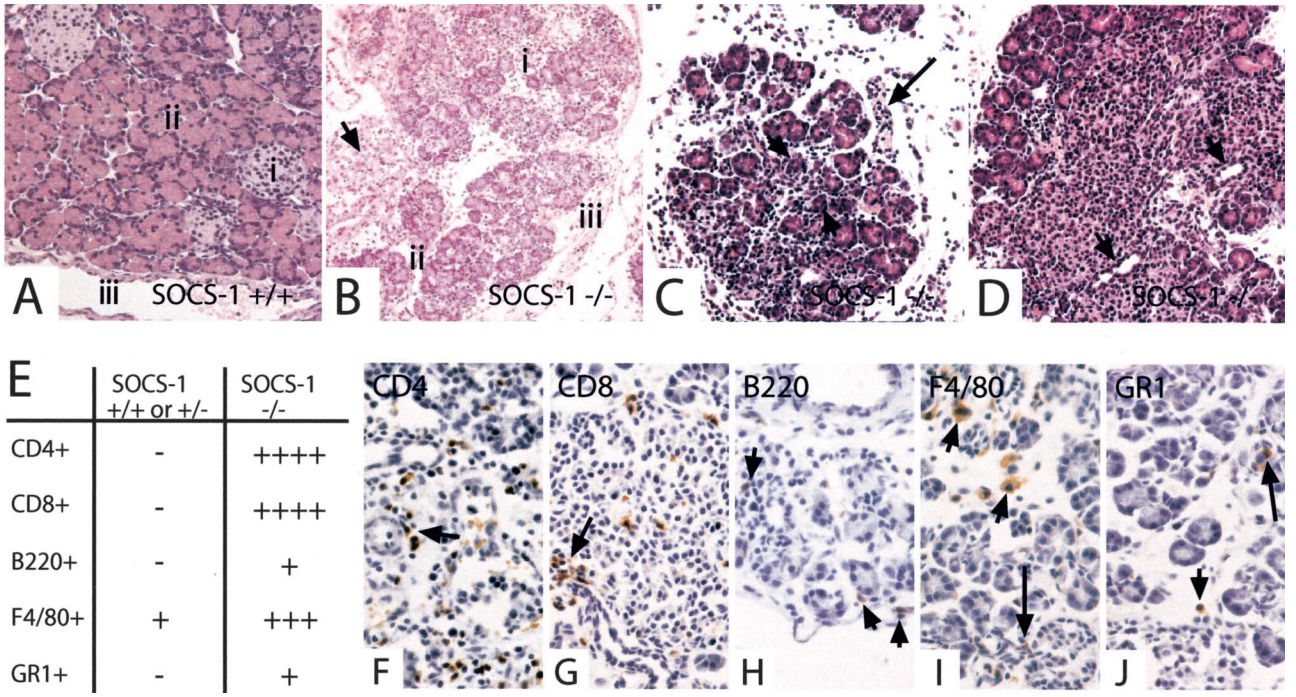


Figure 1. Histopathology of SOCS-1̃ pancreata. **A:** Pancreas from a littermate control (SOCS-$1^{+/+}$) with an endocrine islet (i), exocrine acini (ii), and a blood vessel indicated (iii). **B:** SOCS-1̃ pancreata typically displayed interstitial (i), interlobular (ii), and subcapsular edema (iii). Note the hyperplastic fibrous supporting tissue (**arrow**). **C:** Numerous extravasating cells (**long arrow**) and shrunken acini (**short arrow**) were observed such as those indicated in this severely inflamed SOCS-$1^{-/-}$ pancreatic lobule. Note the loss of eosin staining of acinar cells, which is indicative of reduced zymogen content. **D:** Islets in a SOCS-1̃ pancreas frequently displayed shape irregularities, such as closeness to ductules (**arrows**) and a blurring of islet boundary. **E–J:** Pancreatic sections were stained for the hematopoietic markers CD4, CD8, B220, F4/80, and GR1 by immunohistochemistry to determine the identity of infiltrating cells. The relative frequencies of different hematopoietic populations are indicated in **E**. Examples of SOCS-1̃ pancreas sections stained for hematopoietic markers are shown in **F–J**. **F:** Numerous $CD4^+$ T cells were found, particularly surrounding vessels (**arrow**). **G:** $CD8^+$ T cells were similarly prominent, particularly surrounding vessels (**arrow**). **H:** $B220^+$ B cells were only found occasionally (**arrows**). **I:** $F4/80^+$ macrophages were frequently found within the fibrous septa between lobules (**short arrows**) and near blood vessels (**long arrow**). **J:** Very few $GR1^+$ neutrophils were found in the parenchyma of SOCS-1̃ pancreata (**short arrow**). Most were found within blood vessels (**long arrow**). All pancreatic sections shown are from 10- to 14-day-old mice. Original magnifications: ×200 (**A**, **C**, **D**); ×100 (**B**); ×400 (**E–J**).

Leica DMIRE2 inverted microscope (Leica Microsystems, Heidelberg, Germany).

Morphometric Analysis

For each pancreas, two sections from planes separated by at least 300 μm were covered by accumulating images from nonoverlapping fields. Images were captured by Axiocam attached to an Axioplan 2 microscope (Zeiss, Oberkochen, Germany). Insulin-staining cells within ductal epithelia were counted on blinded sections using the ImageJ software (U.S. National Institutes of Health, *http://rsb.info.nih.gov/ij/*).

Cytokine Enzyme-Linked Immunosorbent Assays (ELISAs)

Cytokine concentrations were determined using standard sandwich ELISAs. IFN-γ was measured using the purified monoclonal antibody R4-6A2 (capture) and the biotinylated monoclonal antibody XMG1.2 (detection) (BD Pharmingen). TNF (DY410) and IL-12p40 (DY499) were measured using the DuoSet ELISA Development System antibodies (R&D Systems, Minneapolis, MN).

Results

SOCS-1̃ Mice Develop Severe Pancreatitis

SOCS-1̃ mice were sacrificed for analysis at 10 to 14 days of age, when they became moribund. The pancreas of SOCS-1̃ mice exhibited extensive interlobular and interstitial edema, with the exudate distending the perilobular and periacinar fibrous tissue (Figure 1B). A mononuclear infiltrate was found between acini, around islets, within fibrous septa, and occasionally within the islets (Figure 1C). The infiltration appeared to show a gradation from the blood vessels with parenchyma nearest to the blood vessels being most infiltrated and edematous. Acini in SOCS-1̃ pancreata were loosely packed, with interacinar spaces filled with inflammatory cells and fibroblasts. Acinar cells appeared atrophied and showed less eosinophilic cytoplasmic staining, suggesting a reduced zymogen content (Figure 1C). In more severely affected pancreata, the islets were disrupted and their boundary with the exocrine tissue obscured by leukocytic infiltration (Figure 1D). A greater number of intralobular ducts were observed in the SOCS-1̃ pancreata than littermate controls (Figure 1D). No features of inflammation were present in the littermate controls (Figure 1A).

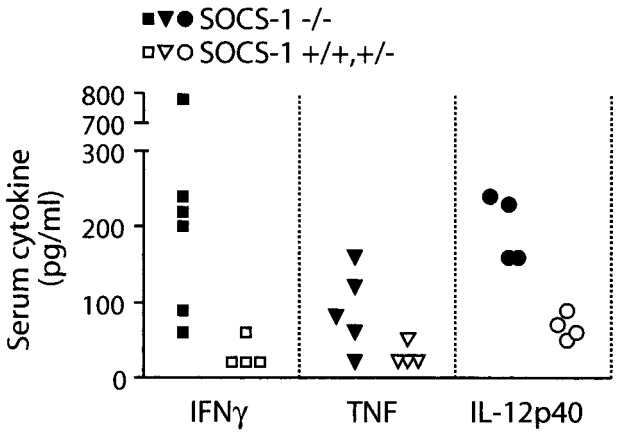


Figure 2. Increased circulating levels of inflammatory cytokines in SOCS-$1^{-/-}$ mice. Serum from moribund SOCS-$1^{-/-}$ mice and littermate controls were analyzed for cytokine levels by ELISA.

The SOCS-1̃ Pancreas Is Infiltrated with T Cells and Macrophages

To characterize the cell types infiltrating the SOCS-1̃ pancreas, immunohistochemical staining for hematopoietic cell markers was performed on frozen pancreas sections. Most of the infiltrating cells were found to be $CD4^+$ T cells, $CD8^+$ T cells, and $F4/80^+$ macrophages (Figure 1E). $CD4^+$ and $CD8^+$ T cells were found within the interacinar spaces and around islets but rarely within islets (Figure 1, F and G). They were mostly concentrated within the thick connective tissue surrounding lobules, vessels, and ducts. Numerous $F4/80^+$ macrophages were also found in the perilobular connective tissue, and to a lesser extent in the interacinar and peri-islet spaces (Figure 1I). Very few infiltrating B cells or neutrophils were found. No infiltrating cells were detected in control pancreata, although resident macrophages were often seen.

The Expression of Inflammatory Markers Is Up-Regulated in the SOCS-1̃ Pancreas

A consequence of aberrant T-cell activation[19] and multiorgan inflammation in SOCS-$1^{-/-}$ mice could be the elevation of circulating levels of inflammatory cytokines. Increased levels of IFN-γ,[19] TNF,[14] and IL-12[26] have all been reported. We too have found increased levels of these cytokines in the serum of moribund SOCS-$1^{-/-}$ mice (Figure 2), but undetectable levels of IL-1β (not shown).

Increased circulating levels of inflammatory cytokines as well as local production by infiltrating T cells and macrophages are likely to induce the expression of inflammatory molecules in the pancreas of SOCS-$1^{-/-}$ mice. The expression of class I MHC and iNOS in pancreatic cells are known to be regulated by IFN-γ and TNF, among other cytokines.[7,8,27–29] All cell types in the SOCS-1̃ pancreas, including exocrine, endocrine, ductal, endothelial, and infiltrating cells, showed intense immunohistochemical staining for class I MHC (Figure 3B). iNOS expression was examined with immunofluorescence, which provided greater sensitivity. Cytoplasmic iNOS staining was found in exocrine structures (acini, ducts) and islets of SOCS-1̃ pancreata (Figure 3D). Unlike for class I MHC, the exocrine tissues stained more strongly for iNOS than the islets. Littermate control pancreata showed no class I MHC or iNOS staining (Figure 3, A and C).

The generation of the SOCS-1̃ mice involved the replacement of the SOCS-1 gene with a LacZ reporter gene, which encodes for bacterial β-galactosidase.[18] Therefore, β-galactosidase expression serves as a reporter of SOCS-1 promoter activity. β-Galactosidase expression was detected in the SOCS-1̃ pancreas (Figure 3F), indicating attempted expression of SOCS-1, presumably in response to local and systemic cytokine levels. As such, staining was most intense around foci of infiltrating cells. The exocrine and endocrine cells displayed different staining patterns. Although exocrine cells showed a diffuse and more intense cytoplasmic staining, endocrine cells displayed a weaker punctate staining pattern. β-Galactosidase expression was not detected in SOCS-$1^{+/+}$ or SOCS-$1^{+/-}$ littermate control pancreata (Figure 3E).

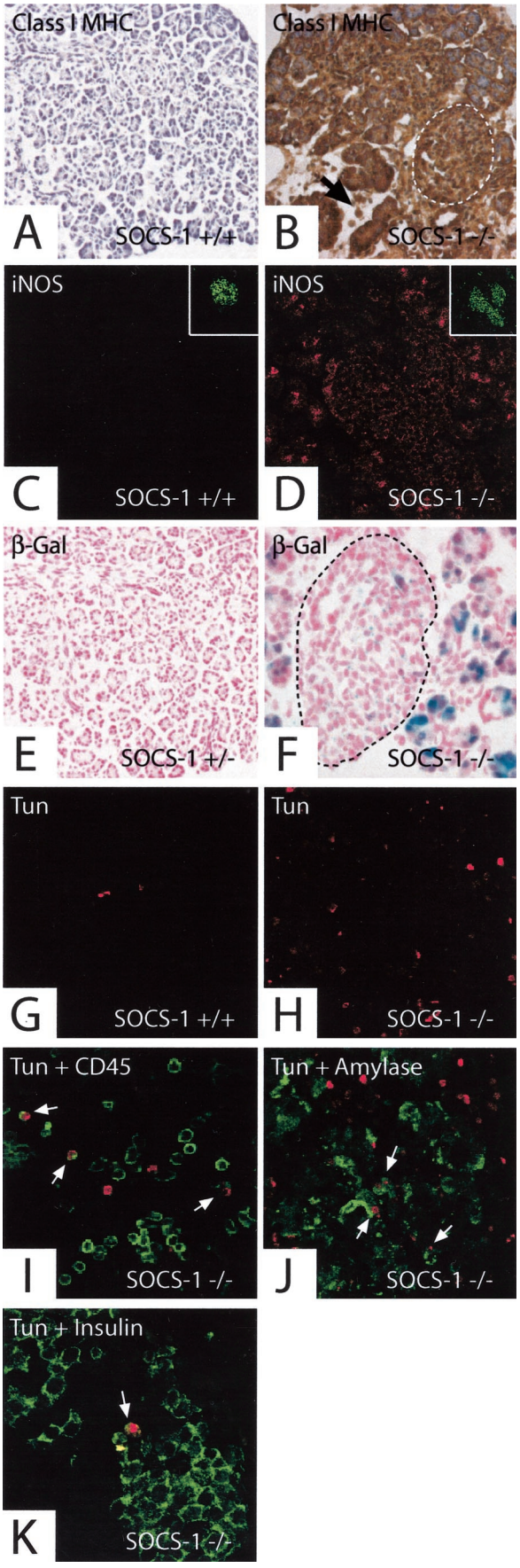
Increased Apoptosis Is Evident in the SOCS-1̃ Pancreas

The combination of TNF and IFN-γ is known to induce apoptosis of pancreatic islet cells[8,30] and exocrine cells.[6] We have previously shown that SOCS-1 deficiency causes hypersensitivity to TNF in pancreatic β cells.[8] SOCS-1 also regulates IFN-γ signaling.[23] The severe pancreatitis and increased levels of and sensitivity to TNF and IFN-γ could result in increased apoptosis of pancreatic cells in SOCS-1̃ mice. Apoptosis was examined by TUNEL staining, which detects DNA strand breaks. Colocalization with CD45 (leukocytes), amylase (acinar cell), and insulin (β cell) were performed to identify the apoptotic cells.

Few apoptotic cells were found in the pancreata of littermate controls (Figure 3G). Some apoptosis was expected because in the neonatal mice, organ growth and remodeling, particularly of the exocrine tissue, is still occurring.[31,32] Substantially more apoptotic cells were detected in the SOCS-1̃ pancreas (Figure 3H). Most apoptotic cells were found to be infiltrating $CD45^+$ cells (Figure 3I) or acinar ($amylase^+$) cells (Figure 3J). Only a few apoptotic β cells were found (one or two per pancreatic section) (Figure 3K).

The Architecture of Islets Is Perturbed in SOCS-$1^{-/-}$ Pancreata

Hormone immunohistochemistry was performed to investigate the effects of inflammation on the organization and morphology of the endocrine islets in the SOCS-1̃ pancreas. In the pancreata of SOCS-1̃ mice, no alterations in the organization of the islet were found. As in wild-type mice, α and δ cells were in the periphery of the islet, with β cells residing in the core. On closer inspection, the staining



intensity for glucagon and somatostatin was reduced in 10 of 12 SOCS-1̃ pancreata examined (Figure 4, D and F), while the staining intensity of insulin was comparable to the littermate controls (Figure 4, A and B). Furthermore, the islets in SOCS-1̃ mice often appeared irregular in shape and many were found adjacent to ducts. Insulin-expressing single cells or cell clusters interspersed within the exocrine tissue or within ductal epithelia were observed in the pancreata of both SOCS-1̃ and littermate control mice. However, these were more frequent in SOCS-$1^{-/-}$ pancreata (Figure 4B and Figure 5, A and B). In particular, there was a fourfold increase in the frequency of insulin-expressing ductal cells in SOCS-1̃ mice compared with littermate controls (Figure 5C).

Increased β-Cell Neogenesis from Ductal Precursors Is Evident in the SOCS-1̃ Pancreas

The increased frequency of insulin-staining cells within ductal epithelia of SOCS-$1^{-/-}$ pancreata is suggestive of increased islet neogenesis. As already mentioned, pancreas growth and remodeling, including islet neogenesis, occurs in wild-type neonatal mice up to 3 to 4 weeks of age.[31,33,34] In neonatal wild-type mice, new islet cells are thought to develop from the proliferation and differentiation of ductal precursors.[35] Suggestive of ductal cell proliferation was the presence of mitotic figures in the ductal walls of SOCS-1̃ pancreata (Figure 5, A and D). Previously, more intralobular ducts were observed in the SOCS-1̃ pancreas (Figure 1D). This is also suggestive of increased ductal proliferation. To ascertain whether ductal cells were proliferating, mice were given a single intraperitoneal injection of the thymidine analogue 5′-bromodeoxyuridine (BrdU). BrdU incorporation was then examined by immunofluorescence on pancreatic sections. Sections were also co-stained for CD45, insulin, and the ductal cell marker, cytokeratin 18. As expected, substantial numbers of $BrdU^+$ cells were found in the pancreas of littermate controls (Figure 5E) because of ongoing organ growth and remodeling. However, many more $BrdU^+$ cells were found in the pancreata of SOCS-1̃ mice (Figure 5F). Of these, few were found to be islet β cells (Figure 5G). Most $BrdU^+$ cells in the pancreas of

Figure 3. Expression of inflammatory markers in the pancreas of SOCS-$1^{-/-}$ mice. SOCS-$1^{+/+}$ (**A**) and SOCS-1̃ (**B**) pancreata were analyzed for class I MHC expression by immunohistochemistry. Note the high level of class I MHC expression on inflammatory cells (**arrow**), stroma, acini, and islets (**dashed boundary**). SOCS-$1^{+/+}$ (**C**) and SOCS-1̃ (**D**) pancreata were analyzed for iNOS expression by immunofluorescence (red). The same sections were also stained for insulin (green), and are shown in the **insets**. SOCS-$1^{+/-}$ (**E**) and SOCS-1̃ (**F**) pancreata were analyzed for β-galactosidase by X-gal staining (blue). Note the different staining patterns evident in SOCS-1 pancreata, punctate stainting in islets (**dashed boundary**) compared with diffuse staining in exocrine cells. SOCS-$1^{+/+}$ (**G**) and SOCS-1̃ (**H**) pancreata were analyzed apoptosis by TUNEL fluorescence staining (red). Note the increased frequency of $TUNEL^+$ cells in SOCS-1̃ pancreata. **I–K:** SOCS-1̃ pancreata were co-stained for the pan-leukocyte marker CD45, amylase, or insulin (green) to determine the identity of apoptotic cells. The **arrows** indicate examples of double-staining cells. Most apoptotic cells in SOCS-1̃ pancreata were infiltrating $CD45^+$ (**I**) cells or acinar ($amylase^+$) cells (**J**). **K:** Some apoptotic cells in SOCS-1̃ pancreata were found to be $insulin^+$. Note that TUNEL stains apoptotic nuclei while anti-CD45, amylase, and insulin stain the cytoplasm of cells, and therefore does not completely co-localize. Original magnifications: ×200 (**A–E**); ×400 (**F**); ×600 (**G–K**).

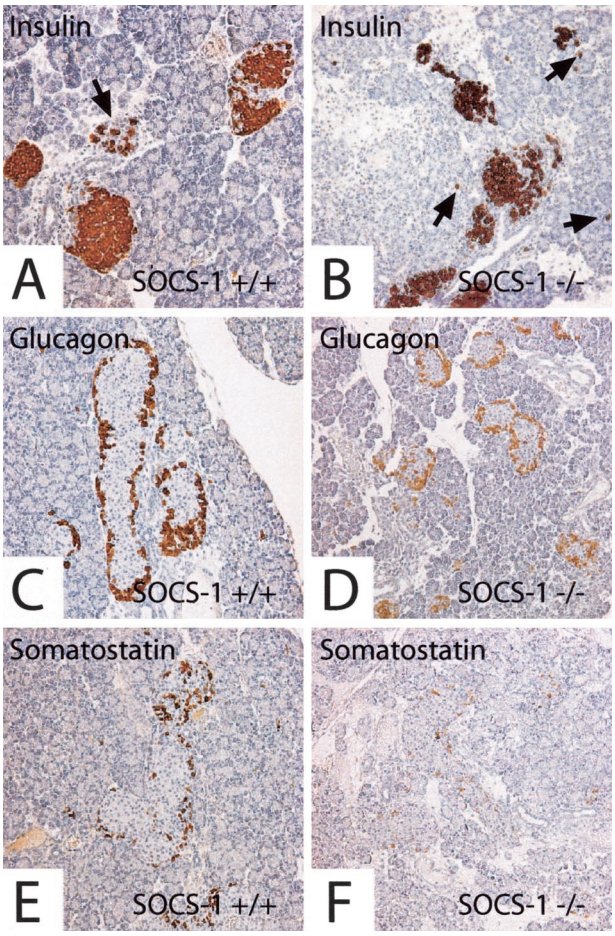


Figure 4. Perturbed endocrine islet morphology in the pancreas of SOCS-1̃ mice. SOCS-$1^{+/+}$ (**A**, **C**, **E**) and SOCS-$1^{-/-}$ (**B**, **D**, **F**) pancreata were stained for insulin (**A**, **B**), glucagon (**C**, **D**), or somatostatin (**E**, **F**) expression by immunohistochemistry. One islet in **A** displays punctate insulin staining typical of a newly formed islet (**arrow**). **B:** Note the abundance of insulin staining single cells or cell clusters (**arrows**) in SOCS-$1^{-/-}$ pancreata. Original magnifications, ×100.

SOCS-1̃ mice were found to be proliferating $CD45^+$ hematopoietic cells or cytokeratin 18^+ ductal cells (Figure 5, I and K). In the pancreas of wild-type mice, fewer, but detectable numbers of cytokeratin 18^+ ductal cells were found to be $BrdU^+$ (Figure 5M).

Discussion

The inflammation in the SOCS-1̃ pancreas is probably mediated by the inflammatory cytokines IFN-γ and TNF. The importance of IFN-γ is suggested by the fact that SOCS-1̃ mice that are also IFN-γ-deficient do not develop pancreatic pathology.[21] The presence of inflammatory cytokines is also suggested by the high-level expression of class I MHC, iNOS, and attempted SOCS-1 transcription (β-galactosidase staining) in the infiltrated SOCS-1̃ pancreas. IFN-γ and TNF are well-characterized regulators of class I MHC and iNOS expression in pancreatic endocrine and exocrine cells.[7,8,27–29] Class I MHC expression is important for $CD8^+$ T-cell-mediated cytotoxicity. iNOS catalyzes the production of the free radical NO, which is cytotoxic to islet cells[7–9] and exocrine cells.[36]

The SOCS-1̃ pancreatitis resembles the pancreatitis that develops in mice injected with IFN-γ and TNF.[37] Indeed, circulating levels of IFN-γ and TNF are elevated in SOCS-1̃ mice, and SOCS-1 deficiency has been shown to cause hypersensitivity to both these cytokines.[8,14,23] Like SOCS-1̃ mice, mice injected with IFN-γ and TNF develop pancreatitis with edema and generalized infiltration affecting the exocrine tissue more than the endocrine tissue. However, unlike the SOCS-1̃ pancreas, neutrophils are the predominant infiltrating cell type in IFN-γ/TNF injected mice, with prominent hemorrhagic necrosis and ductal dilation. This may reflect a difference in time frame because these mice were exposed to cytokines for a longer period than SOCS-1̃ mice. Different sensitivities to cytokines may also account for the differences in pancreatic pathology because cytokine signaling is dysregulated in SOCS-1̃ mice.

Ins-IFN-γ mice, which constitutively express IFN-γ in their islets, develop a pancreatitis with features strikingly similar to the SOCS-1̃ pancreas, including a mainly mononuclear infiltrate and interstitial edema.[11] Like SOCS-1̃ mice, peri- and intraislet infiltrating cells are commonly found in Ins-IFN-γ mice at a young age. In Ins-IFN-γ mice up to 3 weeks of age, the severity of pancreatitis is similar to that of moribund SOCS-1̃ mice, whereas pancreatic destruction is more severe in older mice (6 to 10 weeks). It may be that the premature mortality of SOCS-1̃ mice prevented the pancreatic pathology from progressing to that seen in older ins-IFN-γ mice. The resemblance of the pancreatitis in IFN-γ-injected mice and ins-IFN-γ mice to that in SOCS-1̃ mice is consistent with IFN-γ playing a major role in the neonatal SOCS-1̃ inflammatory disease.

The level of inflammation and apoptosis appeared to be most severe in the exocrine tissue of SOCS-1̃ pancreata. Large numbers of infiltrating hematopoietic cells were found within the exocrine tissue, but not within the islets. Although class I MHC was strongly up-regulated in both exocrine and endocrine cells, the expression of iNOS and β-galactosidase were more prominent in the exocrine tissue. The frequency of apoptotic exocrine cells was also much greater compared with endocrine cells. The relatively mild damage of the islets compared with acini in SOCS-$1^{-/-}$ mice may reflect the degree of infiltration by inflammatory cells. It is possible that exocrine and endocrine cells have different sensitivities to cytokines and regulate the expression of iNOS and SOCS-1 differently. Islets may also be less prone to damage than acini because of their greater blood supply.[38] Furthermore, exocrine cells can perpetuate the inflammation because of their store of lipolytic enzymes and their ability to produce cytokines.[6] Indeed, it may be these reasons that the acini are also damaged preferentially to islets in human pancreatitis.[39]

The pancreatitis in SOCS-1̃ mice resembles human chronic pancreatitis in several other aspects. The inflammatory cell types are similar in both, consisting of primarily T cells and macrophages. The loss of zymogen granules by acinar cells, known as acinar regression,[40]

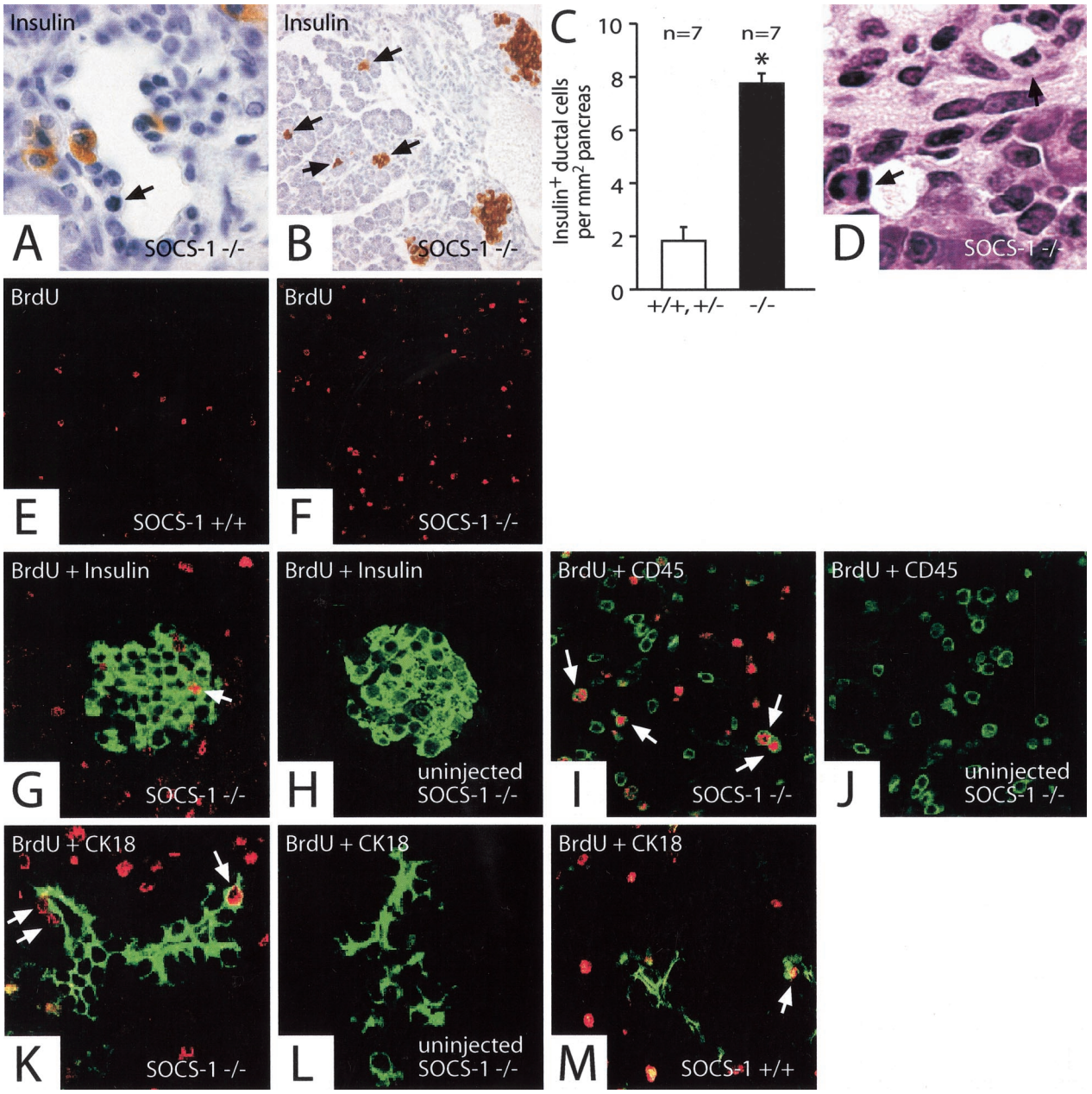


Figure 5. The pancreas of SOCS-1̃ mice display features of increased islet neogenesis. **A:** Insulin-expressing ductal cells (detected by immunohistochemistry) were frequently found in SOCS-$1^{-/-}$ pancreata. A mitotic figure is seen in a nearby ductal cell (**arrow**). **B:** Numerous insulin-staining single cells or cell clusters were present in SOCS-1̃ pancreata. **C:** SOCS-$1^{-/-}$ pancreata display a significant increase in the frequency of insulin-staining cells within ductal epithelia. Shown are the means ± SD. Statistical significance, $P < 0.0001$ (unpaired *t*-test). **D:** Mitotic figures (**arrows**) were frequently found in epithelium of intralobular ducts of SOCS-1̃ pancreata. SOCS-$1^{+/+}$ (**E**) and SOCS-1̃ (**F**) pancreata were analyzed with proliferating cells by measuring the incorporation of BrdU (red immunofluorescence) after an intraperitoneal injection of BrdU at 100 μg g/g body weight. Note the increased frequency of $BrdU^+$ cells in SOCS-1̃ pancreata. **G–L:** SOCS-1̃ pancreata were co-stained for insulin, CD45, or the ductal cell marker cytokeratin 18 (green) to determine the identity of proliferating cells. The **arrows** indicate examples of double-staining cells. **G:** Only a few β cells were found to be $BrdU^+$. Most proliferating cells were $CD45^+$ (**I**) or cytokeratin 18^+ (**K**) ductal cells. **H**, **J**, and **L:** As a negative control, uninjected mice were also analyzed for BrdU staining. **M:** Few cytokeratin 18^+ ductal cells were $BrdU^+$ in SOCS-$1^{+/+}$ pancreata. Note that anti-BrdU stains nuclei while anti-CD45, insulin, and cytokeratin 18 stain the cytoplasm of cells, and therefore does not completely co-localize. Original magnifications: ×1000 (**A**, **D**); ×200 (**B**, **E**, **F**); ×600 (**G–L**).

appears to occur in both. A major difference is that the extensive interstitial fibrosis often found in human chronic pancreatitis is not apparent in the SOCS-1̃ pancreas. Nevertheless, we observed greater than normal numbers of fibroblasts within septal regions. The lack of extensive fibrosis may reflect the shorter time frame in which the SOCS-1̃ pancreatitis develops (within 2 weeks of birth).

Somewhat surprisingly, we found evidence of increased islet neogenesis in SOCS-1̃ mice. This is despite the massive inflammation that is occurring. Increased islet neogenesis was suggested by the large increase in insulin-staining ductal cells in the SOCS-1̃ pancreas. A possible source of this increased neogenesis may be the increased number of proliferating ductal cells that were

found. Ductal epithelial cells of the pancreas are thought to the source of neoislets.[35] Endocrine cells first appear in duct walls,[41] later forming endocrine cell clusters,[42] which ultimately become islets. SOCS-1$^{-/-}$ IFN-$\gamma^{-/-}$ mice did not show features of increased islet neogenesis (data not shown), suggesting that inflammation, and not intrinsic SOCS-1 deficiency, was responsible for stimulating the ductal proliferation and differentiation into islet cells. It is thought that IFN-γ and TNF can trigger the secretion of growth factors by macrophages that infiltrate the inflamed pancreas,[43] and this may be an explanation for this increased islet neogenesis in the SOCS-1$^{-/-}$ pancreas. These growth factors, including transforming growth factor-α and epidermal growth factor, can induce ductal cell proliferation and differentiation into endocrine cells.[44–46] IFN-γ itself may also enhance ductal precursor cell responsiveness to epithelial growth factors.[47]

Increased ductal proliferation and differentiation into islets have also been found in other systems in which IFN-γ and/or TNF are elevated. In the Ins-TNF mice bearing a TNF transgene driven by the insulin promoter, a severe lymphocytic insulitis is accompanied by the development of intraislet ductules. Insulin-positive cell have been found within ductule walls (ie, not derived from the original islet).[48] Ins-IFN-γ mice from 10 weeks of age display extensive ductal proliferation with nearly half of all ductal cells incorporating BrdU.[43] Many of these ducts were also found to have endocrine cells budding into the ductal lumen. This ductal proliferation is greatly reduced after the administration of anti-IFN-γ antibody, suggesting that the proliferation is IFN-γ-dependent.[49] The ductal proliferation in the SOCS-1$^{-/-}$ pancreas was much milder, again perhaps reflecting the much shorter time for which the pancreas was exposed to inflammatory cytokines.

Finally, although there has been much focus on the liver to understand the mechanism behind the neonatal lethal phenotype of SOCS-1$^{-/-}$ mice, in this study we have shown that the pancreas is also severely affected by SOCS-1 deficiency. Therefore in addition to the liver failure, the loss of pancreatic function may also be an important contributor to the neonatal lethality in SOCS-1$^{-/-}$ mice.

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