Pancreatic Secretory Trypsin Inhibitor 1 Reduces the Severity of Chronic Pancreatitis in Mice Over-expressing Interleukin-1β in the Pancreas

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Short title: PSTI reduces pancreatitis in IL-1β transgenic mice

Abbreviations: IL-1 (interleukin-1), PSTI (pancreatic secretory trypsin inhibitor), MPO (myeloperoxidase)

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Abstract

Interleukin-1β (IL-1β) is believed to play a pathogenic role in the development of pancreatitis. Expression of human IL-1β in pancreatic acinar cells produces chronic pancreatitis characterized by extensive intrapancreatic inflammation, atrophy and fibrosis. In order to determine if activation of trypsinogen is important in the pathogenesis of chronic pancreatitis in this model, we crossed IL-1β transgenic mice with mice expressing a trypsin inhibitor that is normally produced in rat pancreatic acinar cells (pancreatic secretory trypsin inhibitor I (PSTI-I)). We had previously demonstrated that transgenic expression of PSTI-I increased pancreatic trypsin inhibitor activity by 190%. Tg(IL1β) mice were found to have marked pancreatic inflammation characterized by histological changes including acinar cell loss, inflammatory cell infiltration and fibrosis as well as elevated myeloperoxidase activity and elevated pancreatic trypsin activity as early as six weeks of age. In contrast to Tg(IL1β) mice, dual transgenic mice [Tg(IL1β)-Tg(Psti1)] expressing both IL-1β and PSTI-I in pancreatic acinar cells had significantly less severe pancreatitis. These findings indicate that over-expression of PSTI-I reduces the severity of pancreatitis and that pancreatic trypsin activity contributes to the pathogenesis of an inflammatory model of chronic pancreatitis.
Introduction

Chronic pancreatitis is a disease of multiple etiologies but is characterized by repeated or long-standing injury resulting in chronic inflammation of the gland. Genetic studies of human chronic pancreatitis have demonstrated the involvement of specific genes that either cause or increase the risk of developing the disease. Mutation of the cationic trypsinogen gene (PRSS1) confers a gain of function for trypsin activation and is a high penetrance risk factor for hereditary pancreatitis (29, 32). Loss of function mutations of the pancreatic secretory trypsin inhibitor (SPINK1) gene, which is believed to protect against intrapancreatic trypsin activity, increases the risk of developing chronic pancreatitis. Recently, mutations in the chymotrypsinogen C (CTRC) gene that predispose to chronic pancreatitis have been described. Together, these findings suggest that excessive protease activity plays a central role in hereditary forms of pancreatitis (6).

Animal models of chronic pancreatitis have been developed to explore the pathogenic mechanisms involved in disease progression. One of the most widely used is based on repeated caerulein injections over a period of several weeks (21). Caerulein-induced injury causes many pathological processes of acute pancreatitis including trypsinogen activation, lysosomal fusion, dysregulated exocytosis, and subsequent inflammation. Long-term caerulein administration produces acinar cell destruction, atrophy, tissue fibrosis, and chronic inflammatory cell infiltration. Recently, we demonstrated that targeted expression of the rat PstI1 gene in pancreatic acinar cells confers protection from chronic pancreatitis in the murine caerulein model implying that trypsin plays a role in repeated caerulein-induced injury. However, the role of trypsin in the evolution of pancreatitis from other causes is not known. An inflammatory model for chronic pancreatitis has been described recently and is based on the targeted
expression of the human *IL1β* gene in pancreatic acinar cells (15). IL-1β is a proinflammatory cytokine primarily expressed in blood monocytes, tissue macrophages and dendritic cells (8). It participates in the inflammatory response of the innate immune system to viral or bacterial agents or to cytotoxic molecules released from damaged cells by activating the NF-κB signal transduction cascade and the JNK and p38 mitogen-activated protein kinase pathways (MAPK) (31). IL-1β plays an active role during acute pancreatitis; it becomes expressed in the pancreas early in the course of caerulein-induced acute pancreatitis and the CDE model of pancreatitis (10, 24). In addition, blood circulating IL-1β is also increased (10). Elimination of neutrophils before initiating acute pancreatitis in the caerulein model significantly reduces the severity of pancreatitis (9). Moreover, blockade of IL-1β during experimental acute pancreatitis with the IL-1β receptor antagonist (IL-1ra) (23, 26) or by genetic deletion of the IL-1 receptor 1(IL-1 R1) (22) have been shown to attenuate the severity of pancreatitis.

Recently, using the rat elastase promoter human IL-1β was over-expressed in pancreatic acinar cells (15). Mice over-expressing IL-1β developed severe chronic pancreatitis characterized by inflammatory infiltrates, pancreatic atrophy and extensive fibrosis. Most experimental models of pancreatitis are generated by injuring the pancreas. The IL-1β transgenic mouse model is unique because it does not involve an antecedent pancreatic injury. Therefore, to examine the role of trypsin in the pathogenesis of an inflammatory model of chronic pancreatitis, we sought to determine whether PSTI-I protected against IL-1β-induced pancreatitis.
Materials and Methods

Animal Care

Transgenic mice expressing the rat PstI gene (19) or the human IL1β gene (15) were maintained in a C57BL/6J background. These animals were crossed under the following scheme. Male mice B6.Cg-Tg(Cela1-IL1β)L123Tcw over-expressing the human IL-1β protein [referred to hereafter as Tg(IL1β)] were mated with Tg(Cela1-Spink3)#Rali [referred to hereafter as Tg(PstI)] female mice expressing the rat PSTI-I protein. Male offspring expressing both transgenes were mated with female Tg(PstI) mice. Offspring of these matings generated dual transgenic mice [Tg(IL1β)-Tg(PstI)] that were used in experiments. The Tg(IL1β) mice were maintained as heterozygotes; male transgenic mice were mated with wild type C57BL/6J mice. All mice were genotyped for confirmation of IL-1β and PSTI-I gene expression. Mice were housed in a 12:12-hour light-dark cycle environment and given water and chow ad libitum. Studies were approved by the Institutional Animal Care and Use Committee of Duke University.

Biochemical measurements

Serum or pancreatic amylase was measured as previously described (19) with the following modification when pancreatic tissue was used. Pancreata were homogenized in MOPS buffer: 250 mM sucrose, 5 mM MOPS pH 6.5, 1 mM MgSO₄ followed by centrifugation for 15 minutes at 4°C at 25,000 x g. Supernatants were adjusted to a protein concentration of 1 mg/mL.

Myeloperoxidase (MPO) assay: Following euthanasia, a portion of the pancreas was immediately frozen and stored at -80°C until further use. Tissue preparation and MPO assays
were performed using the substrate tetraethylbenzidine as previously described (3, 7). MPO activity was expressed as units per mg protein. Protein concentration was determined using the micro BCA protein assay (ThermoScientific, Rockford, IL). Purified human MPO (Sigma Aldrich, St Louis, MO) was used as a standard.

Trypsin assay: Pancreatic extracts were prepared as previously described (19, 29). Trypsin activity was measured by fluorescence following the method described by Kawabata et al. 1988 (12). The Boc-Gln-Ala-Arg-MCA substrate at a final concentration of 40 µM (Peptide International Inc, Louisville, KY) was added to 250 µg of protein extract. The fluorescence emitted at 440 nm after excitation at 380 nm was monitored over 10 minutes and compared to the fluorescence emitted by pure bovine trypsin (15,000 BAEE units/mg) at concentrations ranging from 18.7 pM to 1 nM.

Histological staining

Pancreatic specimens were fixed overnight in 10% neutral buffer formalin at 4°C. The fixed tissue was embedded in paraffin, sectioned (10 µm), stained with hematoxylin and eosin and coded for examination by a blinded investigator. The investigator graded the parameters following the criteria as previously described by Nathan et al. 2005 (20) and Van Laethem et al. 1996 (30). Total histological score was the combined scores of inflammatory infiltrate, atrophy and fibrosis. Estimation of the number of inflammatory cells was done by counting infiltrating inflammatory cells (polymorphonuclear neutrophils, macrophages, etc.) present in a field of tissues at using a 40X objective. Ten different fields were used for each slide.

Sirius red staining was used to quantify fibrosis. After overnight fixation in 10% formalin the tissue specimens were embedded in paraffin. Sections (10 µm) were stained with 0.1% Sirius red F3B in saturated picric acid (both from Sigma, Aldrich, St Louis, MO) as previously
described (28). Photographs were taken at random using a 40X objective; three pictures per slide were obtained. Twelve animals per group were analyzed. Collagen density was quantified using a computerized image analysis system (Metavue, Universal Imaging Corp., Downingtown, PA).

Immunohistochemistry

Formalin fixed tissues were incubated with a rabbit monoclonal α smooth muscle actin (E184) antibody (Abcam, Massachusetts) at a dilution of 1:1000 and stained with 3,3’ diamino benzidine tetrahydrochloride (Dako, California). Pictures were taken with a 40X objective.

Quantitative real time PCR

RNAs from fresh pancreatic tissues were prepared using the Ribopure kit following the manufacturer’s recommendations (Life Technologies Corporation, California). Five μg of RNA was reverse transcribed to cDNA using random hexamers and SuperScript II Reverse Transcriptase (Life Technologies Corporation, California). Amplification was performed using a SYBR Green PCR master mix (Bioline, Tauton, MA). Each sample was analyzed in duplicate. Target gene levels in tissues from transgenic mice are shown as a ratio to levels detected in corresponding control tissue, according to the $2^{-\Delta\Delta CT}$ method (14). S9 was used as a housekeeping control gene.

Statistical analyses

Data were analyzed using one-way ANOVA analysis of variance with the Tukey post-test. Results were expressed as the mean ± standard error of the mean (SEM). Statistical significance
was set at $P < 0.05$. A confidence interval of 95% was used for trypsin activity determination using QuickCalcs Software (GraphPad Software Inc., San Diego, CA).
Results

Targeted expression of IL-1β to the pancreas produced marked inflammatory changes of the gland which were evident as early as six weeks of age in Tg(IL1β) mice. In order to assess the impact of disease on parameters typically associated with chronic pancreatitis, we evaluated pancreatic size, serum and pancreatic amylase levels, histological changes, tissue fibrosis and trypsin activity.

Pancreatic weight

In six week old mice the ratio of pancreas weight to body weight demonstrated that mice expressing IL-1β in pancreatic acini had much smaller pancreata compared to wild type mice or mice expressing PSTI-I (Fig. 1). The reduced pancreatic weight is consistent with pancreatic atrophy occurring in these animals. Animals expressing both IL-1β and PSTI-I also had reduced pancreatic weight. The pancreas/body weight ratio for the Tg(IL1β)-Tg(Psti1) co-expressing mice was slightly higher than for the mice expressing only IL-1β, however this difference was not statistically significant.

Pancreatic histology and acini density

Pancreata from Tg(IL1β) mice exhibited extensive changes in histoarchitecture (Fig. 2A). There was a substantial loss of acini and only a few acini appeared intact. Moreover, acini appeared to be replaced by marked fibrosis. In addition, a large number of inflammatory cells were present. These changes were less severe in pancreata from dual transgenic Tg(IL1β)-Tg(Psti1) mice where there were more intact acini, fewer inflammatory cells and reduced fibrosis. Quantitation of surface area occupied by acini in histologic sections of wt and Tg(Psti1)
mice was 94% and 92% of the pancreatic tissue. In contrast, the percentage of acini was reduced to 25% in Tg(IL1β) mice \( (P < 0.001 \text{ vs. wt or Psti1}) \) but only to 42% in the dual transgenic Tg(IL1β)-Tg(Psti1) mice, respectively \( (P < 0.001 \text{ vs. wt or Psti1 Tg IL1 β}) \) (Fig. 2B).

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**Serum and pancreatic amylase levels**

Serum amylase levels are often elevated in acute pancreatitis and reflect pancreatic injury (11). In chronic pancreatitis, however, amylase levels can be variable and may be influenced by the amount of acinar tissue residing in the pancreas (13). Amylase levels in the blood of six week old mice are shown in Figure 3A. Serum amylase levels were significantly reduced in Tg(IL1β) mice when compared with wild type or Tg(Psti1) mice \( (P < 0.05; n=18) \).

Pancreatic amylase was also reduced Tg(IL1β) and Tg(IL1β)-Tg(Psti1) pancreatic tissues, albeit to a lesser extent in the dual transgenic mice (Fig. 3B). Amylase levels in these mice were one-half of those of wild type mice while the Tg(IL1β) amylase levels were only 35% of the wild type levels. These results correlate with the observation of pancreatic tissue by H&E staining that showed significant reductions of acini in Tg(IL1β) and Tg(IL1β)-Tg(Psti1) mice (see Fig. 2A) and with the measurements of acinar area in the pancreatic tissues of the different strains of mice (see Fig. 2B).

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**Trypsin activity**

Since chronic pancreatitis causes a marked reduction in the amount of acinar tissue, we calculated the amount of active trypsin in relation to acinar area (Table 1). There was a
significant 3.5 fold increase in trypsin activity in Tg(IL1β) pancreata compared to wild type animals. In contrast, active pancreatic trypsin levels in the dual transgenic Tg(IL1β)-Tg(Psti1) mice were also elevated versus wild type mice – but significantly less than the active trypsin present in Tg(IL1β) pancreata. Interestingly, active trypsin was significantly reduced in pancreata of Tg(Psti1) mice compared with wild type animals. These data indicate that PSTI expression confers effective endogenous trypsin inhibition. Transgenic expression of PSTI-I in the pancreata of Tg(Psti1) mice did not alter the mRNA levels for the cationic trypsin (Prss1), the anionic trypsin (Prss2), the chymotrypsin (Ctrb2) and the elastase (Cela3b) genes (results not shown).

Pancreatic myeloperoxidase

To assess the extent of pancreatic inflammation we measured myeloperoxidase activity and inflammatory cell infiltration. Myeloperoxidase (MPO) is a commonly used indicator of neutrophil activity (Fig. 4A). Tg(IL1β) mice had a 150 fold increase in MPO levels in the pancreas compared to wild type pancreas. PSTI-I expression in pancreata of mice over-expressing IL-1β significantly reduced the MPO levels by two fold when compared with mice expressing only IL-1β (P < 0.001). Direct counting of inflammatory cells in tissue sections yielded very similar results to MPO measurement. Twice as many inflammatory cells were presents in pancreatic tissue from Tg(IL1β) mice compared to pancreata from the dual transgenic Tg(IL1β)-Tg(Psti1) mice (Fig. 4B).

Pancreatic collagen and alpha smooth muscle actin (αSMA)
Anti α-SMA immunostaining (Fig. 5A, a,b,c,d) and Sirius Red staining (Fig. 5A, e,f,g,h) were performed on sections from the same area of the pancreas. Abundant Sirius red staining was observed in pancreatic tissue from Tg(IL1β) mice (g). Alpha Smooth muscle actin was also present in locations where collagen was deposited (c) and staining was concentrated in diseased areas around ductal cells and between acinar cells. Reduced Sirius red staining (h) and limited α-SMA staining (d) were observed in pancreata of Tg(IL1β)-Tg(Pstl) mice.

In addition, gene expression of α smooth muscle actin (Acta2) and collagen (Col1a1) was assessed by real time PCR (Fig. 5B). Acta2 mRNA levels were significantly increased in Tg(IL1β) pancreata and were 2.3 greater than in the dual transgenic Tg(IL1β)-Tg(Pstl) pancreata (P < 0.05). The expression level of the Acta2 mRNA from Tg(IL1β)-Tg(Pstl) mice was not significantly different from the wild type animals. Col1a1 mRNA was also elevated in Tg(IL1β) pancreata. Col1a1 mRNA was increased over 20 fold in the pancreata of Tg(IL1β) mice compared to wild type animals. Col1a1 mRNA levels were 45% lower in Tg(IL1β)-Tg(Pstl) mice (P < 0.05) although they were increased over wild type mice. These results are consistent with the data obtained from quantification of Sirius red staining.

Image analysis of histological sections demonstrated that 45.2 ± 2.4% of the surface area in the pancreata of Tg(IL1β) mice stained for collagen compared to 31.8 ± 1.7% in the pancreata of Tg(IL1β)-Tg(Pstl) mice (P < 0.001) (Fig. 6). These findings indicate that pancreatic collagen deposition was reduced in mice expressing both PSTI-I and IL-1β and suggest that PSTI-I reduced the fibrotic changes elicited by IL-1β over-expression.
In the current study we utilized a novel model of pancreatitis in which the IL-1β gene was targeted to acinar cells in the mouse pancreas. This transgene produced marked inflammation in the pancreas and mice developed changes of chronic pancreatitis characterized by loss of acinar cells, inflammatory cell infiltration, and substantial fibrosis. The onset of pancreatic damage was rapid, occurring within four weeks, and was uniform in all mice examined by six weeks of age. This model appears to be a valuable contribution to the experimental repertoire available to pancreatic investigators, particularly because it offers a unique method for inducing pancreatic inflammation. We considered the model to be useful for these studies because it allowed us to evaluate the potential role of trypsin inhibitor in an experimental model that previously had not been described as trypsin dependent. Two major lines of evidence suggest that IL-1β-induced pancreatitis involves trypsin activation. First, increased levels of pancreatic active trypsin were found in the pancreas in this model. Second, PSTI-I over-expression caused a decrease in pancreatic trypsin activity in this same model.

Cytokine expression appears to trigger neutrophil infiltration in the pancreas (1, 2, 4). Interestingly, neutrophil depletion was shown to reduce the activity of intra-pancreatic trypsin (9) suggesting that neutrophils interact with the acinar compartment and play a role in the pathogenesis of acute pancreatitis. IL-1β and TNF-α are two major cytokines expressed early during acute pancreatitis (10, 25). It has been demonstrated that IL-1β is responsible for amplification of the inflammatory response through activation of other cytokines, cellular adhesion molecules and leukocyte recruitment in models of sterile inflammation (5, 17). It is likely that IL-1β expression and signaling involved in the initiation of pancreatitis and prolonged expression may maintain or amplify ongoing inflammation.
In the current study, we confirmed that Tg(IL-1\(\beta\)) mice develop severe chronic pancreatitis with marked pancreatic atrophy. Loss of pancreatic tissue was apparent in both Tg(IL1\(\beta\)) mice and dual transgenic [Tg(IL1\(\beta\)) - Tg(Pst1)] mice indicating that IL-1\(\beta\) transgene expression induced severe pancreatic damage. A slight improvement in pancreatic weight loss was conferred by PSTI-I expression. All other parameters of pancreatitis severity that were measured were improved by PSTI-I over-expression. It is particularly worth noting that pancreatic MPO levels were extremely high in Tg(IL1\(\beta\)) mice. In our experience, these are the highest MPO levels that we have observed in any model of experimental pancreatitis. The observation that PSTI-I expression reduced MPO levels suggests that ongoing trypsin activity plays a role in neutrophil recruitment in chronic pancreatitis.

Serum amylase levels are one of the most commonly used measurements in evaluating the development of pancreatitis. In experimental models and clinical pancreatitis, serum amylase indicates pancreatic injury that can be either acute or ongoing. Production of amylase, however, requires release or dysregulated secretion from acinar cells. When substantial acinar cell mass is lost it is reasonable to expect that serum amylase levels will not be elevated even with ongoing pancreatic disease. Therefore, serum amylase may not be a reliable marker of chronic pancreatitis. In the current study, serum amylase levels were significantly reduced in IL-1\(\beta\)-induced chronic pancreatitis. These findings likely reflect the loss of acinar cell mass that was suggested by measurements of pancreatic weight and confirmed by histological evaluations of pancreatic tissue sections and by measurement of the amount of pancreatic tissue occupied by acinar cells.

Pancreatic histology is considered to be the most sensitive measure of chronic pancreatitis and is characterized by acinar cell destruction, chronic inflammation, and tissue
fibrosis. Alpha smooth muscle actin is a marker for activation of pancreatic stellate cells (27). In cultured pancreatic stellate cells pro-inflammatory cytokines, in particular IL-1, increase levels of α-SMA (18) and type 1 collagen expression upon stellate cell activation (16). We observed that α-SMA and collagen type 1 mRNA were elevated in Tg(IL1β) mice and were more abundant than in dual transgenic Tg(IL1β)-Tg(Psti1) mice. This finding indicated that pancreatic stellate cells were activated in Tg(IL1β) mice but to a significantly lesser extent in Tg(IL1β)-Tg(Psti1) mice. Although PSTI-I expression reduced the extent of pancreatic fibrosis, our in vivo findings cannot distinguish between a direct effect of active trypsin on stellate cell activation or an indirect effect through other possibly inflammatory pathways.

All of the features of chronic pancreatitis that we measured were induced by IL-1β expression and were ameliorated in PSTI-expressing mice. Pancreatic fibrosis is responsible for many of the complications of chronic pancreatitis in humans including pancreatic duct and bile duct strictures and is a topic of intense investigation. The current study suggests that inhibition of trypsin activity could have a significant impact on the generation of pancreatic inflammation and development of pancreatic fibrosis. It is conceivable that strategies to reduce pancreatic fibrosis could be of clinical therapeutic benefit in chronic pancreatitis.
Acknowledgements

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Table legend

Table 1. Pancreatic trypsin activity. Trypsin activity of pancreatic extracts was measured as described in Materials and Methods and results were expressed as pmole/mg protein/acinar area.
Figure legends

**Figure 1. Effect of acinar cell expression of IL-1β on pancreatic size**

The ratios of pancreatic weight to body weight in wild type, Tg(PstI), Tg(IL1β), and Tg(IL1β)-Tg(PstI) mice are shown. All animals were six weeks old. Results are expressed as mean ± SEM (n=12). *** = P < 0.001 vs. wild type or Tg(PstI) mice.

**Figure 2. Effect of IL-1β expression on pancreatic histoarchitecture and acinar density**

A: Representative histological sections of mouse pancreas stained with hematoxylin and eosin from in (a) wild type, (b) Tg(PstI), (c) Tg(IL1β), and (d)Tg(IL1β)-Tg(PstI) mice are shown. Photographs were taken with a 10X objective. Bar = 500 μm.

B: Acinar density. Acinar density was determined by measuring the percentage of area covered by acini in a specific field. 6 independent fields were analyzed per animal. Results are expressed as the mean ± SEM (n=3-6). *** = P < 0.001 vs. wild type, Tg(PstI) or Tg(IL1β). # = P < 0.001 vs. wild type, Tg(PstI) or Tg(IL1β)-Tg(PstI).

**Figure 3. Effect of IL-1β expression on serum and pancreatic amylase levels**

A: Serum amylase levels in wild type, Tg(PstI), Tg(IL1β), and Tg(IL1β)-Tg(PstI) mice at six weeks of age are shown and are expressed as mean ± SEM (n=18 for wt, Tg(PstI) and Tg(IL1β); n=14 for Tg(IL1β)-Tg(PstI)). * = P < 0.05 vs. wild type or Tg(PstI) mice.
B: Pancreatic amylase levels in wild type, Tg(Psti1), Tg(IL1β), and Tg(IL1β)-Tg(Psti1) mice at six weeks of age are expressed as mean ± SEM (n=6). *** = \( P < 0.001 \) vs. wild type or Tg(Psti1). # = \( P < 0.001 \) vs. wild type. ** = \( P < 0.01 \) vs. Tg(IL1β).

**Figure 4. Effect of pancreatic IL-1β expression on pancreatic MPO activity and inflammatory cells infiltration**

A: MPO activity in pancreatic extracts is expressed as units/mg protein. Results are shown as mean ± SEM (n=12). *** = \( P < 0.001 \) vs. wild type, Tg(Psti1) or Tg(IL1β)-Tg(Psti1) mice. # = \( P < 0.001 \) vs. wild type, Tg(Psti1) or Tg(IL1β) mice.

B: Inflammatory cell abundance in pancreatic tissues stained with H&E. Ten independent fields per slide were analysed for presence of inflammatory cells using a 40X objective. Results are expressed as mean ± SEM (n=2 for Tg(Psti1) mice; n=3 for wild type; n=6 for Tg(Psti1) or Tg(IL1β)-Tg(Psti1) mice). *** = \( P < 0.001 \) vs. wild type, Tg(Psti1) or Tg(IL1β)-Tg(Psti1) mice. ** = \( P < 0.01 \) vs. wild type and Tg(Psti1) mice.

**Figure 5. Effect of IL-1β expression on pancreatic α-SMA and collagen**

A: Representative pictures from pancreas sections stained with anti α-SMA from: (a) wild type, (b) Tg(Psti1), (c) Tg(IL1β), and (d) Tg(IL1β)-Tg(Psti1) mice are shown. Photographs were taken with a 40X objective. Bar = 100 μm. Sections were stained for collagen with Sirius red: (e) wild type, (f) Tg(Psti1), (g) Tg(IL1β), and (h) Tg(IL1β)-Tg(Psti1).
B: Quantitative real time PCR of Acta2 mRNA (left panel) and Col1a1 mRNA (right panel) using the \( \Delta \Delta C_T \) method. Results are expressed as mean ± SEM (n=3).

Left panel: * = \( P < 0.05 \), \( IL1\beta \) vs. \( IL1\beta\)-Psti1; ** = \( P < 0.01 \), \( IL1\beta \) vs. wt and Psti1.

Right panel: * = \( P < 0.05 \), \( IL1\beta \) vs. \( IL1\beta\)-Psti1; ** = \( P < 0.01 \), \( IL1\beta\)-Psti1 vs. wt and Psti1; *** = \( P < 0.001 \), \( IL1\beta \) vs. wt and Psti1.

Figure 6: Histomorphometric analyses of Sirius red staining

Histomorphometric analyses were performed as described in Methods and represent surface area stained red. There was no appreciable Sirius red staining in wild type or Tg(Psti1) mouse pancreas. Results are expressed as mean ± SEM (n=12). *** = \( P < 0.001 \), \( IL1\beta \) vs. wt, Psti1 and \( IL1\beta\)-Psti1; \( \delta\delta\delta \) = \( P < 0.001 \), \( IL1\beta\)-Psti1 vs. wt, Psti1 and \( IL1\beta \).
References


A

MPO (Units/mg protein)

wt  Psti1  IL1β  IL1β

wt  Psti1  IL1β  IL1β

B

Inflammatory cells/mm²

wt  Psti1  IL1β  IL1β

Psti1
Col1a1 mRNA (fold increase)

Acta2 mRNA (fold increase)
<table>
<thead>
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<th>genotype</th>
<th>Trypsin activity (pmole x mg⁻¹)/% acinar area mean (95% CI)</th>
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<tr>
<td>wt</td>
<td>1.59 (1.42-1.76)</td>
</tr>
<tr>
<td>Tg(Psti1)</td>
<td>0.47 (0.32-0.62)</td>
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<tr>
<td>Tg(IL1β)</td>
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<tr>
<td>Tg(IL1β)-Tg(Psti1)</td>
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