Endogenous interleukin-10 modulates fibrosis and regeneration in experimental chronic pancreatitis

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Endogenous interleukin-10 modulates fibrosis and regeneration in experimental chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 282: G1105–G1112, 2002; 10.1152/ajpgi.00431.2001.—Interleukin (IL)-10, a potent anti-inflammatory cytokine, limits the severity of acute pancreatitis and downregulates transforming growth factor (TGF)-β release by inflammatory cells on stimulation. Proinflammatory mediators, reactive oxygen species, and TGF-β can activate pancreatic stellate cells and their synthesis of collagen I and III. This study evaluates the role of endogenous IL-10 in the modulation of the regeneration phase following acute pancreatitis and in the development of pancreatic fibrosis. IL-10 knockout (KO) mice and their C57BL/6 controls were submitted to repeated courses (3/wk, during 6 wk, followed by 1 wk of recovery) of cerulein-induced acute pancreatitis. TGF-β1 release was measured on plasma, and its pancreatic expression was assessed by quantitative RT-PCR and immunohistochemistry. Intrapancreatic IL-10 gene expression was assessed by semiquantitative RT-PCR and immunohistochemistry. Intrapancreatic collagen content was assessed by picrosirius staining. Activated stellate cells were detected by immunohistochemistry. S phase intrapancreatic cells were marked using tritiated thymidine labeling. After repeated acute pancreatitis, IL-10 KO mice had more severe histological lesions and fibrosis (intrapancreatic collagen content) than controls. TGF-β1, plasma levels, intrapancreatic transcription, and expression by ductal and interstitial cells, as well as the number of activated stellate cells, were significantly higher. IL-10 KO mice disclosed significantly fewer acinar cells in S phase, whereas the opposite was observed for pseudotubular cells. Endogenous IL-10 controls the regeneration phase and limits the severity of fibrosis and glandular atrophy induced by repeated episodes of acute pancreatitis in mice.

experimental chronic pancreatitis; pancreatic fibrosis; transforming growth factor-β; pancreatic stellate cells

VARIOUS OVERLAPPING MECHANISMS have been shown to be involved in the pancreatic fibrogenesis. They included the necrosis-fibrosis sequence (19), a direct inflammatory pathway (4, 8, 38), the ischemia-reperfusion phenomenon (30), alcohol toxicity (directly and/or via reactive oxygen intermediates and acetaldehyde) (1, 25), and T lymphocyte-induced cytotoxicity (7, 16, 37). The pancreatic stellate cells (PSC), similar to hepatic stellate cells, also play a major role in this process (6). These intrapancreatic resident periacinar and interlobular fibroblastlike cells disclose the characteristics of myofibroblasts (2, 6). On stimulation, they are able to express α-smooth muscle actin (α-SMA), to have contractile properties, to increase their synthesis of type I (and also types III and IV) collagen, laminin, and fibronectin, to form microfilaments, and to increase their own cytokine synthesis leading to autocrine regulation (2, 6). So far, identified major activators of PSC are transforming growth factors (TGF-α and -β1), platelet-derived growth factor, reactive oxygen intermediates, ethanol, and acetaldehyde (3, 4, 6). Activated PSC have been identified in human and experimental fibrotic areas from chronic pancreatitis tissues (15, 17) and seem to play a major role in the development of fibrosis in chronic pancreatitis (2–4, 6).

On the other hand, TGF-β1, a direct PSC activator, is expressed by ductal and interstitial cells in chronic pancreatitis (20, 38) and seems to be involved in glandular atrophy through an antiproliferative effect on acinar cells (3, 6) observed in chronic pancreatitis. Interestingly, TGF-β1 is also released during the early course of acute pancreatitis and during its regeneration phase, playing a major role in the regulation of repair mechanisms (12, 14, 32).

Interleukin (IL)-10 is a potent anti-inflammatory cytokine (27) released during the course of experimental acute pancreatitis, which is known to limit its severity by downregulating proinflammatory mediator release (21, 33, 39, 40). IL-10 also has direct antiproliferative and antifibrotic properties (24, 27, 31, 36).

The present study aims to assess the potential role of endogenous IL-10 in modulating the pancreatic repair phase following acute pancreatitis and the develop-
ment of chronic pancreatic lesions and fibrosis in an experimental murine model of chronic lesions induced by repeated acute pancreatitis.

METHODS

Animals and Experimental Design

Eight- to ten-week-old C57BL/6 (IFFA-CREDO, Brussels, Belgium) and IL-10 knockout (KO)-targeted mutant (IL-10 KO) female mice (specific pathogen free) were used for these experiments. All animals were maintained in our animal facilities on standard laboratory chow and received care in compliance with the national legal requirements and the National Institutes of Health guidelines.

Acute pancreatitis was induced by five intraperitoneal injections of 50 μg/kg cerulein (Pharmacia-Ujphor, Brussels, Belgium) at hourly intervals. Its severity was assessed 6 h (T6) after the beginning of cerulein challenge.

For studying the regeneration phase after acute pancreatitis, mice were killed by cervical dislocation before [day 0 (D0)] and at days 1 (D1), 2 (D2), 7 (D7), and 10 (D10) after a single cerulein-induced acute pancreatitis.

Chronic lesions were induced by repeated acute pancreatitis episodes as previously described (28). Mice were submitted to three episodes of acute pancreatitis per week, during six consecutive weeks, and then killed by cervical dislocation 1 wk later.

Blood samples were obtained by direct intracardiac puncture. Pancreata were immediately removed, embedded in Optimum Cutting Temperature compound (Tissue-Tek, Sakura, Zoeterwoude, the Netherlands), then frozen in liquid nitrogen and stored at −80°C until assayed (collagen measurements). Pancreatic tissues were also fixed in formaldehyde for histology, autoradiography after thymidine labeling and immunohistochemistry, or directly harvested for RT-PCR assays.

Blood Assays

Serum amylases and lipases were measured by using automated chromogenic and turbidimetric assays at 37°C. Results were expressed in international units per liter. Active TGF-β1 levels were determined by using a commercially available Elisa kit (TGF-β1, Kit, Genzyme, Cambridge, MA) of which detection limits were 50 pg/ml.

Histological Grading of Pancreatic Lesions

Hematoxylin-eosin (H&E) staining was performed on 6-μm pancreatic sections. Severity of acute pancreatitis was blindly graded at T6, by a semiquantitative assessment of edema, inflammatory cell infiltrate, and acinar necrosis according to the score previously described (40).

Severity of the chronic lesions was graded by a semiquantitative scoring system. Within pancreatic sections, areas of abnormal pancreatic tissue architecture were observed. These areas were more or less numerous, and this first parameter was graded as follows: 0 = absent, 1 = rare, 2 = minimal (<10%), 3 = moderate (<50%), and 4 = major (>50%) of the total parenchyma affected. Within these areas, glandular atrophy (0 = absent, 1 = minimal, 2 = moderate, and 3 = severe), presence of pseudotubular complexes (0 = absent, 1 = minimal, 2 = moderate, and 3 = major) and fibrosis (0 = absent, 2 = only within areas, and 4 = diffuse) were observed and graded as described. A total score was calculated for each pancreatic section.

Immunohistochemical Staining of Pancreatic TGF-β1 and Stellate Cells

Immunohistochemistry was performed on pancreatic tissue samples fixed in formaldehyde by using the streptavidin-peroxidase method (29, 37), with some modifications. The primary antibodies were affinity-purified rabbit anti-mouse TGF-β1 (V) polyclonal antibody (IgG; Santa Cruz Biotechnology, Santa Cruz, CA) for TGF-β1 staining, purified mouse anti-pig desmin monoclonal antibody (IgG1; Sigma, Saint Louis, MO) for desmin staining, purified mouse anti-pig glial fibrillary acidic protein (GFAP) monoclonal antibody (IgG1; BioGenex, San Ramon, CA) for GFAP staining, and purified mouse anti-mouse α-SMA monoclonal antibody (IgG2a; Sigma) for α-SMA staining. Paraffin-embedded sections were incubated in fresh xylene for 2 × 8 min and then dipped in 100% ethanol for deparaffinization. Endogeneous peroxidases were blocked with 1% H2O2 for 30 min. To block nonspecific binding, sections were incubated in PBS (Optimax Wash Buffer, BioGenex), 1% BSA, and normal sheep serum (1:20) for 60 min. Primary antibodies (1:50 dilution for desmin and α-SMA, 1:25 dilution for GFAP, and 1:200 dilution for TGF-β1) were added overnight at 4°C. Slices were then rinsed with PBS. Contacts with secondary biotinylated antibody [donkey anti-rabbit Igg or sheep anti-mouse Igg, species-specific, 1:200 diluted (Amersham Pharmacia Biotech, Little Chalfont, UK)] were maintained for 60 min. After PBS rinsing, slices were incubated with 1:650 peroxidase solution (Streptavidin biotinylated horseradish peroxidase complex, Amersham Pharmacia Biotech) for 30 min. Peroxidase activity was visualized by using diaminobenzidine H2O2 (Liquid DAB Substrate, BioGenex) as substrate with a reaction time of 10 min at room temperature. Counterstaining was performed with Mayer’s hematoxylin. Specificity controls consisted of substituting the primary antibody by irrelevant monoclonal antibody from the same isotype on an adjacent section of the sample studied. Positive GFAP and desmin staining were used to identify PSC within cells showing a positive α-SMA immunostaining, achieved by staining of serial sections. Results for TGF-β1 and α-SMA immunostaining were expressed as a semiquantitative score. On the one hand, the number of foci with positive immunostained cells was evaluated as follows: 0 = absent, 1 = rare, 2 = <10%, 3 = <50%, and 4 = >50% of the total parenchyma on the section, and on the other hand, the intensity of staining per focus was noted: 1 = minimal, 2 = moderate, and 3 = strong.

Tritiated Thymidine Pulse Labeling

To identify intrapancreatic S phase cells, mice were injected intraperitoneally with 1 μCi/g body wt (diluted in 100 μl saline total volume) [methyl-3H]thymidine (Amersham Life Science, Little Chalfont, UK) 1 h before being killed (13). Pancreas samples were fixed in formalin during 6 h. Exposure time for autoradiography was 21 days. Sections were then counterstained with H&E and mounted using Depex mounting medium (DAKO, Merelbeke, Belgium) (13). Positive nuclei (showing silver grains) were counted in 10 consecutive (nonoverlapping) high-power fields (HPF) of 2,500 μm2 each, on the same section, at a ×400 magnification. Results were expressed as number of positive cells (acinar or pseudotubular or both) per 10 HPF.
**TGF-β1 and IL-10 Gene Expression in Pancreata**

Total RNA was isolated from fresh pancreata with 5 ml TRIzol (Boehringer Mannheim, Mannheim, Germany) and was quantified by ultraviolet (UV) absorbance at 260 nm. To prime the RT, 500 ng of RNA were incubated with 0.2 μg oligo(dT) at 60°C during 10 min. The prepared complementary DNA was subjected to RT-PCR with murine-specific primers for β-actin (sense primer: TGG-AAT-CCT-GTG-GCA-TCC-ATG-AAA-C; antisense primer: TAA-AAC-GCA-GCT-TAG-TAA-CAG-TCC-G), for TGF-β1 (sense primer: ACC-GCA-ACA-ACG-CCA-TCT-AT; antisense primer: GTA-ACG-CCA-GGA-ATT-GTT-GC), and for IL-10 (sense primer: ATG-CAG-GAC-TTT-AAG-GGT-TAC-CTG; antisense primer: CTA-GAC-ACC-TTG-GTC-TTG-GAG-CTT-A). The RT products were amplified respectively for 34 cycles with each specific primer. Electrophoresis was performed on a 2% agarose gel added with ethidium bromide and then photographed under UV illumination.

For TGF-β1, quantitative RT-PCR was then performed using the Quantitative Competitive PCR Protocol (mouse TGF-β, Maxim Biotech, San Fransisco, CA). Results were expressed in attomoles of TGF-β1/H11021, Maxim Biotech, San Francisco, CA). Results were expressed as means ± SE. Each experiment was performed using Wilcoxon, ANOVA, and Newman-Keuls correction for multiple comparisons, when appropriate. P values < 0.05 were considered as significant.

**Intrapancreatic Collagen Quantification**

Sirius red staining of collagen (18, 24) was used for quantification of total intrapancreatic collagen content.

From each pancreas, three cryostat sections of 10 μm were cut and air dried for 20 min. After the slices were rinsed three times for 3 min in PBS, they were fixed in Susa solution (4.5 g HgCl, 0.5 g NaCl, 10 ml 20% trichloroacetic acid, 4 ml acetic acid, 20 ml formalin, and 70 ml distilled water) for 5 h at room temperature and then rinsed three times for 1 min in distilled water. They were then stained in 0.1% Sirius red F3BA (chroma) in saturated picric acid solution for 45 min at room temperature. Slices were rinsed once for 40 s with water and then for 40 s in a 0.01 M HCl solution. After 2 h air drying, slices were mounted using Depex mounting medium (DAKO).

Quantitative analysis of collagen was performed by morphometric analysis (11, 18). A digitized picture of each pancreatic section, viewed through a Zeiss Axioplan microscope (Carl Zeiss, Iena, Germany) equipped with a ×4 objective lens, was transmitted by a Sony 3-CCD video camera to a Dell 300-MHz PC equipped with a KS 300 imaging system (Kontron Elektron, Munich, Germany). The total amount of collagen stained on each submitted section was calculated by the computer via the digitalized image as follows. In the first step, pancreas was distinguished from the background according to a difference in light density, and a measurement of the total pancreatic tissue area was performed. In the second step, the amount of collagen (stained in red) was also measured and was finally expressed as a percentage of the total pancreatic surface.

**Statistical Analysis**

Data are expressed as means ± SE. Each experiment relies on 15 mice per group and per time point. Statistical analysis was performed using Wilcoxon’s, ANOVA, and Newman-Keuls correction for multiple comparisons, when appropriate. P values < 0.05 were considered as significant.

**RESULTS**

**Modulation of the Repair Phase After a Single Course of Acute Pancreatitis**

To investigate whether a modulation of regeneration and repair phase was already present after a single course of acute pancreatitis, mice were killed before (D0) and 6 h and 1, 2, 7, and 10 days after cerulein-induced acute pancreatitis.

As previously shown, IL-10 KO mice disclosed more severe acute pancreatitis 6 h after starting cerulein injections [severity score of histological lesions calculated as previously described (40); 6.3 ± 0.2 vs. 4.9 ± 0.3 for IL-10 KO and control mice, respectively; P < 0.05] and serum hydrolase levels [amylasses 27302.8 ± 1008.6 vs. 17110.7 ± 1008.0 IU/ml (P < 0.05) and lipases 3657.2 ± 200.1 vs. 2130.2 ± 145.6 IU/ml (P < 0.05) for IL-10 KO and control mice, respectively].

**TGF-β1 Expression During Regeneration Phase**

The baseline (D0) plasma levels of TGF-β1 were similar in controls and IL-10 KO mice (42 ± 1.7 vs. 41.7 ± 0.6 ng/ml; P > 0.05; Fig. 1), and the protein was not detected within the pancreas by immunohistochemistry.

Seven days after acute pancreatitis, TGF-β1 plasma levels were significantly higher in IL-10 KO mice (108.3 ± 5.2 vs. 83.6 ± 5.3 ng/ml for IL-10 KO and control mice, respectively; P < 0.05; Fig. 1). Similarly, immunohistochemical expression of TGF-β1 in both acinar and ductal cells was more prominent (scores were 6.2 ± 0.2 and 4.2 ± 0.2 in IL-10 KO and control mice, respectively; P < 0.05). Return to baseline values was observed for both groups of mice on D10.

**Intrapancreatic Collagen Deposition During the Regeneration Phase**

Total amount of pancreatic collagen was similar in the two groups at the basal state (4.2 ± 0.3 vs. 3.9 ± 0.4% in IL-10 KO and control mice, respectively). An increase in collagen content (compared with basal val-
Regeneration Phase

on D10. Return to baseline values was observed for both groups of mice.

values) was observed at D7 and was significantly higher in IL-10 KO (11.8 ± 0.6 vs. 9.1 ± 0.3% in IL-10 KO and control mice, respectively; P < 0.05; Fig. 2). Return to baseline values was observed for both groups of mice on D10.

Acinar Cell Proliferation During the Regeneration Phase

At the basal state, the number of [3H]thymidine-labeled acinar cells in the S phase of mitosis was comparable in IL-10 KO and control mice (3.2 ± 0.6/10 HPF vs. 3.0 ± 0.4/10 HPF, respectively). At D7, a significant increase in this number was observed in controls, whereas it was dramatically lower in IL-10 KO mice: 12 ± 0.9/10 HPF vs. 4.0 ± 0.3/10 HPF for controls and IL-10 KO, respectively (P < 0.05; Fig. 3). Return to baseline values was observed for both groups of mice on D10.

Endogenous IL-10 Limits Fibrosis and Modulates Pancreatic Cell Proliferation After Repeated Courses of Acute Pancreatitis

IL-10 KO mice disclosed more severe pancreatic histological lesions, collagen deposition, and TGF-β1 expression than controls. No death was observed in both groups of mice. After 6 wk of repeated courses of acute pancreatitis, histological lesions (atrophy, focal lesions, and pseudotubular complexes) and fibrosis were observed in both groups of mice but were significantly more severe in IL-10 KO mice [with regard to the histological score: 10.9 ± 0.7 vs. 7.9 ± 1.2 (P < 0.05; Fig. 4, A and B); with regard to the total amount of pancreatic collagen content: 19.7 ± 1.3 vs. 15.0 ± 0.6% (P < 0.05; Fig. 4, C and D) for IL-10 KO vs. controls, respectively]. TGF-β1 plasma levels were significantly increased compared with basal values (D0) in both groups of mice, with significantly higher values for IL-10 KO mice (53.3 ± 1.5 vs. 47.6 ± 1.2 ng/ml; P < 0.05).

Similarly, intrapancreatic TGF-β gene expression was higher for IL-10 KO (14 × 10⁻⁷ ± 3 × 10⁻⁷ vs. 4 × 10⁻⁷ ± 1 × 10⁻⁷ amol/β-actin; P < 0.05) by using quantitative RT-PCR (Fig. 5). By immunohistochemistry, TGF-β1 expression in interstitial and ductal cells was clearly more prominent in the group of IL-10 KO mice than in controls: grading scores were, respectively, 4.7 ± 0.7 vs. 2.6 ± 0.6 (P < 0.05; Fig. 4, E and F).

Endogenous IL-10 intrapancreatic gene transcription is upregulated in control mice after repeated acute pancreatitis. IL-10 gene transcription was clearly increased after repeated courses of acute pancreatitis, compared with basal state in wild-type mice [0.052 ± 0.002 vs. 0.342 ± 0.018 (P < 0.05), respectively, at baseline and after repeated acute pancreatitis].

Endogenous IL-10 controls the activation of stellate cells. In both groups, a positive α-SMA staining was observed, demonstrating the presence of activated stellate cells (previously identified by double-staining desmin and GFAP on serial sections). α-SMA staining was stronger for IL-10 KO mice pancreata, in which it was predominantly located around acini and pseudotubular complexes and in focal areas of chronic lesions. Grading scores were 4.1 ± 0.6 for IL-10 KO mice vs. 2.4 ± 0.2 for controls (P < 0.05; Fig. 4, G and H).

Endogenous IL-10 downregulates the proliferation of acinar cells and upregulates that of pseudotubular cells. After 6 wk of repeated acute pancreatitis courses, both groups of mice disclosed higher numbers of S phase-labeled acinar cells compared with basal state (3- and 5-fold for IL-10 KO and controls, respectively). [3H]thymidine-labeling index was significantly higher for controls when measuring the total number of intrapancreatic-labeled cells (acinar + pseudotubular cells: 20.8 ± 0.5/10 HPF vs. 16 ± 1.5/10 HPF; P < 0.05) and the number of acinar-labeled cells [18.1 ± 1.6/10 HPF vs. 10.1 ± 1.1/10HPF; P < 0.05; Fig. 6, A and B] in control vs. IL-10 KO mice, respectively.

DISCUSSION

The major message to be taken from this study is that, after repeated acute pancreatitis in mice, endogenous IL-10 limits fibrogenesis, collagen deposition, and TGF-β1 expression. This is associated with a modulation of the regeneration phase consisting of a dramatic decrease of acinar cell proliferation of IL-10 KO.
IL-10 KO mice also disclose more activated stellate cells in the pancreatic parenchyma.

It was known from previous studies that both the endogenous production of IL-10 as well as its exogenous administration downregulate the severity of experimental acute pancreatitis. They limit acinar necrosis, parenchymal infiltration by polymorphonuclear cells and macrophages, and release of proinflammatory mediators [including IL-1, IL-6, tumor necrosis factor-α (TNF-α) and chemokines such as MCP-1] (21, 33, 39, 40).

After the acute pancreatitis injury, a regeneration or repair phase of the pancreas is characterized by the decreases of inflammatory cell infiltrate and of the release of proinflammatory mediators (9, 10, 43). A proliferation of acinar cells occurs as soon as 72 h after a cerulein-induced acute pancreatitis in rats and persists for at least 1 wk, with restoration of the acinar cell ratio (10, 43). TGF-β1 production and release by acinar and inflammatory cells starts 24 h after the induction of acute pancreatitis, peaks after 48 h, and returns to basal values only after 1–2 wk (14). Therefore, TGF-β1

Fig. 4. Chronic histological lesions (after repeated AP treatment) stained with hemotoxylin and eosin (H&E) at ×200 magnification. IL-10 KO (A) disclosed more prominent glandular atrophy, fibrosis, and tubular complexes than controls (B). Picrosirius-stained pancreas (collagen is stained red, noncollagen tissue is stained yellow) after repeated AP in controls (D) and in IL-10 KO mice (C) is shown at ×200 magnification. TGF-β1 immunohistochemical staining (brown staining) of interstitial and ductal cells in chronic pancreatic lesions in control mice (F) and in IL-10 KO mice (E) is shown at a ×200 magnification. α-Smooth muscle actin immunohistochemical staining (orange-brown staining) of interstitial cells in chronic pancreatic lesions in control mice (H) and in IL-10 KO mice (G) is shown at a ×200 magnification.
probable role in the synthesis of collagen, which occurs during the repair phase of the pancreas, with peaking pancreatic values at 1 wk. After a single episode of acute pancreatitis, this collagen is destroyed and the pancreatic content returns to preinjury values within 2 wk (10, 43). PSC are key cells responsible for the production of collagen (essentially types I and III) in the pancreas, and their activity is modulated in vitro by oxidative stress, TGF-β, and IL-10 (3, 4, 6). In the model of secretagogue acute pancreatitis (cerulein induced), regeneration phase leads to a complete restitution of the parenchyma within 2 wk (even in IL-10 KO mice). The fact that acute pancreatitis episodes were induced three times per week (i.e., mice were rechallenged with cerulein before recovery from the previous episode) led to an accumulation of lesions in a parenchyma still in regeneration and finally to chronic lesions in the model of chronic pancreatitis we used.

In vivo experiments have demonstrated that during that frame of events, endogenous IL-10 downregulates the release and the intrapancreatic expression of TGF-β1 by acinar and interstitial cells. This probably results from the higher severity of the primary pancreatic injury in the absence of endogenous IL-10, leading to a more pronounced stimulation of TGF-β1 release by proinflammatory mediators. Moreover, a direct role of IL-10 can be involved, as this cytokine has a direct inhibitory effect on TGF-β1 production (27).

TGF-β1 is the most potent activator for collagen synthesis by PSC. Other cytokines, such as IL-6, IL-1, or TNF-α, are known to stimulate the growth of hepatic stellate cells. However, their potential effect on PSC has not yet been demonstrated (3, 6). Because TGF-β1 production is upregulated during the repair phase after acute pancreatitis in IL-10 KO mice, it is not surprising to note an increase in pancreatic collagen content of IL-10-deficient mice. However, plasma levels as well as interstitial cell expression of TGF-β1 are higher during the regeneration phase than during the chronic phase. This would suggest that TGF-β1 is not the only mediator implicated in the development of fibrogenesis observed in this model. Therefore, IL-10 can be indirectly implicated in limiting pancreatic collagen deposition following acute pancreatitis. Moreover, it was previously shown that IL-10 also has anti-fibrotic properties on fibroblastic cells by directly downregulating procollagen I and enhancing collagenase gene expression in vitro. It could, thereby, play in addition a direct role in downregulating pancreatic collagen synthesis besides its modulation of TGF-β1 secretion (9, 10, 12, 43).

After cerulein-induced acute pancreatitis, a peak of acinar cell proliferation occurs (9), followed by the restoration of the amount of acinar cells within 1 wk. We presently observed that 7 days after acute pancreatitis induction, the number of acinar cells in S phase was lower in the IL-10-deficient mice despite the increased severity of pancreatitis. After repeated courses of acute pancreatitis, the number of acinar cells in S phase was still higher than at the basal state but statistically lower in IL-10 KO mice. This could be attributed to a different expression of TGF-β1 in these mice. Indeed, it is well established that TGF-β1 has a negative effect on epithelial and acinar cell proliferation (8, 23, 35, 41). This differential effect of IL-10 could participate in the histological remodeling occurring in chronic pancreatitis directly or via the modulation of TGF-β1. In this setting, our results suggest that the inbalance between TGF-β1 and IL-10 is of major importance not only for the transformation of the interstitial cell compartment, but also for the modulation of exocrine cells. Similarly, a loss of acinar cells and the development of tubular structures and fibrosis were also observed in TGF-α and -β1 transgenic mice (22, 34, 42).
Endogenous IL-10 limits the development of glandular atrophy, fibrosis, and pseudotubular complex formation in the present model. We hypothesized that repeated pancreatic infiltration by inflammatory cells and in situ release of proinflammatory cytokines would lead to a persistent activation of PSC. This could be amplified if the TGF-β1 and proinflammatory effects are not counterbalanced by the downregulating action of endogenous IL-10. Moreover, TGF-β1 can be upregulated via an autocrine loop (26), and subsequently self-regulated events, such as inflammation, tissue repair, and extracellular matrix deposition, but also acinar cell proliferation, could evolve to uncontrolled and persistent fibrogenesis and acinar cell atrophy in case of unbalanced regulation and overactivation of PSC, as presently observed in IL-10 KO mice after repeated acute pancreatitis. Mechanisms controlling this sequence are not completely understood, especially regarding the inbalanced regulation of inflammation by TGF-β1 and IL-10. Moreover, through its known direct and indirect effects, IL-10 is probably one major anti-inflammatory and antifibrotic mediator acting on PSC in the setting of a necroinflammatory pathway. The relevance of this model of repetitive acute pancreatitis episodes to human fibrogenesis is unknown. Although the pathophysiology of chronic pancreatitis is surely multifactorial, the necrosis-fibrosis sequence remains one of the most relevant theories, and it is the reason why we chose this model to investigate the possible role of endogenous IL-10 in controlling regeneration and fibrosis. We clearly observed that in the absence of IL-10, acute and chronic regulation of the fibrogenetic process are amplified and may lead to alterations consistent with those of chronic pancreatitis. However, despite the incomplete relevance of the model to human chronic pancreatitis, exogenous administration of IL-10 thus appears as a good candidate able to control excessive fibrogenesis and might be considered in the future for studying its antifibrotic effect in human pancreatic diseases.

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