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Enhanced Expression of TGF-βs and Their Receptors in Human Acute Pancreatitis

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Objectives
To determine which mechanisms are involved in pancreatic remodeling, repair, and fibrosis after acute necrotizing pancreatitis (NP) in humans.

Summary Background Data
Transforming growth factor betas (TGF-βs) are multifunctional polypeptides that have been implicated in the regulation and formation of extracellular matrix and fibrosis. They exert their functions by binding to specific receptors. In this study, we analyze the expression of TGF-β1, TGF-β2, and TGF-β3 and their receptors type I (Tβ-RI [ALK5]), type II (Tβ-RII), and type III (Tβ-RIII) in NP.

Patients
Pancreatic tissue samples were obtained from 6 female and 8 male patients with a median age of 65 years (range, 37 to 77 years) undergoing surgery for NP. The median Ranson score of the patients was 6 (range, 2 to 9). The operation was performed a median 5.5 days (range, 4 to 17 days) after the onset of acute pancreatitis. Pancreatic tissue obtained from 12 previously healthy organ donors (6 male, 6 female; median age of 43 years) served as controls.

Methods
The expression of TGF-β1, TGF-β2, TGF-β3, Tβ-RI (ALK5), Tβ-RII, Tβ-RIII, and collagen type I mRNA was analyzed by Northern blot analysis. In addition, immunohistochemical analysis using polyclonal antibodies was performed to detect TGF-β1, TGF-β2, TGF-β3, Tβ-RI (ALK5), and Tβ-RII.

Results
Northern blot analysis showed an increase in TGF-βs and their receptors in NP tissue samples compared with samples from normal controls. The increase was 3.5-fold for TGF-β1 ($p < 0.05$), 2.7-fold for TGF-β2 ($p < 0.05$), 3.5-fold for TGF-β3 ($p < 0.05$), 10-fold for

Tβ-RI (ALK5) ($p < 0.05$), 5.7-fold for Tβ-RII ($p < 0.05$), and 1.4-fold for Tβ-RIII (not significant). Collagen type I mRNA was also markedly increased in NP samples and correlated with the level of TGF-βs. Immunohistochemical analysis demonstrated intense TGF-β1, TGF-β2, TGF-β3, Tβ-RI (ALK5), and Tβ-RII immunoreactivity in the remaining acinar and ductal cells in most NP samples; in the normal control pancreas, there was weak to moderate immunoreactivity for these factors only in some acinar cells and a few ductal cells.

Conclusion

The marked increase in expression of TGF-βs and their signaling receptors Tβ-RI (ALK5) and Tβ-RII suggests a role for TGF-βs in the repair process after the onset of NP in humans and raises the possibility that TGF-βs might be involved in tissue remodeling and the fibrotic reaction that occurs in the pancreas after necrosis.

Acute pancreatitis is a sudden inflammatory process whose treatment remains a challenge for clinicians.[1,2] Depending on the development of pancreatic or peripancreatic necrosis, the clinical course of acute pancreatitis may be either mild and self-limiting (edematous pancreatitis) or severe and life-threatening (necrotizing pancreatitis [NP]).[1,2] Histomorphologically, acute inflammation of the pancreas is associated with different degrees of autodigestion.[3] Acute edematous pancreatitis is characterized by interstitial edema, vacuolization of acinar cells, and infiltrates of polymorphonuclear leukocytes in the pancreas. In NP, fat necrosis, interstitial hemorrhage, and various degrees of acinar and duct cell necrosis also occur.[4]

The cellular changes that occur during the course of acute pancreatitis have not been studied in humans, but in animal models of acute edematous pancreatitis, morphologic pancreatic damage heals completely, without permanent tissue destruction.[5,6] In animal studies of NP, however, areas of pancreatic and peripancreatic necrosis are sealed off by granulation and scar tissue.[7,8] Although pancreatic morphology cannot be studied in the course of human NP, analysis of exocrine or endocrine pancreatic function indicates that areas of pancreatic necrosis are replaced by fibrotic tissue.[3,9]

Transforming growth factor betas (TGF-βs) are multifunctional polypeptides that can exert stimulatory and inhibitory effects on various cells.[10,11] TGF-βs play a role in the regulation of cell growth and differentiation, angiogenesis, carcinogenesis, cell adhesion, immunosuppression, inflammation, tissue repair, and extracellular matrix formation.[10,11] The stimulation of collagen synthesis and the inhibition of its degradation by TGF-βs suggests a major role for these growth factors in the pathogenesis of disorders associated with fibrosis (*e.g.*, glomerulonephritis, cirrhosis, and pulmonary fibrosis).[12–14]

TGF-βs exert their functions by binding to specific transmembrane receptors.[15,16] Three major TGF-β receptors—TGF-β receptor type I (Tβ-RI), TGF-β receptor type II (Tβ-RII), and TGF-β receptor type III (Tβ-RIII)—have been characterized recently. Although only one subtype of Tβ-RII has been identified, several subtypes of Tβ-RI have been found.[17–19] One of these is ALK5, which participates mainly in mediation of TGF-β signaling. The exact functions of the other Tβ-RI subtypes are unknown.[18,19] The presence of both Tβ-RI and Tβ-RII is required for signal transduction by a serine/threonine kinase located at the internal domain of the Tβ-RII.[20] In contrast, Tβ-RIII (betaglycan) binds TGF-βs but is not believed to be involved directly in signal transduction.[21]

In this study, we examined for the first time the distribution of the three mammalian TGF-β isoforms and their receptors (types I to III) in human acute pancreatitis, using immunohistochemical and Northern blot analysis. We report that in comparison with the normal human pancreas, all three TGF-β isoforms and their signaling type I (ALK5) and type II receptors are overexpressed in the pancreases of patients with NP.

PATIENTS AND METHODS

Normal pancreatic tissue samples were obtained from 12 previously healthy multiorgan donors at the University of Bern. The 6 female and 6 male organ donors ranged in age from 14 to 56 years, with a median age of 43 years.

NP tissue samples were obtained from 14 patients (6 female, 8 male) undergoing surgery for NP. The median age of these patients was 65 years, with a range of 37 to 77 years. The median Ranson score of the patients was 6 (range, 2 to 9). Pancreatic surgery was performed a

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median 5.5 days (range, 4 to 17 days) after the onset of acute pancreatitis. The etiology of acute pancreatitis was alcohol in six patients and gallstones in eight patients; in two patients, the etiology could not be clarified. Standard histopathologic analysis of the NP tissues revealed no morphologic difference between the different etiologic groups.

The pancreatitis tissue samples were taken at the border between necrotic and nonnecrotic pancreatic regions. Normal and NP tissue samples used for our studies were immediately fixed in either Bouin or paraformaldehyde solution for immunohistochemical analysis. Tissue samples destined for RNA extraction were frozen in liquid nitrogen and maintained at −80 C until use.

All studies were approved by the human subjects committees of the University of Ulm, Germany; the University of Bern, Switzerland; and the University of California, Irvine.

IMMUNOHISTOCHEMISTRY

The TGF-β antibodies used in this study were raised against synthetic peptides corresponding to segments of the amino-terminal ends of the respective biologically active TGF-βs.[22,23] These antibodies are isoform specific, as determined by immunoblotting with recombinant TGF-β1 and TGF-β3 and native TGF-β2 and as demonstrated by the complete absence of cross-reactivity among the individual antisera.[22,23] For Tβ-RI (ALK5) and Tβ-RII immunostaining, two highly specific polyclonal antibodies were used (Santa Cruz Biotechnology, Santa Cruz, CA). Anti–Tβ-RI antibodies were raised by immunizing against amino acids 158–179 of Tβ-RI, which are specific for ALK5 and do not cross-react with Tβ-RII. Anti–Tβ-RII antibodies were raised by immunizing against amino acids 246–266 of Tβ-RII, which do not cross-react with Tβ-RI (ALK5).

After the tissue samples were embedded in paraffin, 3-μm sections were subjected to immunostaining using the streptavidin biotin technique (Kirkegaard & Perry Laboratories, Gaithersburg, MD), as previously reported.[22–25] Optimal results were obtained at an antibody dilution of 2.5 μg/mL for TGF-β1, TGF-β2, and TGF-β3 and at a dilution of 1:50 for Tβ-RI (ALK5) and Tβ-RII.

Tissue sections were submerged for 15 minutes in tris buffered saline (TBS) buffer (10 mM Tris-HCl, 0.85% NaCl, pH 7.4) containing 0.1% (vol/vol) Triton X-100 and were washed for 5 minutes in TBS solution, as previously reported.[24,25] Endogenous peroxidase activity was blocked by incubating the slides in methanol and in methanol with 0.6% hydrogen peroxide, followed by washings in methanol and TBS with 0.1% bovine serum albumin (BSA).[24,25] After treatment with hyaluronidase (1 mg/mL in 100 mM sodium acetate and 0.85% NaCl), the sections were incubated for 30 minutes at 23 C with 10% normal goat serum. They were then incubated overnight at 4 C with isoform-specific polyclonal TGF-β1, TGF-β2, and TGF-β3 antibodies that had been diluted in TBS containing 5% normal goat serum and 0.1% BSA.[24,25] Bound antibody was detected with a biotinylated goat anti-rabbit IgG secondary antibody and a streptavidin–peroxidase complex (Kirkegaard & Perry Laboratories). The sections were then incubated with diaminobenzidine tetrahydrochloride (0.05%) as the substrate and counterstained with Mayer's hematoxylin.

To ensure specificity of the TGF-β1, TGF-β2, TGF-β3, Tβ-RI (ALK5), and Tβ-RII antibodies, consecutive sections were incubated either in the absence of the primary antibody or with a nonimmunized rabbit IgG antibody. In both cases, no immunostaining was detected.

Probes

For Northern blot analysis, specific cRNA (TGF-β1, TGF-β2, TGF-β3, Tβ-RI [ALK5], Tβ-RII, Tβ-RIII) or cDNA (collagen, 7S) probes were used, as previously reported. The TGF-β1 cRNA probe consisted of a 280-kb EcoRI-KpnI fragment of human TGF-β1 cDNA, corresponding to nucleotides 997 to 1277.[24,26] The TGF-β2 cRNA probe consisted of a 600-kb HindIII-PstI fragment of human TGF-β2 cDNA, corresponding to nucleotides 253 to 853.[24,26] The TGF-β3 cRNA consisted of a 125-kb XbaI-BglII fragment of human TGF-β3 cDNA, corresponding to nucleotides 917 to 1042.[24,26]

The Tβ-RI (ALK5) cRNA probe consisted of a 377-bp fragment of human Tβ-RI (ALK5) cDNA, corresponding to nucleotides 442 to 818.[27] The Tβ-RII cRNA probe consisted of a 477-bp fragment of human Tβ-RII cDNA, corresponding to nucleotides 42 to 519.[28] The Tβ-RIII cRNA probe consisted of a 721-bp fragment of the human Tβ-RIII cDNA, corresponding to the nucleotides 1734 to 2455 of the corresponding rat cDNA sequence.[28] The collagen cDNA probe consisted of a 1.8-kb EcoRI insert of human collagen type I cDNA (American Type Culture Collection [ATCC], Rockville, MD).

To verify equivalent RNA loading, all filters were rehybridized with a 7S cDNA consisting of a 190-bp BamHI fragment of the mouse 7S cytoplasmic cDNA that cross-hybridizes with human 7S RNA.[24,25,28]

The antisense probes used for Northern blot analysis were prepared with [alpha-^{32}P]-CTP (DuPont, Boston, MA) using a transcription kit (Promega, Madison, WI). [Alpha-^{32}P]-dCTP (DuPont) was incorporated into the cDNA probe using a random primer labeling system (Boehringer-Mannheim, Mannheim, Germany).

Northern Blot Analysis

Total RNA was extracted by the guanidine isothiocyanate method, fractionated on 1.2% agarose with 1.8 M

formaldehyde gels, and stained with ethidium bromide for verification of RNA integrity and loading equivalency.[24,25,28] The RNA was electrotransferred onto nylon membranes (GeneScreen, DuPont) and cross-linked by ultraviolet irradiation.[24,25,28] The blots were then prehybridized, hybridized, and washed under conditions appropriate for antisense riboprobes (TGF-β1, TGF-β2, TGF-β3, Tβ-RI [ALK5], Tβ-RII, Tβ-RIII) or cDNA probes (7S, amylase, collagen), as previously described.[24,25,28]

In the case of antisense riboprobes, the blots were prehybridized overnight at 65 C in 50% formamide, 0.5% sodium dodecyl sulfate (SDS), 5 × SSC, 5 × Denhardt's solution (1 × Denhardt's = 0.02% ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 250 μg/mL salmon sperm DNA, and 50 mM Na_2PO_4 (pH 6.5). The blots were then hybridized for 18 hours at 65 C in the presence of 1×10^6 cpm/mL of the labeled antisense riboprobe, washed twice at 65 C in a solution containing 1 × SSPE (150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA) and 0.5% SDS, washed twice at 65 C in a solution containing 0.1 × SSPE and 0.5% SDS, and washed once at 68 C in 0.1 × SSPE and 0.5% SDS.[24,25,28]

In the case of cDNA probes, blots were prehybridized overnight at 42 C in a prehybridization buffer that contained 50% formamide, 1% SDS, 0.75 M NaCl, 5 mM EDTA, 5 × Denhardt's solution, 100 μg/mL salmon sperm DNA, 10% dextran sulfate, and 50 mM Na_2PO_4 (pH 7.4). The hybridization was carried out at 42 C for 18 hours with the labeled cDNA probe (1×10^6 cpm/mL), which was then washed two times at room temperature in 2 × SSC and three times at 55 C in 0.2 × SSC and 2% SDS.[24,25,28]

Blots were exposed at −80 C to Fuji x-ray film (Fuji, Tokyo, Japan) with intensifying screens, and the intensity of the radiographic bands was quantified by video densitometry (BioRad 620, BioRad, Hercules, CA), as previously reported.[24,25,28]

STATISTICAL ANALYSIS

The results are expressed as median and range or as mean ± standard error. For statistical analysis, the unpaired Student's t test was used. Significance was defined as $p < 0.05$.[29]

RESULTS

Immunohistochemistry

In the normal human pancreas, weak to moderate TGF-β1 (Fig. 1A), TGF-β2 (Fig. 1C), and TGF-β3 (Fig. 1E) immunoreactivity was present in a few acinar and ductal cells in a focal pattern. A similar pattern of immunostaining was observed for Tβ-RI (ALK5) (Fig. 2A) and Tβ-RII (Fig. 2B) in the pancreatic acinar and ductal cells of the normal pancreas.

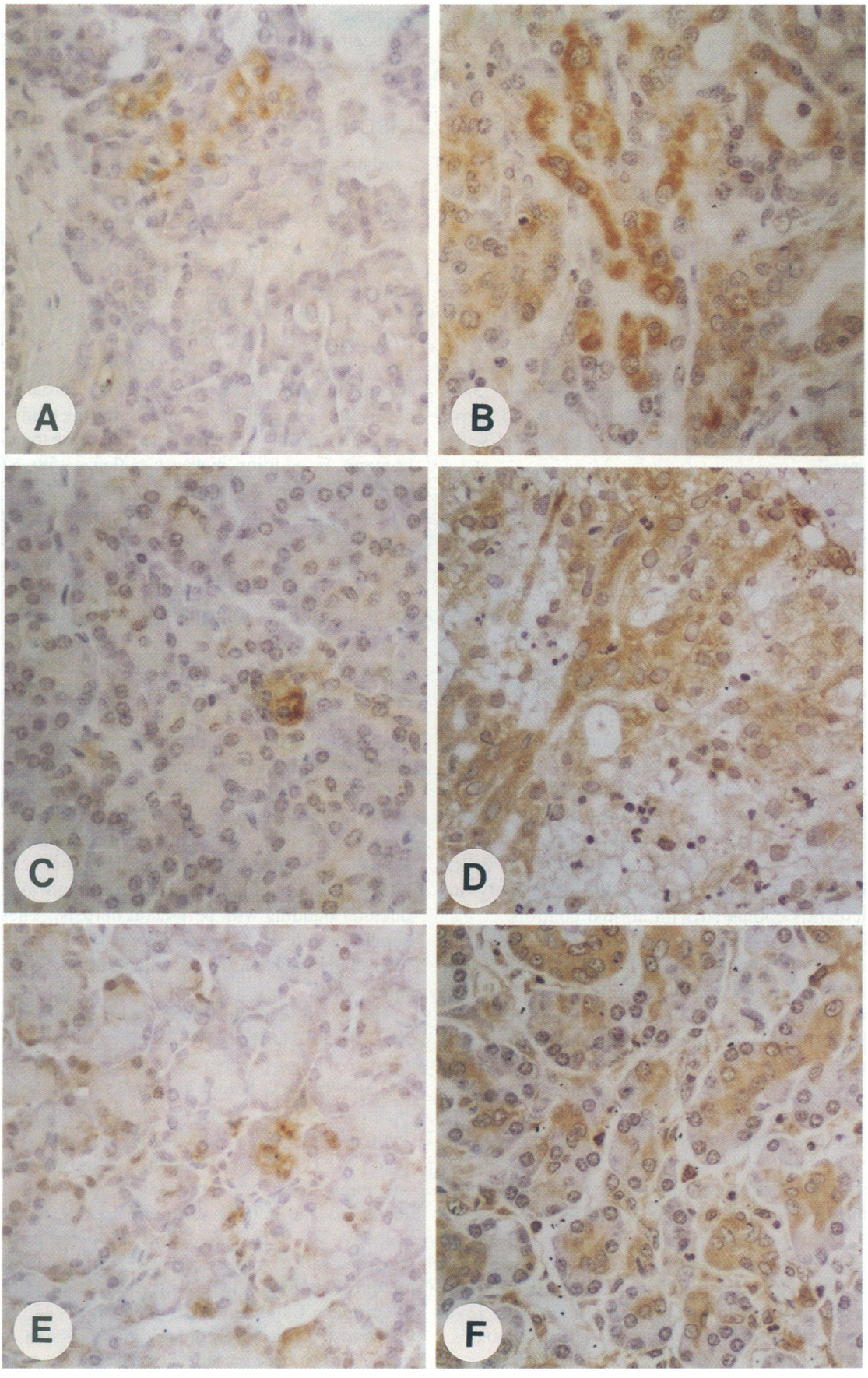
Tissue samples obtained from patients with NP showed huge areas of pancreatic necrosis (Figs. 1F, 2D, 2E, 2F). The largest areas of necrosis were present primarily in the periphery of pancreatic lobules. Between the necrotic areas were regions with vital exocrine pancreatic parenchyma exhibiting single cell necrosis and various degrees of interstitial edema (Figs. 1B, 1F). Immunohistochemical analysis of TGF-β1 (Fig. 1B), TGF-β2, and TGF-β3 in NP tissues revealed moderate to intense immunoreactivity of all three TGF-β isoforms in most remaining acinar and ductal cells. The strongest immunohistochemical signals were present in the acinar and ductal cells adjacent to the necrotic areas. In contrast, in the center of the lobules, which were spared by necrosis and which exhibited less interstitial edema, weaker and less frequent TGF-β immunostaining was detectable. The immunohistochemical distribution pattern of the TGF-βs isoforms in NP was comparable.

Immunohistochemical analysis of Tβ receptors revealed a distribution of immunoreactivity similar to that of TGF-βs. Moderate to intense immunoreactivity of Tβ-RI (ALK5) (see Figs. 2C and 2E) and Tβ-RII (see Figs. 2D and 2F) was also present in the remaining acinar and ductal cells in NP. The immunohistochemical pattern of Tβ-RI (ALK5) and Tβ-RII paralleled that of the TGF-βs. Areas that were less affected by the inflammatory reaction exhibited weaker immunoreactivity for both receptors than did areas with major pancreatic damage. The strongest immunoreactivity for Tβ-RI (ALK5) and Tβ-RII was present in the pancreatic acinar and ductal cells adjacent to the necrosis (see Figs. 2E and 2F).

Northern Blot Analysis

Low levels of TGF-β1, TGF-β2, and TGF-β3 mRNA expression were present in the normal human pancreas

Figure 1. Immunohistochemical analysis of TGF-β1 (A, B), TGF-β2 (C, D) and TGF-β3 (E, F) in the normal pancreas (A, C, E) and in the pancreases of patients with acute necrotizing pancreatitis (B, D, F). TGF-β1 (A), TGF-β2 (C), and TGF-β3 (E) immunoreactivity was present in a few acinar and ductal cells in the normal pancreas. In contrast, in acute necrotizing pancreatitis, moderate to strong TGF-β1 (B), TGF-β2 (D), and TGF-β3 (F) immunoreactivity was present in most surviving acinar and ductal cells. (×400)


A
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(Fig. 3). In all normal pancreatic tissue samples, TGF-β2 mRNA expression was visible only on the original autoradiographs (see Fig. 3). In tissue samples obtained from patients with NP, mRNA expression of all TGF-β isoforms was markedly increased. For the NP tissues, 10 of 14, 9 of 14, and 7 of 14 exhibited overexpression of TGF-β1, TGF-β2, and TGF-β3 mRNA, respectively. All NP samples exhibited marked overexpression of at least one TGF-β moiety. Densitometric analysis of the Northern blots of all 14 acute pancreatitis samples revealed a 3.5-fold ($p < 0.05$), a 2.7-fold ($p < 0.05$), and a 3.5-fold ($p < 0.05$) increase of TGF-β1, TGF-β2, and TGF-β3 mRNA expression, respectively, compared with the normal controls. When densitometry was carried out only in samples with increased mRNA expression, levels of TGF-β1, TGF-β2, and TGF-β3 mRNA were increased 5.4-fold ($p < 0.05$), 4.5-fold ($p < 0.05$), and 6.1-fold ($p < 0.05$), respectively, in acute pancreatitis compared with normal controls.

Northern blot analysis of total RNA demonstrated low to moderate levels of Tβ-RI (ALK5), Tβ-RII, and Tβ-RIII mRNA in the normal human pancreas (Fig. 4). In the pancreatic tissue samples obtained from patients with NP, however, there was a marked increase in Tβ-RI (ALK5) and Tβ-RII mRNA expression in 9 of 14 patients (see Fig. 4). Tβ-RIII mRNA levels were increased in only four NP samples compared with the normal controls. Densitometric analysis of the Northern blots of all acute pancreatitis samples revealed a 10-fold ($p < 0.05$), a 5.7-fold ($p < 0.05$), and a 1.4-fold (not significant) enhanced expression of Tβ-RI (ALK5), Tβ-RII, and Tβ-RIII, respectively, compared with the normal controls. When densitometry was carried out only in samples with increased mRNA expression, levels of Tβ-RI (ALK5), Tβ-RII, and Tβ-RIII mRNA were increased 17-fold ($p < 0.05$), 9.8-fold ($p < 0.05$), and 3.7-fold, respectively, in acute pancreatitis compared with normal controls.

In addition to analyzing the expression of TGF-βs and their receptors, we analyzed collagen mRNA expression in the normal pancreas and in NP (see Fig. 3). In the normal human pancreas, no mRNA expression for collagen was detectable by Northern blot analysis. In contrast, in the tissue samples from patients with NP, collagen mRNA expression was present in all samples at various levels. In all acute pancreatitis samples, the signal corresponding to the collagen mRNA was visible on the original autoradiographs. Because of the lack of detectable mRNA expression in the normal pancreas, the increase in NP compared with the normal controls could not be calculated.

Correlation analysis between TGF-β and collagen mRNA levels in NP tissue samples revealed a significant correlation for TGF-β1 ($r = 0.64$, $p < 0.05$), TGF-β2 ($r = 0.83$, $p < 0.05$), and TGF-β3 ($r = 0.68$, $p < 0.05$). In addition, there were significant positive correlations between the mRNA levels of the TGF-β isoforms and the two signaling Tβ receptors.

DISCUSSION

Acute pancreatitis is defined as acute inflammation of the exocrine pancreas. The morphologic changes that occur during acute pancreatitis can be determined in clinical practice by contrast-enhanced computed tomography (CT),[1,2] and the CT findings can be used to separate acute pancreatitis into an acute edematous and an acute necrotizing course.[2] Most patients with edematous and necrotizing pancreatitis respond well to conservative treatment, and in general only a few patients with NP require surgical treatment.[30] Therefore, few pancreatic tissue samples are surgically removed and available for the study of remodeling and repair mechanisms after acute pancreatitis in humans. In addition, because patients with NP undergo pancreatic surgery at different time points after the onset of acute pancreatitis, it is impossible to study remodeling and repair of the pancreas in a time-dependent manner.[30,31] However, based on autopsy and animal studies, it is hypothesized that relatively little necrosis can be phagocytosed and the inflammation resolved to leave a normal pancreas.[3] However, in widespread panlobular necrosis, the pancreas cannot be restored to normal, and scar formation is presumed to take place.

This hypothesis is supported by morphologic studies in which edematous and necrotizing pancreatitis were induced in rats.[4] Fourteen days after the induction of acute edematous pancreatitis, histopathology showed no difference between pancreases of diseased animals and controls.[4] In contrast, the pancreases of animals with NP showed widespread chronic inflammation, acinar dilation and atrophy, marked reactive stromal proliferation, and ductular budding with periductal fibrosis.[4] These findings support the theory that the degree of pancreatic recovery after acute pancreatitis is closely correlated to the initial trauma.

In the present study, we analyzed the expression of TGF-β1, TGF-β2, and TGF-β3 and their receptors in the normal human pancreas and after NP. TGF-β1, TGF-β2, and TGF-β3 belong to a gene superfamily that also includes TGF-β4, TGF-β5, activins, inhibins, müllerian inhibiting substance, and the bone morphogenetic proteins.[10–12] TGF-βs bind with high affinity to specific transmembrane receptors; three major Tβ-receptors have been identified and can be differentiated by their molecular weight.[15] Tβ-RI and Tβ-RII are involved in signal transduction, and Tβ-RIII is involved in ligand storage and the presentation of TGF-βs to the signaling Tβ-RI/TβR-II complex. Using Northern blot analysis and immunohistochemistry, we found enhanced expression of TGF-β1,

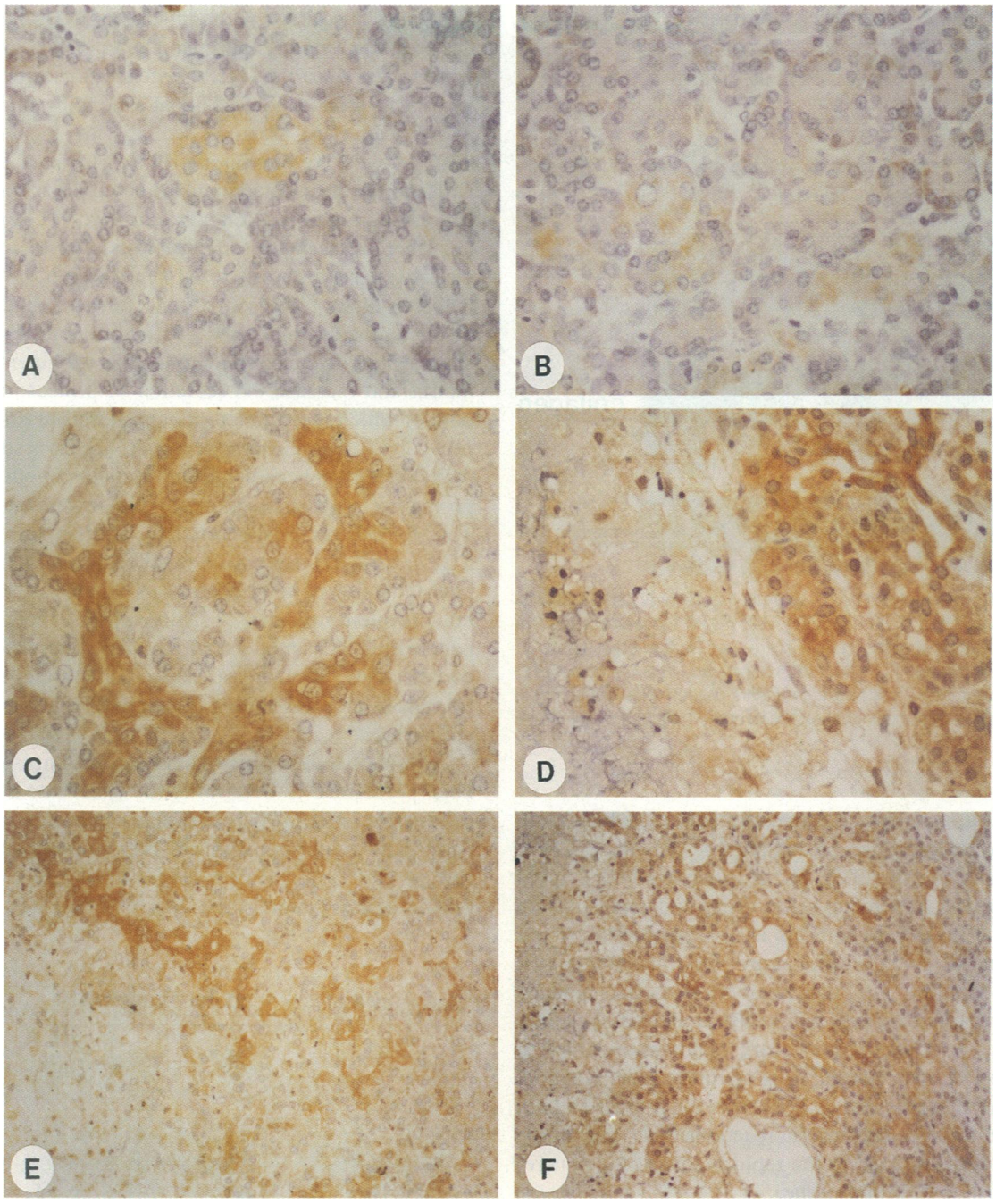

Figure 2. Immunohistochemical analysis of Tβ-RI (ALK5) (A, C, E) and Tβ-RII (B, D, F) in the normal pancreas (A, B) and in the pancreases of patients with acute necrotizing pancreatitis (C–F). In the normal pancreas, Tβ-RI (ALK5) (A) and Tβ-RII (B) immunoreactivity was present in a few acinar and ductal cells. In contrast, in acute necrotizing pancreatitis, intense Tβ-RI (ALK5) (C, E) and Tβ-RII (D, F) immunoreactivity was present in most surviving acinar and ductal cells. The intensity of the immunohistochemical signals for both Tβ-receptors was highest in the surviving acinar and ductal cells adjacent to the necrosis (E, F). (A–D, ×400; E, F, ×200)

TGF-β2, TGF-β3, and the receptors Tβ-RI (ALK5) and Tβ-RII mRNA in the pancreases of patients with NP compared with normal controls. In contrast, Tβ-RIII mRNA expression in NP was comparable with that found in the normal pancreas. Interestingly, the highest levels of TGF-βs, Tβ-RI, and Tβ-RII were found in the periphery of vital pancreatic lobules adjacent to the necrosis. In contrast, weaker immunoreactivity of these factors was

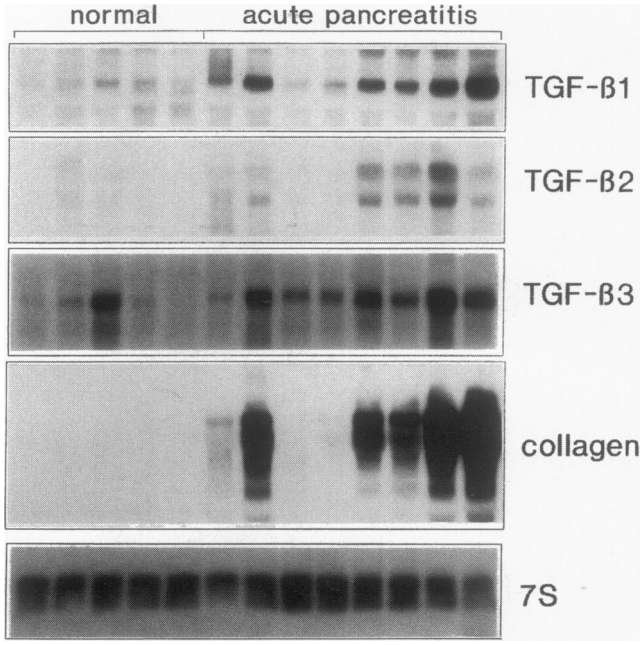

Figure 3. Northern blot analysis of TGF-β1, TGF-β2, and TGF-β3. Low levels of TGF-β1, TGF-β2, and TGF-β3 mRNA were present in the normal pancreas. In contrast, there was a marked increase in TGF-β1, TGF-β2, and TGF-β3 mRNA expression in acute necrotizing pancreatitis. Acute necrotizing pancreatitis tissues with higher levels of TGF-β mRNA expression also exhibited higher collagen type I mRNA levels.

present in the centers of pancreatic lobules, which were unaffected by necrosis and exhibited less damage. These findings indicate that TGF-βs and their receptors might be involved in pancreatic remodeling and repair during and after NP. This hypothesis is also supported by our finding that collagen mRNA expression was upregulated in the NP samples. After pancreatic necrosis, the damaged tissue is replaced by granulation tissue and subsequently by fibrosis. Because TGF-βs are major stimulators of collagen synthesis and inhibitors of collagen degradation, the enhanced expression of TGF-βs in the remaining pancreas might induce the fibrotic reactions that occur in the necrotic areas and lead to the replacement of the pancreatic necrosis by fibrotic tissue.

TGF-βs mediate their functions by binding to specific transmembrane receptors. Interestingly, levels of both TGF-βs and the two signaling Tβ-receptors were increased in the acinar cells adjacent to pancreatic necrosis. This concomitant expression of ligands and receptors indicates an autocrine or paracrine stimulation of the Tβ-receptors in the pancreas after acute NP in humans.

Previous experimental studies have reported that TGF-βs are potent regulators of growth and differentiation of normal and malignant cells.[10-12] They also stimulate gene expression of extracellular matrix components and inhibit extracellular matrix degradation, which leads to tissue repair and fibrosis.[10,11] Upregulation of TGF-β expression has been reported in experimental myocardial infarction,[32] bone fracture healing,[33] liver regeneration after major resection in rats,[34] and excisional wounding and burn injury.[35,36] In bone fracture healing, the highest TGF-β mRNA levels are present after 5 and 15 days, coinciding with the onset of osteomembranous bone formation and endochondral ossification, respectively.[33] TGF-βs are potent chemoattractants for fibroblasts, and they stimulate gene expression and increase synthesis and secretion of collagen types I and III, fibronectin, and other proteins of the extracellular matrix.[37] In addition, TGF-βs inhibit enzymes involved in the degradation of the extracellular matrix and increase the secretion of protease inhibitors, both of which result in a pronounced fibroblastic stimulation.[10-12,38,39] These characteristics of TGF-βs suggest that they are potential candidates for inducing, modifying, and promoting fibrotic reactions in NP in humans as well. This hypothesis is supported by data in rats in which TGF-β1 mRNA expression was enhanced 24 to 48 hours after induction of acute edematous pancreatitis by intravenous cerulein infusion.[40,41] Although this model of acute pancreatitis does not lead to major pancreatic damage and necrosis, an increase occurs in mitotic activity of fibroblasts and stimulation of synthesis and deposition of collagen.[42,43]

Although immunohistochemistry has not previously been performed to determine the exact site of TGF-β1 production, *in situ* hybridization has indicated that the pancreatic acinar cells are the major source of TGF-β1 mRNA expression after pancreatic repair in experimental edematous pancreatitis.[40] As in human NP, enhanced

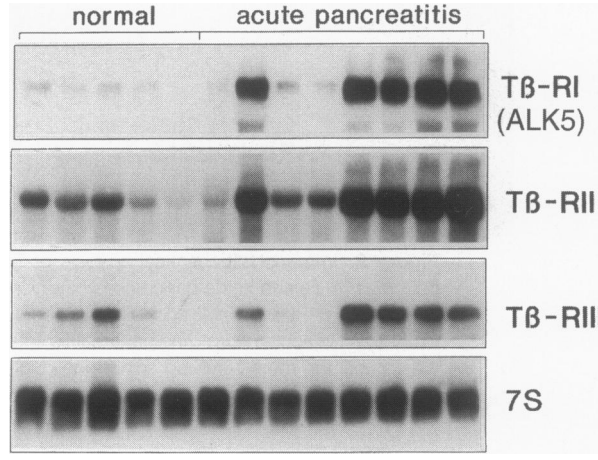

Figure 4. Northern blot analysis of Tβ-RI (ALK5), Tβ-RII, and Tβ-RIII. Low levels of Tβ-RI (ALK5) and moderate levels of Tβ-RII and Tβ-RIII mRNA were present in the normal pancreas. In acute necrotizing pancreatitis, a marked overexpression of Tβ-RI (ALK5) and Tβ-RII, but not of Tβ-RIII, was present. The samples are blotted in the same order as in Figure 3.

expression of TGF-β in experimental pancreatitis has been associated with upregulation of collagen, indicating that all three TGF-β isoforms are important stimulators of extracellular matrix formation after minor and major pancreatic damage. Furthermore, in conjunction with locally concentrated levels of chemotactic and inflammatory mediators, TGF-βs might stimulate monocytes to undergo enhanced phagocytic and lysosomal activity by modulating the expression of Fc receptors,[44] which in turn would begin the process of removing the necrotic areas, an initial step in pancreatic remodeling and repair. The release of TGF-βs from the remaining pancreatic acinar and ductal cells adjacent to the necrotic areas suggests that the intensity of the inflammatory reaction at the border between the necrotic and nonnecrotic pancreas is mainly regulated and modified by the surviving pancreatic parenchyma.

Taken together, these are the first data analyzing tissue repair and remodeling in NP in humans. Upregulation of TGF-βs and their signaling type I (ALK5) and type II receptors seems to stimulate extracellular matrix synthesis and contribute to tissue remodeling and repair.

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