Pancreatic stellate cells contribute to regeneration early after acute necrotising pancreatitis in humans

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Background and aim: The aim of this study was to systematically analyse the pattern of regeneration in human acute pancreatitis by testing whether pancreatic stellate cells, their myofibroblastic offspring, and pancreatic ductules are involved in the regenerative process.

Patients and methods: Between January 1994 and November 2000, 24 necrosectomy specimens containing vital tissue were obtained for pathological examination. Formalin fixed tissue samples were routinely processed and immunostained for cytokeratins 7 and 19, smooth muscle actin, desmin, Ki-67, and CD68. Pancreatic tissue from organ donors served as normal controls.

Results: Necrosectomy specimens were obtained between 11 and 41 days after the onset of symptoms. In vital areas of necrosectomy samples, spherical hypercellular spheres consisting of loose vascular connective tissue occurred, in part showing duct-like profiles which sprouted from remnant exocrine tissue almost perpendicularly to the periphery of the spheres. In normal tissue, only a few stellate cells and myofibroblasts were present around ducts and ductules. In contrast, numerous stellate cells and myofibroblasts were detected in the hypercellular regenerative spheres after acute pancreatitis, both being situated within the loose tissue and forming compact periductular sheaths. Stellate cells/myofibroblasts and ductule cells exhibited increased proliferative activity.

Conclusions: Pancreatic stellate cells and their activated myofibroblastic offsprings may participate in regeneration after acute necrotising pancreatitis in humans. Time course studies are needed to further strengthen this regeneration concept.

Acute pancreatitis encompasses a whole spectrum of inflammatory lesions in the pancreas. In accordance with a recent proposition, these lesions are being described using the terms, severe acute pancreatitis, mild acute pancreatitis, acute fluid collections, pancreatic necrosis, and acute pseudocysts. The aetiology and pathogenesis of necrosis and haemorrhage as hallmarks of severe acute pancreatitis have been studied in detail, and several models of pathogenic pathways have recently been developed. The end result of an acute attack of acute pancreatitis is a well characterised type of pancreatic and peripancreatic tissue breakdown ranging from interstitial oedema and low grade multifocal necrosis of the pancreas (mild acute pancreatitis) to massive haemorrhagic necrosis. In patients who survive, at least some of these lesions are generally considered to be reversible, in contrast with the overall progressive character of chronic pancreatitis. If necrotic tissue is present it may become infected or forms fluid collections containing debris. These acute fluid collections may disappear spontaneously or develop into pseudocysts. What has not been specifically addressed is the question as to whether, during the time period between the initiation of necrosis and necrosectomy, repair and/or regeneration of the exocrine apparatus occurs. Theoretically, regeneration of pancreatic exocrine tissue destroyed by acute pancreatitis requires replacement of both epithelial cell populations and cells forming the matrix, allowing an ordered regrowth of lost epithelia.

Recently, stellate cells representing the homologue of the respective cells occurring in the liver have been identified in the pancreas. Pancreatic stellate cells have been shown to play a significant pathogenic role in fibrogenesis and in particular in mechanisms involved in fibrosis occurring in chronic pancreatitis. In contrast, it has not been established whether pancreatic stellate cells are involved in remodelling and regenerative mechanisms ensuing after acute necrotising pancreatitis in humans.

In the present investigation in patients with acute necrotising pancreatitis, we systematically studied the roles of two cell systems holding a key position in the maintenance of pancreatic tissue homeostasis in regeneration after severe pancreatitis: ductular complexes and pancreatic stellate cells.

PATIENTS AND METHODS

Patients

Between January 1994 and November 2000, 42 necrosectomy specimens from patients with acute necrotising pancreatitis were obtained for pathological examination. Of these specimens, 18 consisted of completely necrotic tissue exclusively. The remaining 24 specimens contained vital tissue structures and were used for the present investigation. These samples were obtained from eight female and 16 male patients (median age 59 years; range 28–71; 95% confidence interval (CI) 50–61.3) between 11 and 41 days after the onset of symptoms. The extent of necrosis was assessed preoperatively as previously described. The pertinent data are summarised in table 1. Pancreatic tissue from organ donors served as controls.

Histology and immunohistochemistry

Randomly chosen samples were fixed in 4% neutral buffered formalin and embedded in paraffin. Standard morphological evaluation was based on haematoxylin and eosain stained sections. For immunohistochemistry, deparaffinised sections were rehydrated through a graded series of ethanol and brought into Tris buffered saline. Depending on the antibody used, sections were subjected to trypsin digestion (Difco, Detroit, Michigan, USA) or to heat induced epitope retrieval in 10 mM citrate buffer, pH 6.0, either in a microwave oven or in

Abbreviations: SMA, α smooth muscle actin; CK, cytokeratin.
a pressure cooker. Primary antibodies were directed against cytokeratin (CK)-7 (clone OV-TL 12/30; Dako Diagnostics AG, Zug, Switzerland; working concentration 2 µg/ml; trypsin pretreatment), CK-19 (clone RCK108; Dako; 0.8 µg/ml; trypsin), desmin (clone D33; Dako; 5 µg/ml; microwave), α smooth muscle actin (SMA, clone 1A4; Sigma, St Louis, Missouri, USA; dilution 1:600; no pretreatment), CD68 (clone PG-M1; Dako; 2.5 µg/ml; microwave), and Ki-67 (clone MIB1; Dako; 1 µg/ml; pressure cooker). After the primary antibody a biotinylated goat-antimouse Ig antibody (Dako) was applied, followed by streptavidin-biotin complex/alkaline phosphatase (Dako). Sections were developed in new fuchsin-naphtol AS-BI (Sigma), counterstained with haematoxylin, and mounted. For double immunohistochemistry, stainings for the respective antibodies employed an avidin-biotin complex/horseradish peroxidase system (Vector, Burlingame, California, USA) and 3,3-diaminobenzidine as chromogen, and a streptavidin-biotin complex/alkaline phosphatase system as outlined above, respectively. For immunohistochemistry, positive control sections were processed simultaneously. For estimation of the proliferation index (in per cent; labelled nuclei/all nuclei counted×100) of stellate cells and ductule cells, in acute pancreatitis and in normal controls, Ki-67/SMA and Ki-67/CK-7 double immunostains were used. In these preparation, five areas were randomly chosen, and in each area 300 nucleated cells of interest were counted and analysed for the presence of labelled nuclei.

**Statistics**

All clinical data were collected prospectively and entered into a statistical package program (SPSS Statistical Software, Chicago, Illinois, USA) on a personal computer. Data were analysed using Fisher’s exact test or the Mann Whitney U test where appropriate.

**RESULTS**

**Normal pancreatic tissue: qualitative analysis**

Control tissue was structurally normal. Reactivity for CK-19 was markedly present in epithelia of large and small ducts.
In this study we have shown that regeneration after acute necrotising pancreatitis in humans evolves in a distinct and highly ordered fashion and seems to be independent of the type of pancreatic injury causing the acute disease. The regenerative process involves pancreatic stellate cells, their differentiated myofibroblastic offspring, and pancreatic ductules or ductules originating from remnant lobules, suggesting that stellate cells/myofibroblasts and pilot ductules represent a structural and functional unit growing in parallel.

Recent evidence suggests that pancreatic stellate cells represent a key cell type for pancreatic fibrogenesis and remodelling, but their role in acute pancreatitis has not yet been clarified. Vitamin A storing cells in the pancreas were originally observed in 1982 in mice fed an excess of this vitamin but these cells were first described in the human pancreas eight years later. A potential role of this cell system in pancreatic fibrogenesis was suggested via isolation of myofibroblast-like cells from the human pancreas, and it has
been demonstrated that vitamin A storing cells from pancreas can in fact differentiate in primary culture into myofibroblasts producing extracellular matrix proteins. It has been demonstrated that pancreatic stellate cells, similar to their hepatic counterpart, play a pathogenic role in fibrosis. In chronic alcoholic pancreatitis, active synthesis of collagen by stellate cells appears to co-localise with lipid peroxidation derived aldehydes, and exposure to ethanol or acetaldehyde led to cell activation in cultured rat pancreatic stellate cells. Mechanisms involved in the activation of pancreatic stellate cells have been shown to include transforming growth factor β, in part derived from activated macrophages, interleukin 1β, and tumour necrosis factor α, inducing secretion of interleukin 8, monocyte chemotactic protein 1, and RANTES, and platelet derived growth factors.

It is of particular interest that pancreatic stellate cells and their activated offspring play a pathogenic role in fibrosis. In chronic alcoholic pancreatitis, active synthesis of collagen by stellate cells appears to co-localise with lipid peroxidation derived aldehydes, and exposure to ethanol or acetaldehyde led to cell activation in cultured rat pancreatic stellate cells. Mechanisms involved in the activation of pancreatic stellate cells have been shown to include transforming growth factor β, in part derived from activated macrophages, interleukin 1β, and tumour necrosis factor α, inducing secretion of interleukin 8, monocyte chemotactic protein 1, and RANTES, and platelet derived growth factors.

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Thus they mimic their hepatic analogues where it has recently been shown that myofibroblasts in the rat liver reflect the degree of hepatic remodelling rather than cirrhosis inasmuch as the myofibroblast volume fraction inversely reflects hepatocyte volume bimodality, suggesting that ductular complexes and stellate cells act as pacemakers in tissue remodelling.

The mechanisms operational in the phenomena observed in the present study are not known.

In conclusion, the results of this study suggest that pancreatic stellate cells and their activated myofibroblastic offspring may participate in regeneration after acute necrotising pancreatitis. Time course studies are needed to further strengthen this regeneration concept.

References