Redox-sensitive modulation of CD45 expression in pancreatic acinar cells during acute pancreatitis.

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CD45, a transmembrane protein tyrosine phosphatase required for signal transduction in leukocytes, has recently been found in pancreatic acinar cells. The authors have investigated the relationship between kinetic expression of CD45 on acinar cells during acute pancreatitis (AP) and the ability of these cells to produce tumour necrosis factor-alpha (TNF-alpha) through mechanisms sensitive to the cellular redox state. Flow cytometric analysis showed a significant decrease in the constitutive expression of CD45 in acinar cells from six hours onwards after inducing AP by bile-pancreatic duct obstruction (BPDO) in parallel with a significant increase in acinar TNF-alpha production. Changes in protein expression on the acinar cell surface preceded CD45 mRNA down-regulation, which was not found until 12 hours after BPDO. N-acetylcysteine treatment delayed and reduced the down-regulation of CD45 expression induced by AP and prevented acinar cells from producing TNF-alpha. These results show that CD45 expression is down-regulated in acinar cells during acute pancreatitis by redox-sensitive mechanisms, and they support the notion that CD45 negatively controls the production of cytokines in pancreatic acinar cells.
Pancreatic regenerating protein (reg I) stimulates pancreatic regeneration after pancreatectomy and is mitogenic to ductal and beta-cells. This suggests that reg I and its receptor may play a role in recovery after pancreatic injury. The authors hypothesized that reg I and its receptor are induced in acute pancreatitis. Acute pancreatitis was induced in male Wistar rats by retrograde injection of 3% sodium taurocholate into the pancreatic duct. Pancreata and serum were collected 12, 24, and 36 h after injection and from normal controls (4 rats/group). Reg I receptor mRNA, serum reg I protein, and tissue reg I protein levels were determined by Northern analysis, enzyme-linked immunosorbent assay (ELISA), and Western analysis, respectively. Immunohistochemistry was used to localize changes in reg I and its receptor. Serum amylase levels and histology confirmed necrotizing pancreatitis in taurocholate treated rats. There was no statistically significant change in serum reg I concentrations from controls. However, Western blot demonstrated increased tissue levels of reg I at 24 and 36 h. This increase was localized primarily to the acinar cells and the ductal cells by immunohistochemistry. Northern blot demonstrated a significant increase in reg I receptor mRNA expression with pancreatitis. Immunohistochemistry localized this increase to the ductal cells, islets, and acinar cells. In conclusion, acute pancreatitis results in increased tissue reg I protein levels localized to the acinar and ductal cells, and a parallel threefold induction of reg I receptor in the ductal cells, islets, and acinar cells. These changes suggest that induction of reg I and its receptor may be important for recovery from acute pancreatitis.
affected by epidural analgesia. Lactate and interleukin-6 levels increased in untreated pancreatitis, which was prevented in the treatment groups (P<0.05). Epidural analgesia increased 7-day survival from 33% to 73% (P<0.05). In conclusion, thoracic epidural analgesia attenuated systemic response and improved survival in severe acute pancreatitis. These effects might be explained by improved mucosal perfusion.

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Cellular immune reaction in the pancreas is induced by constitutively active IKK2.


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Activation of the NF-kappaB system is a major event in acute and chronic inflammatory processes. NF-kappaB cascades are comprised of IkappaB-kinases, IkappaB alpha, and NF-kappaB dimers. Little is known about the individual roles of these proteins in organ-specific inflammation. The aim of the present study was to analyze the consequences of ectopic IkappaB Kinase-2 (IKK2) activation in the pancreas of mice. Transgenic mice were generated using an inducible genetic system (tet-system) to conditionally over-express a gain of function mutant of IKK2 (tetO-IKK2-EE) in the pancreas. To achieve transgene expression in the pancreas these animals were crossed to CMV-rTATA mice that show expression of the rTATA protein in the pancreas. In these double-transgenic animals doxycycline treatment induced the expression of IKK2-EE (IKK2CA) in pancreatic acinar cells resulting in a moderate activation of the IkappaB kinase-complex as measured by immune-complex kinase assay, and an up to 200-fold activation of the transgene expression cassette as detected by luciferase assay. IKK2CA expression in the pancreas had a mosaic appearance. Ectopic IKK2CA mostly activated the classical NF-kappaB pathway. The activation level of the NF-kappaB cascade induced by IKK2CA was considerably lower compared to that observed after supramaximal caerulein stimulation, but still led to the formation of leukocyte infiltrates first observed after 4 weeks of doxycycline stimulation with a maximum after 8-12 weeks. The infiltrates were mainly composed of B-lymphocytes and macrophages. Increased mRNA levels of TNF-alpha and RANTES were detected in pancreatic acinar cells. However, only minor damage of the pancreatic tissue was observed. A combination of supramaximal caerulein stimulation with induction of IKK2CA led to an increased tissue damage compared to either IKK2CA or caerulein alone. In conclusions, these observations suggest that the role of IKK2 activation in pancreatic acini is to induce leucocyte infiltration, however, at a moderate level of activation it is not sufficient to induce pancreatic damage in mice. The IKK2CA-induced infiltrations resemble those observed in autoimmune pancreatitis, indicating a role for IKK2/NF-kappaB in this disease. IKK2CA in pancreatic acinar cells increases tissue damage of secretagogue-induced experimental pancreatitis underlining the pro-inflammatory role of the IKK/NF-kappaB pathway in this disease.

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Effects of immunosuppressive and immunostimulative treatment on pancreatic injury and mortality in severe acute experimental pancreatitis.

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Acute pancreatitis is associated with substantial alterations of the immunologic
host response which has been claimed to promote remote organ dysfunction, septic complications, and mortality. Treatment with immunomodulating substances has been subject of few experimental studies with still conflicting results. The authors used the taurocholate-induced model of severe acute pancreatitis (SAP) in rats which were assigned to different treatment regimen: isotonic saline (SAP-S) for nontreated controls, recombinant rat interferon-gamma for immunostimulation (SAP-IFN-gamma), and FK506 for immunosuppression (SAP-FK506). Animals were killed after 3, 6, and 24 hours as well as 3 and 7 days, and parameters of local and systemic severity were assessed. Treatment with IFN-gamma and FK506 attenuated the progression of intrapancreatic necrosis within the first 24 hours after pancreatitis induction along with a substantial reduction of subsequent neutrophil tissue infiltration as shown by decreased myeloperoxidase activity. Enhanced cell death by apoptosis during the postacute course was reduced in FK506-treated animals only. Pancreatic interleukin (IL) 1beta messenger RNA up-regulation occurred early and was slightly suppressed in both treatment groups; tumor necrosis factor alpha (TNF-alpha) and IL-2 messenger RNA expression paralleled the onset of apoptosis and was markedly decreased in IFN-gamma- and FK506-treated rats. The two therapeutic regimens had similar effects on intrapancreatic and systemic IL-1beta and TNF-alpha protein release; however, the profiles of both cytokines were differently influenced. Whereas IFN-gamma and FK506 treatment lead to an enhanced intrapancreatic and systemic TNF-alpha protein release during the early course, IL-1beta concentrations were significantly reduced within the late intervals. Seven-day mortality was 44% in saline-, 29% in IFN-gamma-, and 25% in FK506-treated rats (P not significant). In conclusions, severe acute pancreatitis is associated with early alterations of the immune response comprising overt T-cell activation and impaired monocyte/macrophage function alike. Targeting either immunologic derangement improves local pancreatic damage and systemic severity. However, because mortality was not improved, a therapeutic benefit of immunomodulating substances in clinical SAP remains to be defined.

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**Hemoconcentration and pancreatic necrosis: further defining the relationship.**

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In the setting of acute pancreatitis, an admission hematocrit greater than or equal to 44% and/or a failure of hematocrit to drop at 24 hours have been reported as useful markers to predict subsequent necrosis. The authors aimed to validate the use of hemoconcentration as a marker to predict necrosis in adult patients presenting with acute pancreatitis. Patients admitted to the Dartmouth-Hitchcock Medical Center from 1990 to 2003 with a first presentation of acute pancreatitis were identified. Charts were abstracted for baseline demographic and clinical information, including admission and 24-hour hematocrit, and subsequent hospital course. Necrosis was determined based on computed tomography scan. The authors calculated the sensitivity, specificity, positive and negative predictive values (NPV) for different admissions, and 24-hour hematocrit levels in predicting the subsequent development of necrosis. Two hundred thirty patients were identified. Admission hematocrit (greater than or equal to 44%) was a poor predictor of subsequent necrosis with a sensitivity of 52.9%. The absence of hemoconcentration at admission or a drop in 24-hour hematocrit level was reliable in predicting that patients would not develop necrosis (NPV of 94.7% for hematocrit greater than or equal to 44%). Results were
similar when the authors compared a range of admission and 24-hour hematocrit values. In conclusions, in a community setting with low rates of necrosis, admission and 24-hour hematocrit levels were not helpful in predicting subsequent necrosis. The absence of admission hemoconcentration had strong NPV for necrosis. However, the actual clinical utility of this test to direct clinical decision making may be limited.


**Effect of thoracoscopic splanchnic denervation on pain processing in chronic pancreatitis patients.**

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Central sensitisation due to visceral pancreatic nociceptive input may play an important role in chronic pancreatitis pain. Using quantitative sensory testing (QST), this study investigates whether thoracoscopic splanchnic denervation (TSD), performed to reduce nociceptive visceral input, affects central sensitisation in chronic pancreatitis patients. The authors studied 19 chronic pancreatitis patients (11 men, 8 women on stable opioid medication) and 18 healthy volunteers as preoperative controls. Preoperatively and 6 weeks after TSD, pain numeric rating scores, opioid medication, and thresholds to electric skin stimulation and pressure pain (measured in dermatomes T10 (pancreas), C5, T4, L1, L4) were documented. Treatment success was defined as cessation of opioids 6 weeks after TSD. Six weeks after TSD, there was a trend towards lower pain scores, only 10 patients were still on opioids (P<0.05 vs. preoperatively) and thresholds overall were significantly higher than preoperatively (pressure pain: +25%, P<0.001; electric: sensation +55%, pain detection +34%, pain tolerance +21%, P<0.05). Gender-specific differences in hypoalgesia patterns were seen. Preoperatively, TSD treatment successes consumed significantly less opioids than failures, without significant differences in preoperative patterns of neuroplasticity. In conclusions, TSD for chronic pancreatitis pain resulted in fewer patients on opioids and overall increases in pain thresholds. These results suggest that TSD for reducing visceral nociceptive input may be effective in reducing resulting central sensitisation. Although patients benefiting from TSD consume less opioids preoperatively, the authors were unable to clearly link treatment success with specific perioperative patterns of neuroplasticity such as the presence or absence of hyperalgesia.

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**Cyclooxygenase-2 is required for activated pancreatic stellate cells to respond to pro-inflammatory cytokines.**


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Cyclooxygenase-2 (Cox-2) mediates various inflammatory responses and is expressed in pancreatic tissue from patients with chronic pancreatitis. To examine the role of Cox-2 in chronic pancreatitis, the authors investigated its participation in regulating functions of pancreatic stellate cells (PSCs), using isolated rat PSCs. Cox-2 was expressed in culture-activated PSCs but not in freshly isolated quiescent PSCs. TGF-beta1, interleukin (IL)-1beta, and IL-6 enhanced Cox-2 expression in activated PSCs, concomitantly increasing the expression of alpha-smooth muscle actin (alpha-SMA), a parameter of PSC activation. The Cox-2 inhibitor NS-398 blocked culture-activation of freshly isolated quiescent PSCs. TGF-beta1, interleukin (IL)-1beta, and IL-6 enhanced Cox-2 expression in activated PSCs, concomitantly increasing the expression of alpha-smooth muscle actin (alpha-SMA), a parameter of PSC activation. The Cox-2 inhibitor NS-398 blocked culture-activation of freshly isolated quiescent PSCs. NS-398 also inhibited the enhancement of alpha-SMA expression by TGF-beta1, IL-1beta and IL-6 in activated PSCs. These data
indicate that Cox-2 is required for the initiation and promotion of PSC activation. The authors further investigated the mechanism by which cytokines enhance Cox-2 expression in PSCs. Adenovirus-mediated expression of dominant-negative Smad2/3 inhibited the increase in expression of Cox-2, alpha-SMA, and collagen-1 mediated by TGF-beta1 in activated PSCs. Moreover, dominant-negative Smad2/3 expression attenuated the expression of Cox-2 and alpha-SMA enhanced by IL-1beta and IL-6. Anti-TGF-beta neutralizing antibody also attenuated the increase in Cox-2 and alpha-SMA expression caused by IL-1beta and IL-6. IL-6 as well as IL-1beta enhanced TGF-beta secretion from PSCs. These data indicate that Smad2/3-dependent pathway plays a central role in Cox-2 induction by TGF-beta1, IL-1beta, and IL-6. Furthermore, IL-1beta and IL-6 promote PSC activation by enhancing Cox-2 expression indirectly through Smad2/3-dependent pathway via increasing TGF-beta1 secretion from PSCs.


Fibrogenesis in alcoholic chronic pancreatitis: the role of tissue necrosis, macrophages, myofibroblasts and cytokines.

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Myofibroblasts and cytokines such as transforming growth factor-beta1 (TGF-beta1) and platelet-derived growth factor (PDGF)-B have been found to play an important role in pancreatitis-associated fibrogenesis. It is still unclear, however, where in the inflamed pancreas and when these fibrogenic cells and cytokines can be detected. In this study the authors examined pancreatic tissue from patients with alcoholic chronic pancreatitis to determine the localization and distribution of myofibroblasts and the expression of cytokines in relation to the tissue damage and the activity of the inflammatory process. In tissue from pancreatic specimens from 59 patients with alcoholic chronic pancreatitis the inflammatory process was histologically staged. Myofibroblasts and the cytokines latency-associated peptide, a TGF-beta propeptide, TGF-beta receptor II, PDGF-B and the alpha-isoform of the PDGF receptor were immunohistochemically identified in 10 selected cases representing the four defined stages of alcoholic chronic pancreatitis. In stage I, the stage with overt tissue injury, myofibroblasts were numerous and especially associated with macrophages around areas of necrosis. In stage II, the stage with cellular fibrosis, myofibroblasts were the main component of the interlobular tissue. In stage III, the stage with dense fibrosis, myofibroblasts were rare, and in stage IV, when calculi were present, myofibroblasts were only detected adjacent to duct ulcerations caused by calculi. Latency-associated peptide and TGF-beta receptor II as well as PDGF-B and PDGF receptor-alpha were mainly expressed by macrophages, myofibroblasts and epithelial cells in stages I and II. The results suggest that the fibrogenic process in alcoholic chronic pancreatitis is initiated by a cytokine-based interplay of macrophages and myofibroblasts that follows tissue injury.

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Oncogenic K-Ras down-regulates Rac1 and RhoA activity and enhances migration and invasion of pancreatic carcinoma cells through activation of p38.


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Activating mutations in the K-ras gene are genetic alterations frequently found in human
carcinomas, particularly in pancreatic adenocarcinomas. Mutation of the K-ras gene is thought to be an early and important event in pancreatic tumor initiation, but the precise role of the mutant K-Ras proteins in neoplastic progression is still unknown. In the present study, the authors have characterized the influence of oncogenic K-Ras on the phenotype and on the signal transduction of epitheloid PANC-1 pancreatic carcinoma cells by generating PANC-1 cell clones, which stably express EGFP(enhanced green fluorescent protein)-K-Ras (V12). EGFP-K-Ras (V12)-expressing cells exhibited a more fibroblastoid cellular phenotype with irregular cell shape and disorganized cytokeratin filaments. Moreover, these cells showed a marked enhancement of their migratory and invasive properties. Stable expression of EGFP-K-Ras (V12) down-regulated the activity of Rac1 and RhoA, resulting in reduced subcortical actin filaments and stress fibers, which might contribute to the epithelial dedifferentiation. Characterization of the activity of mitogen-activated protein kinases revealed that EGFP-K-Ras (V12) enhanced the activity of p38, but did not affect the activities of the Raf/MEK/ERK cascade and JNK. While inhibition of either MEK or JNK activity had no effect on EGFP-K-Ras (V12)-induced migration, inhibition of p38 activity markedly reduced EGFP-K-Ras (V12)-induced migration. Collectively, the results suggest that oncogenic K-Ras enhances the malignant phenotype and identify the mitogen-activated protein kinase p38 as a target to inhibit oncogenic K-Ras-induced pancreatic tumor cell migration.