Pancreatitis

Human pancreatitis and the role of cathepsin B

M M Lerch, W Halangk

Any assumption about the role of the newly detected cathepsin B polymorphisms in pancreatitis must, for now, remain speculative

Acute pancreatitis has long been considered an autodigestive disorder in which the pancreas is destroyed by its own digestive proteases.[1] Under physiological conditions pancreatic proteases are synthesised as inactive precursor zymogens and stored by acinar cells in zymogen granules. Autodigestion of the gland would therefore require premature activation of these zymogens. How and where such a premature and intrapancreatic activation of digestive proenzymes is initiated in the course of pancreatitis has been the subject of several investigations.[2 3] Recent studies strongly suggest that the early pathophysiological events that eventually lead to necrosis of pancreatic tissue originate in acinar cells[3–5] and involve the intracellular presence of active trypsin,[5 6] a serine proteinase capable of activating other pancreatic zymogens. Within pancreatic acinar cells cytoplasmic vesicles have been identified as the subcellular compartment in which premature trypsinogen activation begins within minutes after induction of experimental pancreatitis.[3 7 8]

The molecular mechanisms responsible for the intracellular activation of trypsinogen, however, have remained elusive. One hypothesis predicts that the lysosomal cysteine proteinase cathepsin B (CTSB) plays an essential role in this process.[9] The largely circumstantial evidence for this ''cathepsin B hypothesis'' is based on the following observations: (a) CTSB has been shown to activate trypsinogen in vitro[10]; (b) during the initial phase of acute pancreatitis in several animal models, redistribution of CTSB into a zymogen granule containing subcellular compartment was detected by density gradient centrifugation[11]; and (c) in the same pancreatitis models lysosomal enzymes were detected by immunogold electron microscopy in secretory organelles that also contained digestive enzymes (for example, trypsinogen).[12] Experimental approaches to show an essential role for CTSB in premature zymogen activation by inhibition of this lysosomal enzyme with synthetic inhibitors rendered contradictory results either increasing[13] or decreasing premature zymogen activation,[14] or failing to improve the course of experimental pancreatitis.[15] To test the cathepsin B hypothesis more directly and to overcome the shortcomings of lysosomal enzyme inhibitors, which have only limited specificity for CTSB, a CTSB deficient mouse strain that was generated by targeted disruption of the *ctsb* gene was studied in an experimental animal model of pancreatitis.[16] The results of these studies were unequivocal: 90% of intrapancreatic trypsinogen activation during pancreatitis depends on the presence of cathepsin B.[16] While the reduction in local and systemic complications of pancreatitis that were conveyed by deletion of *ctsb* were not nearly as impressive, the experiments answered the question about the pathophysiological role of CTSB in premature digestive enzyme activation during experimental pancreatitis with a resounding yes and settled all arguments about this issue.

The relevance for human disease, however, is another matter. First attempts to establish the relevance of the cathepsin B-pancreatitis hypothesis in humans focused on the capacity of the lysosomal enzyme to activate human trypsinogen, and specifically varieties of human trypsinogen, into which disease relevant mutations had been introduced that were identified in the context of hereditary pancreatitis studies. Hereditary pancreatitis is a type of pancreatitis that follows an autosomal dominant inheritance pattern, is associated with an early diseases onset of chronic pancreatitis (usually in children and young adults), and is associated with various germline mutations in the cationic trypsinogen (*prss1*) gene.[5] When recombinant trypsinogen with hereditary pancreatitis mutations was subjected to activation by CTSB in vitro it was, indeed, found that some trypsins behaved differently from their wild-type counterpart,[17 18] an observation that clearly supported the cathepsin B hypothesis of pancreatitis. On the other hand, the most common PRSS1 mutations, such as R122H and N29I, did not convincingly vary from wild-type trypsin in their activation kinetics by CTSB.[19] The same study also demonstrated that CTSB is abundantly secreted from the human exocrine pancreas, plentifully contained in pancreatic secretory zymogen granules (rather than in lysosomes), as well as active within the secretory pathway.[19] Thus all cellular conditions for the cathepsin-B-pancreatitis hypothesis to be operative in humans were met. Moreover, the proposed requirement for a subcellular redistribution of CTSB into the secretory compartment[11] could finally be put to test because most CTSB in the pancreas was found to already reside in the secretory compartment under physiological conditions[19 20] rather than having to be redistributed there from lysosomes. Nevertheless, no direct evidence for active involvement of CTSB in the onset of human pancreatitis—at least not in hereditary pancreatitis caused by the most common mutations—could be produced from these studies.

At this stage, Mahurkar and colleagues[21] entered the fray with a study published in the present issue of *Gut* ***(see page 1270)***. This group from Hyderabad had been instrumental in characterising the genetic basis of tropical pancreatitis,[22 23] a disease variety that was previously thought to be linked to dietary components (for example, cassava) or selenium deficiency and is now known to be linked to mutations in the pancreatic secretory trypsin inhibitor gene (*spink1* gene).[4] Because *spink1* mutations explain only about half of the cases with tropical pancreatitis, Mahurkar *et al* sequenced the entire coding region of the *ctsb* gene from 51 South Indian patients with tropical pancreatitis and speculated that *ctsb* germline changes may explain the rest of cases. When they compared their *ctsb* sequencing data with that of 25 healthy controls they found 23 different polymorphisms and increased the number of patients to 140 (that of controls to 155) to genotype all of them for the four most interesting of these polymorphism. They found a significant difference between patients and controls only for a C76G polymorphism that results in a leucine to valine mutation at amino acid 26 (allele frequency in patients 0.46 versus 0.30 in controls). To rule out a chance finding, they went further south in India and recruited a second cohort of tropical pancreatitis patients (n = 166) and controls (n = 175) from Calicut and genotyped them for the same four polymorphism as the first group. Again, only the Leu26Val mutation

was about twice as common among patients than controls.

So far the data would suggest that carrying a C76G polymorphism in the *ctsb* gene (that is, a leucine to valine mutation in the CTSB protein) would double the risk of developing tropical pancreatitis provided one is ethnically Drawidian and hail from southern India. The study then went further. The fact that the most common *spink1* mutation associated with tropical pancreatitis (N34S) has no measurable effect on the trypsin inhibiting capacity of the SPINK1/PSTI protein[24] led to the speculation that, rather than causing tropical pancreatitis in India[5] and idiopathic pancreatitis elsewhere,[25 26] SPINK1 may act as a modifier gene for other genetic changes. That this was not the case for mutations in the cationic trypsinogen (*prss1*) gene had already been shown[27] but Mahurkar *et al* also tested it for *ctsb*. As found previously for trypsin, no differences in phenotype of pancreatitis or genotype with regard to N34S positive and N34S negative pancreatitis patients could be found for the L26V mutation. This demonstrates effectively that, whatever the effects of the CTSB mutations, they are unrelated to changes in SPINK1.

Other polymorphisms, those that do not lead to amino acid exchanges and were equally distributed between patients and controls, varied between N34S carriers and non-carriers in further subgroup analyses but these may have been chance findings. Hard evidence was therefore only presented for a twofold pancreatitis risk in carriers or the L26V mutation in CTSB.

What could be the effect of this mutation at the cellular level? As no functional data are presently available, structural consideration must serve as a surrogate and the interpretation will necessarily remain speculative. The L26V mutation affects the propeptide region of CTSB which makes it very unlikely that it has an effect on the catalytic centre and thus on the enzymatic activity of CTSB. The most that could therefore be expected from a mutation at this site would be an effect on CTSB trafficking, but that may be sufficient to be disease relevant because it matters very much whether the mannose-6-phosphate dependent sorting of CTSB ends up in a lysosome or in a zymogen granule where dangerous substrates such as trypsinogens reside.

Little else can be learned from the actual polymorphism: a leucine to valine exchange is about as unexciting as a mutation can get, replacing one nonpolar amino acid with another that differs only in one CH_2 group. A more attractive explanation to emerge from functional studies would be that the mutation affects the capacity of CTSB to activate mesotrypsin, a trypsin variant that preferentially degrades SPINK1,[28] which in turn can inhibit cationic trypsin, which in turn has an established role in at least some variety of pancreatitis. As this complicated chain of events indicates, any assumption about the role of the newly detected *ctsb* polymorphism in the context of pancreatitis must remain wildly speculative. Even a role of CTSB in pancreatitis that is completely unrelated to activation of trypsin must be considered, just as the function of SPINK1 in the pancreas was found in knockout animal studies to involve embryonic pancreas development and not, to the surprise of many, the premature activation of trypsinogen during pancreatitis.[29]

Functional studies that examine the biochemistry, cell biology, and interaction with other proteins for different CTSB variants will ultimately have to provide that answer. Whether other more common varieties of pancreatitis are equally associated with genetic *ctsb* changes will also have to be determined. Until then, Mahurkar *et al* have proven to us that: (1) the 30 year old cathepsin B hypothesis of pancreatitis has entered the phase in which evidence from human studies is finally being presented, and (2) that candidate gene sequencing based on pathophysiological information is still a valid and successful research strategy, particularly when well characterised patient cohorts are available for genotyping. The authors from Hyderabad should be congratulated on these achievements.

ACKNOWLEDGEMENTS

The authors own studies are supported by grants from the DFG HA2080/6-1, Le 625/7-1, and Le 625/8-1, Mildred Scheel Stiftung 10-2031-Le I, and Alfried Krupp von Bohlen und Halbach-Stiftung (Graduiertenkolleg Tumorbiologie).

Gut 2006;**55**:1228–1230.
doi: 10.1136/gut.2006.092114

Authors' affiliations

M M Lerch, Department of Gastroenterology, Endocrinology, and Nutrition, Ernst-Moritz-Arndt Universität Greifswald, Germany
W Halangk, Department of Surgery, Division of Experimental Surgery, Otto-von-Guericke-Universität, Magdeburg, Germany

Correspondence to: Professor M M Lerch, Department of Gastroenterology, Endocrinology, and Nutrition, Ernst-Moritz-Arndt Universität Greifswald, Friedrich-Loeffler-Str 23A, 17487 Greifswald, Germany; lerch@uni-greifswald.de

Conflict of interest: None declared.

REFERENCES

1 **Chiari H**. Über die Selbstverdauung des menschlichen Pankreas. *Z Heilk* 1896;**17**:69–96.
2 **Halangk W**, Krüger B, Ruthenbürger M, *et al*. The role of trypsin in premature, intrapancreatic trypsinogen activation and inactivation of trypsin activity. *Am J Physiol Gastrointest Liver Physiol* 2002;**282**:G367–74.
3 **Kruger B**, Albrecht E, Lerch MM. The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. *Am J Pathol* 2000;**157**:43–50.
4 **Lerch MM**, Saluja AK, Dawra R, *et al*. Acute necrotising pancreatitis in the opossum: earliest morphologic changes involve acinar cells. *Gastroenterology* 1992;**103**:205–13.
5 **Whitcomb DC**, Gorry MC, Preston RA, *et al*. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996;**14**:141–5.
6 **Gudgeon AM**, Heath DI, Hurley P, *et al*. Trypsinogen activation peptides assay in the early prediction of severity of acute pancreatitis. *Lancet* 1990;**335**:4–8.
7 **Hofbauer B**, Saluja AK, Lerch MM, *et al*. Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. *Am J Phys* 1998;**275**:G352–62.
8 **Otani T**, Chepilko SM, Grendell JH, *et al*. Codistribution of TAP and the granule membrane protein GRAMP-92 in rat caerulein-induced pancreatitis. *Am J Physiol* 1998;**275**:G999–1009.
9 **Steer ML**, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987;**316**:144–50.
10 **Figarella C**, Miszczuk-Jamska B, Barrett A. Possible lysosomal activation of pancreatic zymogens: activation of both human trypsinogens by cathepsin B and spontaneous acid activiation of human trypsinogen-1. *Biol Chem Hoppe-Seyler* 1988;**369**:293–8.
11 **Saluja A**, Sadamitsu H, Saluja M, *et al*. Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis. *Am J Physiol* 1987;**253**:G508–16.
12 **Watanabe O**, Baccino FM, Steer ML, *et al*. Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. *Am J Physiol* 1984;**246**:G457–67.
13 **Leach SD**, Modlin IM, Scheele GA, *et al*. Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high does of cholecystokinin. *J Clin Invest* 1991;**87**:362–6.
14 **Saluja AK**, Donovan EA, Yamanaka K, *et al*. Cerulein-induced in vitro activation of trypsinogen inrat pancreatic acini is mediated by cathepsin B. *Gastroenterology* 1997;**113**:304–10.
15 **Steer ML**, Saluja AK. Experimental acute pancreatitis: studies of the early events that lead to cell injury. In: Go VLW, DiMagno EP, Gardner JD, *et al. The pancreas: biology, pathobiology, and disease*. New York: Raven Press, 1993:489–500.
16 **Halangk W**, Lerch MM, Brandt-Nedelev B, *et al*. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 2000;**106**:773–81.
17 **Szilagyi L**, Kenesi E, Katona G, *et al*. Comparative in vitro studies on native and recombinant human cationic trypsins. Cathepsin B is a possible pathological activator of trypsinogen in pancreatitis. *J Biol Chem* 2001;**276**:24574–80.
18 **Simon P**, Weiss FU, Sahin-Tóth M, *et al*. Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122→Cys) that alters autoactivation and autodegradation of cationic trypsinogen. *J Biol Chem* 2002;**277**:5404–10.
19 **Kukor Z**, Mayerle J, Kruger B, *et al*. Presence of cathepsin B in the human pancreatic secretory pathway and its role in trypsinogen activation during hereditary pancreatitis. *J Biol Chem* 2002;**277**:21389–96.
20 **Tooze J**, Hollinshead M, Hensel G, *et al*. Regulated secretion of mature cathepsin B from rat exocrine pancreatic cells. *Eur J Cell Biol* 1991;**56**:187–200.

21 **Mahurkar S**, Idris MM, Reddy DN, *et al.* Association of cathepsin B gene polymorphisms with tropical calcific pancreatitis. *Gut* 2006;**55**:1270–5.
22 **Chandak GR**, Idris MM, Reddy DN, *et al.* Mutations in the pancreatic secretory trypsin inhibitor gene (PSTI/SPINK1) rather than the cationic trypsinogen gene (PRSS1) are significantly associated with tropical calcific pancreatitis. *J Med Genet* 2002;**39**:347–51.
23 **Chandak GR**, Idris MM, Reddy DN, *et al.* Absence of PRSS1 mutations and association of SPINK1 trypsin inhibitor mutations in hereditary and non-hereditary chronic pancreatitis. *Gut* 2004;**53**:723–8.
24 **Kuwata K**, Hirota M, Shimizu H, *et al.* Functional analysis of recombinant pancreatic secretory trypsin inhibitor protein with amino-acid substitution. *J Gastroenterol* 2002;**37**:928–34.
25 **Witt H**, Luck W, Hennies HC, *et al.* Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat Genet* 2000;**25**:213–16.
26 **Weiss FU**, Simon P, Bogdanova N, *et al.* Complete cystic fibrosis transmembrane conductance regulator gene sequencing in patients with idiopathic chronic pancreatitis and controls. *Gut* 2005;**54**:1456–60.
27 **Weiss FU**, Simon P, Witt H, *et al.* SPINK1 mutations and phenotype expression in patients with trypsinogen mutation-associated pancreatitis. *J Med Genet* 2003;**40**:1–5.
28 **Szmola R**, Kukor Z, Sahin-Toth M. Human mesotrypsin is a unique digestive protease specialized for the degradation of trypsin inhibitors. *J Biol Chem* 2003;**278**:48580–9.
29 **Ohmuraya M**, Hirota M, Araki M, *et al.* Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. *Gastroenterology* 2005;**129**:696–705.

Cirrhosis

Role of endothelin in systemic and portal resistance in cirrhosis

P W Angus

Endothelin may be involved in many of the vascular abnormalities in patients with cirrhosis, and its overall effects in different tissues may depend on differential expression of endothelin receptors on smooth muscle and endothelial cells

Many of the complications of cirrhosis result from haemodynamic changes involving the systemic circulation and regional vascular beds. Typically, patients with advanced cirrhosis and portal hypertension have a hyperdynamic vasodilated circulation characterised by high cardiac output and low blood pressure, and this leads to compensatory activation of vasoconstrictor systems, including the sympathetic nervous system and renin-angiotensin-aldosterone systems.[1] Much of this picture can be attributed to vasodilatation of the mesenteric vascular bed which in turn contributes to portal hypertension by increasing portal inflow. While vascular resistance in the mesenteric bed is reduced, another major contributor to portal hypertension is an increase in vascular tone within the liver which is at least partly mediated by hepatic stellate cells. Altered local vascular tone contributes to other important complications of cirrhosis—intrarenal vasoconstriction can result in the hepatorenal syndrome, while in the lung pathological vasodilatation can result in the development of the hepatopulmonary syndrome, and less commonly pulmonary vasoconstriction may result in portopulmonary hypertension. As a result there has been intense interest in understanding the mechanisms and vascular mediators responsible for these systemic and regional changes in vascular tone with the hope that this will lead to the development of new treatments.

Endothelin 1 (ET-1) is a potent endothelium derived vasoactive peptide that plays a central role in regulating vascular tone in healthy individuals but has multiple other actions that may be of importance in disease, including stimulation of cellular growth and proliferation, and involvement in the wound healing response and tissue fibrogenesis.[2 3] For more than a decade there has been major interest in the possible role of ET-1 in the pathogenesis of cirrhosis, its contribution to portal hypertension, and the possibility that endothelin antagonists might be used in the treatment of portal hypertension and other complications of cirrhosis.[4 5] Plasma endothelin levels are increased in cirrhosis, and correlate with the severity of liver disease and portal pressures.[6 7] The hepatosplanchnic circulation, including the splenic vascular bed and the liver itself, appears to be the major source of this increased endothelin production.[8–11] Importantly, while in health the vascular endothelium is the major source of endothelin production, in the cirrhotic liver ET-1 appears to be largely derived from activated stellate cells.[10] Recent studies suggest that in cholestatic liver injury, cholangiocytes are another important source of ET-1.[12]

A number of lines of evidence suggest that this increased ET-1 production may contribute to portal hypertension. Endothelin receptor expression is upregulated in liver disease and hepatic stellate cells express the highest levels of endothelin receptors.[13–15] Furthermore, endothelin induced contraction is enhanced in stellate cells from cirrhotic rat livers and in the intact liver endothelin causes sustained vasoconstriction.[16] Thus it has been proposed that increased hepatosplenic production of endothelin contributes to portal hypertension by mediating intrahepatic stellate cell contraction and an increase in hepatic sinusoidal tone. This concept has been supported by studies in animal models of portal hypertension which have shown that administration of endothelin antagonists reduces portal pressure.[17–19] The weight of evidence is that this effect is largely due to a reduction in hepatic and collateral resistance rather than to changes in mesenteric blood flow.[17 19]

Thus there is strong experimental evidence that endothelin contributes to increased intrahepatic vascular tone in cirrhosis. However, there is also evidence that altered responsiveness to ET-1 may contribute to changes in the systemic and mesenteric circulation. Despite elevation of circulating endothelin and vasopressin levels and activation of the renin-angiotensin system and adrenergic nervous systems, peripheral and mesenteric vascular tone is reduced in patients with advanced liver disease, with the degree of activation of vasoconstrictor responses being greatest in those in whom vasodilatation is most prominent.[20] This suggests vascular responsiveness to these endogenous vasoconstrictors is impaired. Helmy *et al* have shown that peripheral vascular responses to angiotensin II are diminished in cirrhotic patients but can be restored by inhibition of local nitric oxide production.[21] Using a similar experimental approach, these workers observed that in patients with compensated cirrhosis, vasoconstrictor responses to ET-1 were significantly reduced compared with controls.[5 22] We have recently shown that infusion of ET-1 into the forearm of patients with