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Chymotrypsin Reduces the Severity of Secretagogue-Induced Pancreatitis in Mice

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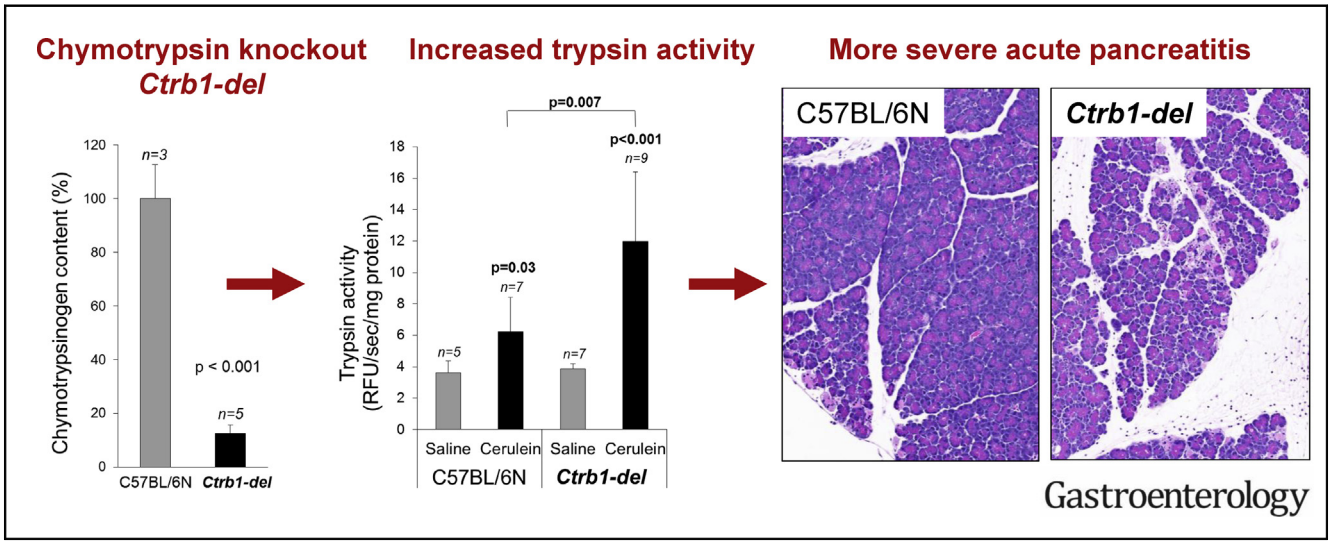

Intrapancreatic activation of the digestive proteases trypsin and chymotrypsin is an early event in the development of pancreatitis. Human genetic studies indicate that chymotrypsin controls trypsin activity via degradation, but there is no evidence of this from animal models. We used CRISPR-Cas9 to disrupt the chymotrypsinogen B1 gene (*Ctrb1*) in C57BL/6N mice and induced pancreatitis in CTRB1-deficient and C57BL/6N (control) mice by administration of cerulein. CTRB1-deficient mice given cerulein had significant increases in intrapancreatic trypsin activity and developed more severe pancreatitis compared with control mice. CTRB1 therefore protects against secretagogue-induced pancreatitis by reducing trypsin activity. Protease inhibitors developed for treatment of pancreatitis should be designed to target trypsin but not chymotrypsin.

Keywords: Mouse Model; Pancreas; Inflammation; Proteolysis.

Activation of digestive proteases inside pancreatic acinar cells is an early event in experimental models of acute pancreatitis in rodents.[1,2] The best-characterized example is the activation of trypsin and chymotrypsin in response to supramaximal stimulation with secretagogues such as cerulein, an analog of cholecystokinin. In the cerulein-induced pancreatitis model, the lysosomal cysteine protease cathepsin B appears to be responsible for activation of trypsinogen to trypsin.[1–3] In turn, trypsin can activate chymotrypsinogen to chymotrypsin, although an ATP-dependent activation mechanism unrelated to trypsin has been also proposed.[4] Trypsin activity has been implicated as a driver of the pathogenic process by inducing acinar cell apoptosis or necrosis either directly[5] or through the cytoplasmic release of cathepsin B.[6] Genetic deletion of cathepsin B or the mouse cationic trypsinogen (T7) moderately protected against pancreatitis, suggesting that trypsin-independent mechanisms also contribute to disease severity.[3,7] The pathogenic role of chymotrypsin in the rodent pancreatitis models has been less well characterized, although the powerful digestive activity of this protease suggests significant harmful potential.

Human genetic studies, together with biochemical investigations, confirmed the pivotal role of trypsin and chymotrypsin in pancreatitis and revealed that chymotrypsin regulates trypsin activity through trypsinogen degradation and thereby exerts a protective function.[8] The

Abbreviations used in this paper: **CTRB1, chymotrypsin B1; CTRC, chymotrypsin C; T7, mouse cationic trypsinogen.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Intra-pancreatic activation of trypsinogen to trypsin is an early pathogenic event in pancreatitis. Chymotrypsin degrades trypsinogen and thereby may protect against pancreatitis.

NEW FINDINGS

The researchers show that genetic disruption of chymotrypsin results in higher intra-pancreatic trypsin levels and increased severity of secretagogue-induced pancreatitis in mice.

LIMITATIONS

Chymotrypsin deficiency did not alter pancreatitis severity in arginine-induced pancreatitis, where trypsin plays a lesser role.

IMPACT

Therapeutic protease inhibition in pancreatitis should spare protective chymotrypsin while targeting harmful trypsin.

clinically most common mutations in the serine protease 1 (*PRSS1*) gene encoding human cationic trypsinogen cause hereditary pancreatitis by rendering trypsinogen resistant to protective degradation by chymotrypsin C (CTRC) and thereby increasing intrapancreatic trypsin activity. Loss-of-function mutations in *CTRC* are independent risk factors for chronic pancreatitis, even in the absence of trypsinogen mutations, demonstrating that chymotrypsin-dependent trypsin control is essential for pancreas health. A regulatory role for chymotrypsin was further corroborated by the recent observations that a common inversion at the *CTRB1-CTRB2* locus (encoding chymotrypsin B1 and B2) has a small but significant impact on pancreatitis risk.[9]

To confirm the presumed protective role of chymotrypsin in a mouse model in vivo, we used CRISPR-Cas9–based genome editing in C57BL/6N mice to disrupt the *Ctrb1* gene. The mouse genome contains a single *Ctrb1* gene, which encodes the functional ortholog of human chymotrypsin B2.[10,11] Genomic manipulation resulted in the surgical deletion of the 79 nucleotides long exon 4 from *Ctrb1* (Supplementary Figure 1). Reverse-transcription polymerase chain reaction of pancreatic messenger RNA and Western blots of pancreas homogenates from homozygous *Ctrb1-del* mice confirmed successful elimination of CTRB1 expression (Figure 1*A*, Supplementary Figure 2). Total chymotrypsinogen content of pancreas homogenates from *Ctrb1-del* mice was reduced by almost 90%, relative to C57BL/6N controls, whereas trypsinogen content was unchanged (Figure 1*B*). Because the C57BL/6N strain is naturally deficient in CTRC, the remaining chymotrypsinogen is likely chymotrypsin-like protease[12,13] or a nonacinar protease with chymotrypsin-like activity such as mast cell chymase. Mice deficient in CTRB1 showed no obvious phenotypic changes relative to their C57BL/6N parent strain; they exhibited normal weight gain and typical breeding behavior. Macroscopic pancreas morphology and pancreas histology, as judged on hematoxylin-eosin–stained sections, was also unchanged. No spontaneous pancreatitis or other pancreatic pathology was apparent up to 1 year of age.

To examine whether the absence of CTRB1 alters pathological responses in experimentally induced pancreatitis, we challenged the mice with 10 hourly injections of cerulein and euthanized them 1 hour after the last injection. Histological analysis of the pancreas indicated increased edema, stronger infiltration of inflammatory cells, and more pronounced acinar cell necrosis in the *Ctrb1-del* mice compared with C57BL/6N controls (Figure 1*C–K*).These findings were confirmed and extended by direct assessment of edema and biochemical assays of pancreatitis severity. Thus, relative to controls, the pancreas of *Ctrb1-del* mice was visibly larger and weighed significantly more (Figure 2*A*). Measurement of pancreatic water content provided direct evidence for the more pronounced edema in *Ctrb1-del* mice (Figure 2*B*). Plasma amylase activity (Figure 2*C*) and pancreas tissue myeloperoxidase content (Figure 2*D*) were significantly elevated in *Ctrb1-del* mice in comparison with controls, indicating more extensive acinar cell injury and inflammation. Systemic inflammation was also higher in *Ctrb1-del* mice, as judged by the lung myeloperoxidase content (Supplementary Figure 3). In conclusion, all parameters of cerulein-induced pancreatitis were significantly worse in the absence of CTRB1.

To demonstrate that increased acinar cell damage and inflammation are due to higher trypsin activity, we measured cerulein-induced intrapancreatic trypsin and chymotrypsin activities in *Ctrb1-del* and C57BL/6N control mice. Protease activities were determined at 30 minutes after a single cerulein injection when trypsin activity peaks[3,7] and inflammatory cells do not influence trypsin activation yet. We found significantly higher trypsin activity in *Ctrb1-del* mice relative to controls (Figure 2*E*). As expected, chymotrypsin activity increased

Figure 1. Chymotrypsin expression and cerulein-induced pancreatitis in *Ctrb1-del* and C57BL/6N control mice. (*A*) Western blot analysis of CTRB1 protein in pancreas homogenates. ERK1/2 was measured as loading control. (*B*) Trypsinogen and chymotrypsinogen content of pancreas homogenates. (*C–F*) Representative hematoxylin-eosin–stained histological sections of the pancreas from mice treated with 10 hourly injections of saline (*C*, *D*) or cerulein (*E*, *F*). (*G*, *H*) Higher magnification of indicated areas. (*I–K*) Histology scoring of hematoxylin-eosin–stained sections for edema (*I*), inflammatory cell infiltration (*J*), and acinar cell necrosis (*K*). Mean values with standard deviation are shown.