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Chymotrypsin Reduces the Severity of Secretagogue-induced Pancreatitis in Mice

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Abstract

Intra-pancreatic 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 intra-pancreatic 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

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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 clinically most common mutations in *PRSS1* encoding human cationic trypsinogen cause hereditary pancreatitis by rendering trypsinogen resistant to protective degradation by chymotrypsin C (CTRC) and thereby increasing intra-pancreatic 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 CTRB2 [10, 11]. Genomic manipulation resulted in the surgical deletion of the 79 nucleotides long exon 4 from *Ctrb1* (Supplementary Figure S1). Reverse-transcription PCR of pancreatic mRNA and Western blots of pancreas homogenates from homozygous *Ctrb1-del* mice confirmed successful elimination of CTRB1 expression (Figure 1A, Supplementary Figure S2). Total chymotrypsinogen content of pancreas homogenates from *Ctrb1-del* mice was reduced by almost 90%, relative to C57BL/6N controls, while trypsinogen content was unchanged (Figure 1B). Because the C57BL/6N strain is naturally deficient in CTRC, the remaining chymotrypsinogen is likely chymotrypsin-like protease (CTRL) [12, 13] or a non-acinar 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 (H&E) 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 sacrificed them 1h 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 to C57BL/6N controls (Figure 1C–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 2A). Measurement of pancreatic water content provided direct evidence for the more pronounced edema in *Ctrb1-del* mice (Figure 2B). Plasma amylase activity (Figure 2C) and pancreas tissue myeloperoxidase (MPO) content (Figure 2D) were significantly elevated in *Ctrb1-del* mice in comparison to controls, indicating more extensive acinar cell injury and inflammation. Systemic inflammation was

also higher in *Ctrb1-del* mice, as judged by the lung MPO content (Supplementary Figure S3). 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 intra-pancreatic trypsin and chymotrypsin activities in *Ctrb1-del* and C57BL/6N control mice. Protease activities were determined at 30 min 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 2E). As expected, chymotrypsin activity increased in C57BL/6N controls in response to cerulein but not in *Ctrb1-del* mice (Figure 2F). Interestingly, baseline chymotrypsin activity was similar in *Ctrb1-del* and C57BL/6N control mice, indicating that this is unrelated to CTRB1.

In contrast to the cerulein model, we observed no effect of *Ctrb1* gene disruption in L-arginine-induced pancreatitis (Supplementary Figure S4). This result is consistent with recent studies showing that arginine acts through mitochondrial injury rather than early trypsin activation [14].

Finally, we performed biochemical experiments using purified proteins to demonstrate that CTRB1 degrades mouse cationic trypsinogen (T7) and thereby limits its activation to trypsin. T7 trypsinogen is the most abundant isoform comprising 40–50% of total mouse trypsinogen, and it autoactivates almost as readily as its human counterpart [15]. When autoactivation of T7 was measured in the presence of CTRB1, final trypsin levels were significantly reduced (Figure 2G). Digestion experiments revealed that CTRB1 cleaved T7 primarily at the Leu149-Ser150 peptide bond (Figure 2H). Mutation of Leu149 protected T7 against chymotryptic degradation and the L149A mutant autoactivated similarly in the absence and presence of CTRB1 (Figure 2I).

In summary, the present study demonstrates that the major mouse chymotrypsin CTRB1 protects against secretagogue-induced pancreatitis by degrading trypsinogen and reducing pathological trypsin levels. The findings should guide the design of anti-protease therapy in pancreatitis, which needs to spare protective chymotrypsin activity while targeting harmful trypsin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CTRB1	chymotrypsin B1
CTRB2	chymotrypsin B2
CTRC	chymotrypsin C
CTRL	chymotrypsin-like protease
H&E	hematoxylin-eosin
MPO	myeloperoxidase
PRSS1	serine protease 1, human cationic trypsinogen
T7	mouse cationic trypsinogen

REFERENCES

1. Steer ML. Pancreas 1998; 17:31–37 [PubMed: 9667517]
2. Saluja AK et al. Annu Rev Physiol 2007; 69:249–269 [PubMed: 17059357]
3. Halangk W et al. J Clin Invest 2000; 106:773–781 [PubMed: 10995788]
4. Thrower EC et al. Am J Physiol Gastrointest Liver Physiol 2006; 290:G894–902 [PubMed: 16339296]
5. Sendler M et al. J Biol Chem 2016; 291:14717–14731 [PubMed: 27226576]
6. Talukdar R et al. Gastroenterology 2016; 151:747–758 [PubMed: 27519471]
7. Dawra R et al. Gastroenterology 2011; 141:2210–2217 [PubMed: 21875495]
8. Hegyi E, Sahin-Tóth M. Dig Dis Sci 2017; 62:1692–1701 [PubMed: 28536777]
9. Rosendahl J. Gut. 2017 Jul 28. [Epub ahead of print].
10. Hudáky P et al. Eur J Biochem 1999; 259:528–533 [PubMed: 9914536]
11. Szabó A, Sahin-Tóth M. FEBS J 2012; 279:4283–4292 [PubMed: 23035638]
12. Reseland JE et al. J Biol Chem 1997; 272:8099–8104 [PubMed: 9065485]
13. Sogame Y et al. Pancreas 2002; 25:378–386 [PubMed: 12409833]
14. Biczo G et al. Gastroenterology 2018; 154:689–703 [PubMed: 29074451]
15. Németh BC et al. J Biol Chem 2013; 288:24049–24062 [PubMed: 23814066]

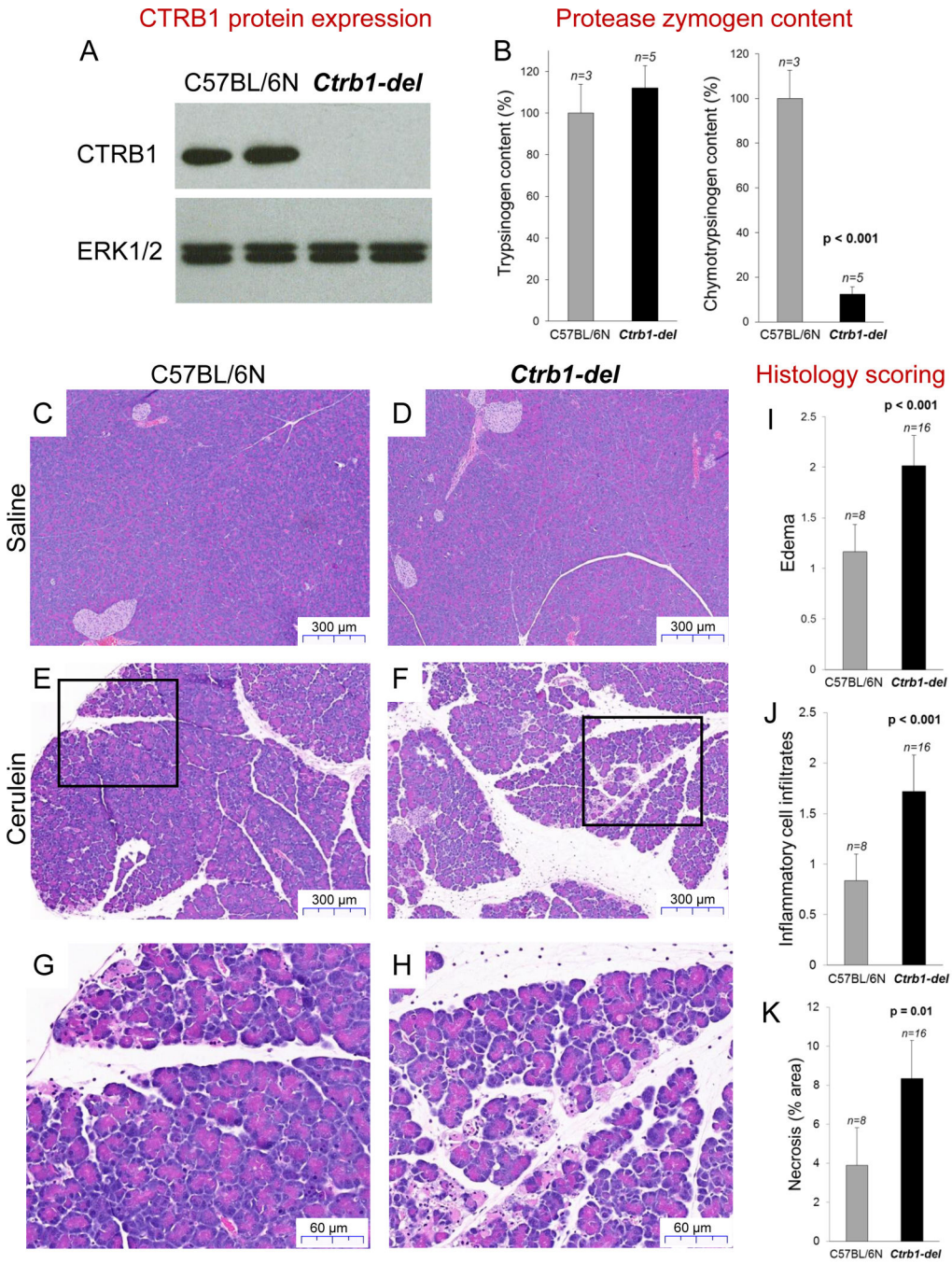

Figure 1.

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 (H&E) 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 H&E stained

sections for edema (I), inflammatory cell infiltration (J) and acinar cell necrosis (K). Mean values with S.D. are shown.

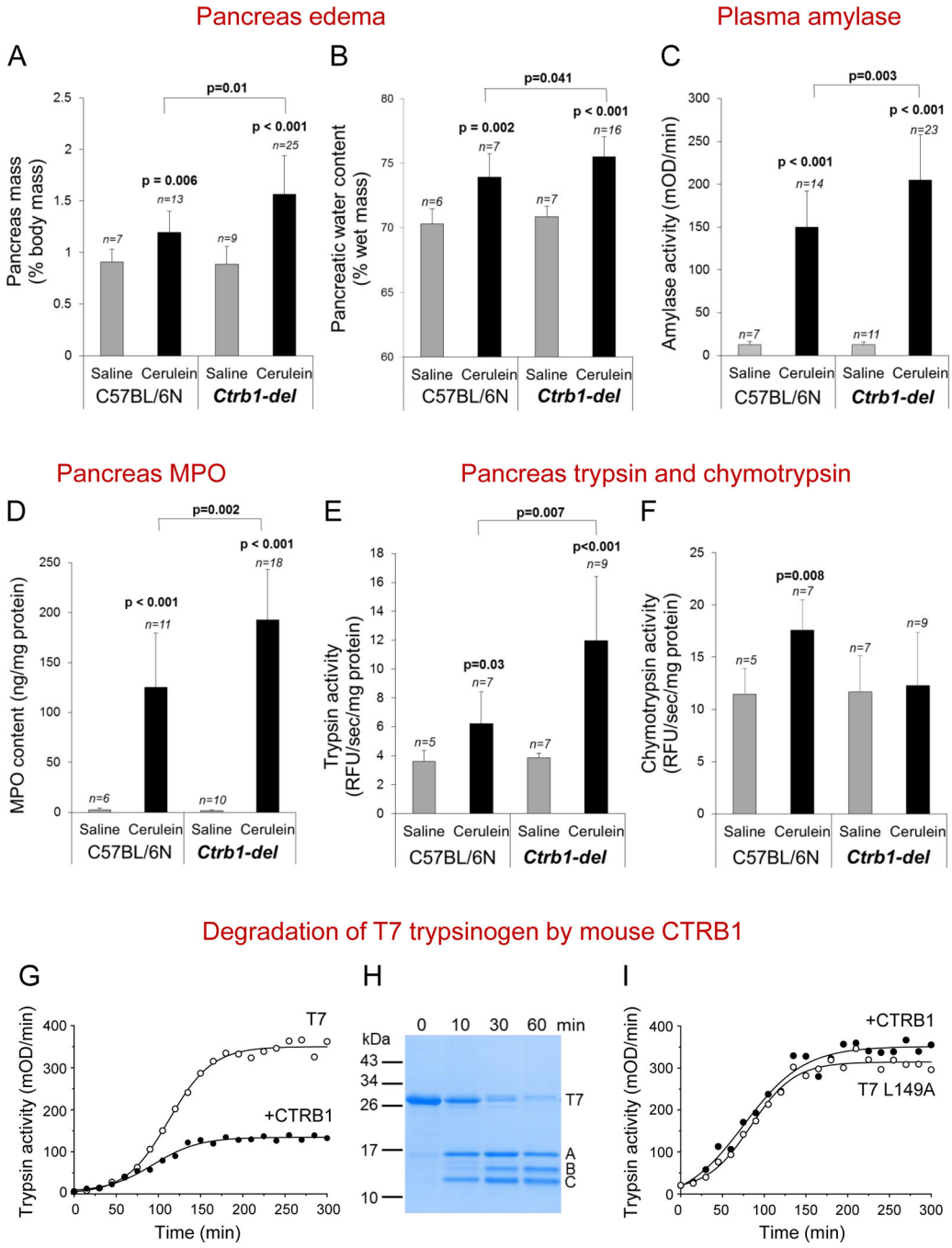

Figure 2.

Cerulein-induced pancreatitis in *Ctrb1-del* and C57BL/6N control mice and degradation of T7 trypsinogen by CTRB1. Mean values with S.D. are shown. See *Methods* for experimental details. **A**, Pancreas mass. **B**, Pancreatic water content. **C**, Plasma amylase activity. **D**, Pancreas MPO content. **E**, **F**, Intra-pancreatic trypsin (E) and chymotrypsin (F) activation 30 min after a single saline or cerulein injection. **G**, Autoactivation of recombinant T7 trypsinogen in the absence and presence of mouse CTRB1. **H**, Digestion of T7 trypsinogen by CTRB1. Bands A and C are generated by cleavage of T7 at Leu149. Band B is the result

of secondary CTRB1 cleavages at Tyr29 and Leu159. **I**, Autoactivation of T7 trypsinogen mutant L149A in the absence and presence of CTRB1.