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Trypsinogen activation in acute and chronic pancreatitis: Is it a prerequisite?

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Summary

Trypsinogen activation is sufficient to induce acute pancreatitis in an experimental model. However, whether it is a requirement for the pathogenesis of acute and chronic pancreatitis remains to be explored.

Keywords

Trypsin; Acute pancreatitis; Chronic pancreatitis

More than a century ago, a German pathologist noted at autopsies of patients who had succumbed to acute pancreatitis that their intra-pancreatic digestive enzymes had been activated. [1]. His fateful observation engendered the surprisingly l-lived belief that pancreatitis is an autodigestive phenomenon resulting from the inappropriate activation of digestive enzymes within the pancreas itself. The last three decades have seen the development of several animal models of pancreatitis and remarkably, early intracellular trypsinogen activation has been observed consistently during the course of pancreatitis in all of them [2-5]. Subsequently, pancreatitis research began to focus on the mechanisms of premature intra-cellular trypsinogen activation. We and others have shown that premature trypsinogen activation takes place in membrane-bound compartments resembling autophagic vesicles within which zymogen and lysosomal contents are colocalized [6-9]. In these colocalization vacuoles, the lysosomal protease cathepsin B activates trypsinogen [10-13]. The occurrence of these colocalization vacuoles has been confirmed both in models of experimental pancreatitis and pathological specimens of human pancreatitis [14,15]. It is thought that that the now-activated trypsin continues the process by activating other digestive enzymes, presumably in the same manner as it normally would in the duodenum. According to this paradigm the process culminates in prematurely active digestive enzymes “digesting” the acinar cell and leading to acute pancreatitis.

So elegant and credible is this trypsin-central paradigm of pancreatitis that the reader often accepts it even before looking at the scientific evidence. It is to be noted that premature trypsinogen activation has only been observed during acute pancreatitis, and that no definitive proof exits for a causal role of trypsinogen activation in the pathogenesis of

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pancreatitis. Inhibition of trypsinogen activation by inhibiting the activity of cathepsin B [11, 12] or by deleting the cathepsin B gene [13] have shown that there is decrease in pancreatic injury during acute pancreatitis, suggesting that trypsinogen activation is important for pancreatic damage. Our own data using trypsinogen-7 knockout mice, which lack pathologic trypsinogen activation, suggest that premature trypsinogen activation contributes directly to only a part of pancreatic injury during acute pancreatitis [16]. Instead, the persistence of a significant degree of pancreatic damage and no change in the measured markers of pancreatic and systemic inflammation in cathepsin B −/− mice [13], or in mice treated with a cathepsin B inhibitor [11,12] or in trypsinogen7 −/− mice [16] all indicate that trypsinogen activation may only be a partial player in the pathogenesis of acute pancreatitis. Based on the persistence of local and systemic inflammation and a considerable, albeit reduced, degree of pancreatic damage in these studies, one could argue, perhaps a little speculatively at this point, that the pathogenesis of several manifestations of acute pancreatitis, at least in these experimental models, does not even require intra-acinar trypsinogen activation to occur.

While whether trypsinogen activation is a prerequisite for the pathogenesis of pancreatitis is a question still awaiting a definitive answer, there is yet another approach to investigate into this: what is intra-acinar trypsinogen activation capable of doing? Recently *in vitro* experiments have provided some mechanistic lines of evidence into acinar cell death induction by persistent intra-acinar trypsinogen activation. Using adenoviral gene transfer techniques, Ji et al showed for the first time in 2009 that intra-acinar expression of active trypsin leads to acinar cell apoptosis [17]. Further, expression of mutated trypsinogens that result in persistently activated trypsin related to hereditary pancreatitis was shown to result in acinar cell death [18,19]. However, it was unclear how these *in vitro* effects of intra-acinar expression of active trypsin translate *in vivo*. In a landmark paper in this issue of *Gut*, Gaiser et al establish *in vivo* for the first time that intra-acinar expression of active trypsin is sufficient to induce acinar death and local and systemic inflammation resulting in acute pancreatitis [20].

In this excellent model developed by Gaiser et al [20], active trypsin can be conditionally expressed within pancreatic acinar cells using a tamoxifen inducible genetic construct. The rat trypsinogen II (PRSS2) was mutated such that the enteropeptidase cleavage site was replaced with a paired basic amino-acid cleaving enzyme (PACE) cleavage site. Upon synthesis, this mutated trypsinogen is cleaved by trans-Golgi-localized PACE leaving activated trypsin within the secretory compartment. This model differs from the traditional experimental models of pancreatitis in one very important aspect: the intra-acinar trypsinogen activation is prolonged over a long time while transient but high levels of trypsinogen activation are observed very early in the other models. Each experimental animal model tends to replicate only certain aspects of pancreatitis while none may completely mimic the human disease, and arguing about the superiority of one model over another may not be very meaningful. Nevertheless, this *in vivo* model is an important tool to study the pathological effects of intra-acinar activated trypsin.

One remarkable observation that has emerged from this important study [20] is that the pancreatic acinar cell seems to be very tolerant of activated trypsin with pathologic effects noticeable only at extremely high levels of intracellular trypsin, levels probably exceeding intrinsic protective mechanisms. However, at these high levels, intra-acinar trypsin caused development of severe acute pancreatitis and the severity was correlated with rate of expression of active trypsin [20]. Severe inflammatory infiltration in the pancreas and lungs was seen indicating local and systemic inflammatory response [20]. The widespread systemic inflammation led to significant mortality as high as 50%. These data clearly

establish that intra-acinar trypsin can induce acute pancreatitis by itself when the intrinsic protective mechanisms are overloaded.

The most surprising data, however, relate to the long-term effects of activated intra-acinar trypsin. Confirming the *in vitro* apoptosis-causing effect, intra-acinar active trypsin led to acinar cell death by both apoptosis and necrosis *in vivo* [20]. Remarkably, sustained intra-acinar trypsin activity resulting from repeated tamoxifen administration every 5th day over 40 days led to massive acinar loss caused by ongoing cell death, and significant fatty replacement was observed [20]. However, there was no evidence of chronic inflammation or of fibrosis - both hallmark features of chronic pancreatitis. Similar pattern of injury lacking any chronic inflammation or fibrosis was also seen 10 weeks after severe episode of acute pancreatitis which was induced by relatively more intense but short term elevation of trypsin activity (in this case, daily tamoxifen administration for five days) [20]. The consequences of repeated episodes of severe acute pancreatitis in this model remain somewhat speculative at this time as these episodes could not be repeated due to high mortality. Nevertheless, it is clear that prolonged and sustained intra-acinar trypsin activity alone is not sufficient to cause chronic pancreatitis.

The discovery that a cationic trypsinogen mutation is associated with hereditary pancreatitis has been the most substantial buttress to the trypsin-central theory of pancreatitis [21]. Subsequently several additional cationic trypsinogen mutations as well as loss-of-function mutations in trypsin inhibitors have been and continue to be identified as factors associated with hereditary and idiopathic chronic pancreatitis [22-25]. Though never proven for any single mutation so far [25], it is believed that these mutations cause or increase the risk of chronic pancreatitis by causing persistent high levels of intra-acinar trypsin activity. In this context, the model of Gaiser et al [20] probably mimics the “persistent intra-acinar trypsin activity” condition required by these hypotheses relating to hereditary pancreatitis. However, this condition alone failed to induce chronic pancreatitis, except leading to acinar atrophy and fatty infiltration [20]. Therefore, the finding of Gaiser et al that persistent intra-acinar trypsin activity alone is not enough to induce chronic pancreatitis clearly challenges the “persistent unchecked intra-acinar trypsin activity” theory of hereditary pancreatitis. Although it might be premature to reach a conclusion regarding this theory, it is clear that the pathogenesis of chronic pancreatitis is more complex than thus far suspected.

Most mutations associated with hereditary pancreatitis are those of cationic trypsinogen (PRSS1) [21, 23-25]. In fact, only one loss-of-function mutation in anionic trypsinogen (PRSS2) has been found to protect against chronic pancreatitis [26]. The biochemical study of PRSS1 and PRSS2 suggest that their activation kinetics are very different and that PRSS2 overexpression during pancreatitis may in fact be a protective mechanism to limit trypsin activity [27]. Interestingly, the model of Gaiser et al uses a PRSS2 construct [20]. However, there is currently no reason to believe that the trypsin activity from PRSS2 may have different consequences than that from PRSS1 [27]. Therefore, the model of Gaiser et al is a valid model to study the effects of intra-acinar trypsin activity *per se*, and has indeed clarified the potential effects of intra-acinar trypsin. However, as discussed previously, another crucial aspect in the pathogenesis of pancreatitis remains to be answered: is trypsinogen activation a prerequisite? The presence of more than one isotype of trypsinogen and the off-target effects of pharmacologic inhibitors of trypsin activity have been important challenges in this field. In our lab, we have generated knockout mice lacking the mouse correlate of the PRSS1 gene. As there is no pathologic trypsinogen activation in these novel knockout mice [16] they are by far the simplest, yet a rigorous, system to study the role of trypsinogen activation. Experiments are currently under way and expected to establish convincingly the role (or lack of it!) of trypsinogen activation in the pathogenesis of acute and chronic pancreatitis. This novel mouse model could also help elucidate trypsin

independent pathways in acute and chronic pancreatitis, such as activation of inflammatory pathways, and the possible role of non-trypsin proteases as hypothesized by some investigators.

We have certainly come a long way from the simplistic description by Chiari in 1896 [1] about the role of digestive enzyme activation and pathogenesis of pancreatitis. This study by Gaiser et al establishes that intra-acinar trypsin activation persisting for a long time and exceeding intrinsic protective mechanisms is sufficient to induce acute pancreatitis in an experimental setting [20]. Further, absence or inhibition of trypsin activity leads to reduced pancreatic injury in experimental models of acute pancreatitis but there is persistence of significant injury and local and systemic inflammation in these models [11-13, 16]. The acinar cell death induced by intra-acinar trypsin activity does not induce chronic pancreatitis [20]. Therefore, it remains to be established whether intra-acinar trypsin activation is a prerequisite for the pathogenesis of pancreatitis, both acute and chronic. Lack of any specific treatment for pancreatitis even to this day is a constant reminder that there is a great deal to be learned about its pathogenesis. Gaiser and colleagues [20] must be congratulated for their landmark study, which has advanced the science of pancreatitis significantly.

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