Molecular and Cellular Changes in Acute Pancreatitis

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Abstract—Acute pancreatitis is a possibly lethal disease with high morbidity and mortality rates. The pathogenesis of acute pancreatitis is not easily understood, this condition having no specific or efficient treatment. It is thought to stem from acinar cells. Typical responses of pancreatitis include hyperamylasemia, intra-acinar activation of digestive enzymes (for example, trypsinogen converted into trypsin), agglomeration of large vesicles in acinar cells leading to generation of proinflammatory mediators and consequent acinar cell death induced by apoptosis and necrosis. These paper summarize the latest research in unveiling the cellular and molecular changes in acute pancreatitis.

Keywords—acute pancreatitis, molecular changes, acinar cells

I. LYSOSOMAL AND AUTOPHAGIC DYSFUNCTIONS IN PANCREATITIS

Based on experimental models, it has been that nonalcoholic pancreatitis has a double effect on autophagy.[1] It activates autophagy but impairs autophagic flux.[2-4] In pancreatitis, impairment of autophagy is determined by defective lysosomal degradation. Findings show that damaged lysosomal function is common in pancreatitis, indicating abnormal functioning of lysosomal hydrolases. There is mainly an early significant decrease in the enzymatic activity of cathepsins in the lysosome-enriched pancreatic subcellular fraction (first demonstrated by Steer and Saluja 20 years ago) in models of pancreatitis. Recent results[5] indicate an impaired cathepsin processing in experimental pancreatitis: decreasing fully mature (“double-chain”) cathepsin forms and accumulation of immature cathepsin forms. These results further note that pancreatitis determines an asymmetry between two major cathepsins, cathepsin B (CatB) and cathepsin L (CatL), with an increase in the ratio of their activities (CatB/CatL) in pancreatitis.[1]

The dramatic decrease in pancreatic levels of lysosomal-associated membrane protein 1 (LAMP-1) and lysosomal-associated membrane protein 2 (LAMP-2) is another significant (and early) lysosomal defect.[6, 7] This decrease was found in 4 dissimilar in vivo models of nonalcoholic pancreatitis induced in rats and mice by CR, L-arginine and CDE diet, as well as in the ex vivo model. Moreover, pancreatic LAMP-2 recorded significant decreases in pancreatitis, generated by ethanol feeding combined with low-dose CR.[8] Other significant decreases in LAMP-1 and -2 have been reported for human pancreatitis.[9] The findings of this study show that LAMP damage in pancreatitis takes place in an acidic compartment, being mediated by cathepsins.[1]

II. AUTOPHAGY PATHWAYS

The continuous renewal of living cells recycles old components and replaces them with new ones. Cellular homeostasis can be maintained through macromolecule and organelle damage generating new “building blocks”. Cellular components are being damaged by two significant systems in eukaryotic cells: the ubiquitin-proteasome system and autophagy. Autophagy particularly alters short-lived proteins, which are tagged by ubiquitin to be recognized and degraded by the proteasome. [10] On the contrary, autophagy degrades long-lived proteins, lipids and cytoplasmic organelles through a lysosome-driven process.[11-14] Autophagy occurs at a basal rate in most cells, acting as a quality control mechanism to remove protein aggregates and damaged or unneeded organelles. Autophagy is also a significant protective mechanism that permits cell survival and adaption to fluctuations in external conditions. Nutrient deprivation is one of the strongest inducers of physiological autophagy, generating and recycling components (for instance, amino acids) which are crucial for cell survival.[15]

Microautophagy is defined by the lysosome itself absorbing small parts of the cytoplasm through inward invagination of the lysosomal membrane.[16, 17] Similarly, in the case of cromophagyy,[18] lysosomes directly fuse with secretory granules and generate their degradation [insulin-containing granules in pancreatic β-cells].[19] There is no knowledge of the molecular signals and mechanisms mediating cromophagy. The main mechanism of autophagy is macroautophagy, which benefited from more extensive studies than the other pathways. It is here referred to as “autophagy”. Macroautophagy has more
steps[20, 23], starting with the development of an autophagosome, a unique double-membrane vacuole that isolates organelles and proteins designed for deterioration. Autophagosomes merge with endosomes and further with lysosomes, producing single-membrane autolysosomes. In the end, the isolated material is deteriorated by lysosomal hydrolases, with the resulting degraded products (amino acids) being recycled back to the cytoplasm. The dynamic character is a major but undervalued aspect of autophagy[24, 25] Modifications of the steps described above alter autophagy progression and function. Therefore, it is of utmost importance to assess the flux by means of this pathway (the turnover rate of autophagic vacuoles), observing its alteration by various stresses and diseases.[15], [26, 27]

III. PHYSIOLOGICAL AUTOPHagy IN THE EXOCRINE PANCREAS

Based on the abundance of GFP-LC3 puncta, the basal level of autophagy is higher in mouse exocrine pancreas than in liver, kidney, heart or endocrine pancreas. Moreover, the number of GFP-LC3 puncta 24 hours after starvation is bigger in the exocrine pancreas than in other organs.[28] The findings of our in vitro studies, based on the comparative analysis of LC3-II levels in both intact and blocked lysosomal deterioration situations, also notice efficient basal level of autophagy in mouse pancreatic acinar cells (Jia et al., unpublished observations). The significantly high rate of protein synthesis of the exocrine pancreas might lead to speculations regarding a greater need to eliminate defective (or excessive) proteins. The basic function of pancreatic acinar cell is to secrete digestive enzymes, mostly produced as inactivezymogens prepacked and stored in zymogen granules (ZGs) and generally activated after being secreted and reaching the intestine.[28] Therefore, macroautophagy might play a role in regulating the number of ZGs, adjusting to the needs of acinar cells and the whole organism. Nevertheless, the impact of autophagy on the regulation of the level and secretion of digestive enzymes has not been described yet. The interrelations between secretory granule (mainly ZGs) synthesis and secretion and their degradation are also not well understood.[15] Autophagosomes consist of various organelles (mitochondria, ER, ZGs) resulting in nonselective basal and starvation-induced autophagy in pancreatic acinar cells.[29, 30]. Starvation highly triggers autophagy in the pancreas, causing a significant decrease in the number of ZGs in acinar cells.[31] This finding is consistent with previous studies[32] showing a decrease in the number of ZGs in starved rodents. Nevertheless, crinophagy [direct fusion of secretory granules with lysosomes], and not autophagy, was believed to mediate ZG degradation during starvation.[33, 34] This aspect should be reconsidered using modern approaches.[15]

IV. IMPACT OF PANCREATITIS ON AUTOPHAGOSOME FORMATION

Transmission electron microscopy (TEM) and immunogold-TEM demonstrate the existence of autophagic vacuoles in acinar cells, accompanied by all their characteristics, that is double membrane, LC3-II and intact load. Genetic, control notes that Atg5 affects autophagosome formation in acinar cells[34, 35], proceeding via canonical pathway. Moreover, data indicate the implication of endoplasmic reticulum (ER) in autophagosome development in pancreatic acinar cells, as heterozygous deletion of X-box binding protein 1, a significant ER mediator of the adaptive “Unfolded Protein Response”, stimulating pancreatic autophagy in mice fed with ethanol.[36]

A new autophagic pathway to selectively remove zymogen granules in pancreatitis is VMP1-mediated “zymophagy”, based on the selective isolation of ZGs (and not other organelles) by autophagosomes in VMP1-overexpressing mice with caerulein-induced pancreatitis.[36] These findings were obtained by immunoisolating autophagic vacuoles and require confirmation of the changes in load composition using quantitative TEM.[37] The existence of a selective autophagic pathway for ZG removal, induced by pancreatitis, is an appealing aspect. Nevertheless, autophagosomes isolate all types of organelles, including mitochondria, ER and ZGs, in wild-type mice with caerulein-induced pancreatitis, as well as in other experimental models[38] and human diseases. Zymophagy is also not triggered by VMP1 overexpression alone, with stimulation of autophagosome formation, but mainly occurs after caerulein-induced pancreatitis in VMP1-overexpressing mice.[39] Therefore, the occurrence of zymophagy implies that ZGs in pancreatitis receive a kind of pathological signal that generates their selective isolation. [40] There is still need to identify these signals. Eventually, the impact of VMP1 overexpression can be more complex, causing cell death in (nonpancreatic) cell lines[39], and VMP1 has other functions besides autophagy, as indicated in Dictyostelium discoideum.[15]

V. MECHANISMS OF INFLAMMATORY PATHWAY ACTIVATION

Activation of nuclear factor kappa beta (NFkB) has been noticed in experimental models complementing trypsinogin activation in terms of time course.[48, 49] Present data indicate that pathologic Ca\(^{2+}\)\(^{2+}\), and activation of novel isoforms of protein kinase C cause NFkB activation. The indication for the role of pathologic Ca\(^{2+}\)\(^{2+}\) comes from experiments using pharmacologic agents to block Ca\(^{2+}\)\(^{2+}\), inhibiting NFkB.[50] The activation of novel protein kinase C (PKC) isoforms ε and ζ has been shown to influence NFkB activation in studies employing caerulein and ethanol based models of pancreatitis. These new PKC isoforms also seem to be involved in zymogen activation.[51, 52] There is not enough knowledge of the effect of pathologic Ca\(^{2+}\)\(^{2+}\), and PKC in the activation of these early pancreatitis responses, and whether they are independent or not. The signal transduction steps causing PKC activation and pathologic Ca\(^{2+}\)\(^{2+}\) are described in depending on the caerulein model of pancreatitis. Downstream targets of PKC and Ca\(^{2+}\)\(^{2+}\) are still not known. Protein kinase D1 has been suggested as the downstream target of PKC isoforms[52] even if PKD1 activation may also take place through other PKC independent mechanisms.[53] Pharmacologic inhibition of PKD1 decreased trypsinogin activation and NFkB activation without affecting cell injury.[54] Calcinerin has also been suggested as
downstream target of Ca\textsuperscript{2+}, even without fully understanding its effects on NFκB activation during pancreatic injury.[1]

Besides Calcium or PKC dependent pathways other mechanisms have also been stated, including angiotensin II acting through AT2 receptors[55] and p38/MAPK pathways.[54] The role of acinar cell injury alone in the activation inflammatory cascades has been examined in a recent study. Cell injury was found to cause activation of damage-associated molecular pattern (DAMP) receptors (TLR-9 and P2X-7 being the most important). DAMP activation triggers the formation of a cytosolic complex, the inflammasome, involved in the initiation of inflammatory cascade during pancreatic injury.[56]

VI. CELL SIGNALING

The initiation of acute pancreatitis is correlated with two pathologic intracellular signals in the acinar cell. Several forms of acute pancreatitis have been strongly associated with acute changes in cytosolic Ca\textsuperscript{2+} signaling. This abnormal signal is characterized by loss of Ca\textsuperscript{2+} oscillations and emergence of continuous high peak levels of cytosolic Ca\textsuperscript{2+}. Even if more mechanisms act to develop this pathologic signal, the ryanodine receptor (RyRs) Ca\textsuperscript{2+} release channel in the endoplasmic reticulum seems to have a notable early contribution.[57] Other studies imply that Ca\textsuperscript{2+} mainly targets calcineurin (CaN), a protein phosphatase 2B.[58] As both RyRs and CaN can be targeted by specific drugs, they might be significant therapeutic targets.

Acute pancreatitis responses are also correlated with another pathologic signal, the activation of specific protein kinase C isoforms, including the stimulation of NFκB and zymogen activation.[59] Activation of protein kinase C (PKC-ε) is noticed following physiologic stimulation. Nevertheless, the activation of both PKC-δ and PKC-ε is observed under acute pancreatitis generating conditions. The stimulating effects of ethanol might be connected with PKC activation. Therefore, in combination with physiologic CCK activation of PKC-δ, ethanol leads to the activation of PKC-ε.[59, 60] This pattern states again the cause generated by supraphysiologic concentrations of CCK. Protein kinase D (PKD), which is downstream from PKC-δ and PKC-ε, seems to mediate some of these responses (Thrower et al., unpublished findings). Even if these observations offer fascinating evidence that specific PKC isoforms may mediate responses in early acute pancreatitis, there is still little knowledge of the molecular targets of these enzymes.

References


