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Genetically Defined Models of Chronic Pancreatitis

See "A mouse model of hereditary pancreatitis generated by transgenic expression of R122H trypsinogen" by Archer H, Jura N, Keller J, Jacobson M, Bar-Sagi D, on page 1844; and "Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis" by Cano DA, Sekine S, Hebrok M, on page 1856.

The concept that acute pancreatitis was not an infection, but was caused by autodigestion of the pancreas through activation of digestive enzymes, was first proposed by Chiari in 1896.[1] It is now generally accepted that, in most cases, acute pancreatitis begins with trypsinogen activation to trypsin within the pancreas. Anti-trypsin protective mechanisms are overwhelmed with further activation of trypsinogen and other zymogens, thereby resulting in pancreatic injury and initiation of an acute inflammatory response.[2] A major breakthrough that strongly supported Chiari's hypothesis of autodigestion and that linked trypsin activity to both acute and chronic pancreatitis was the discovery in 1996 that mutations in the cationic trypsinogen gene (PRSS1) were associated with hereditary pancreatitis.[3]

Hereditary pancreatitis is an uncommon, autosomal-dominant disorder that was first described by Comfort and Steinberg[4] in 1952 in a kindred spanning 3 generations comprising 4 affected persons and 2 others suspected of being obligate carriers of the disease. The typical family member who carries a major PRSS1 gene mutation develops recurrent acute pancreatitis at around 10 years of age, and a majority go on to develop various degrees of chronic pancreatitis within the next 10–15 years; of these who develop chronic pancreatitis, up to 40% develop pancreatic cancer.[5,6] The fact that each manifestation of pancreatic disease is indistinguishable from the sporadic form, except for the family history and the lack of other etiologic factors, has made hereditary pancreatitis a very important human model for investigating disease mechanisms.[7] Indeed, insights from this disease have revolutionized our conceptualization of sporadic pancreatic diseases.

To date, over 25 mutations have been identified in the cationic trypsinogen gene, with the most common being PRSS1 R122H and N29I.[8,9] The mutations generally cluster around the 2 calcium-binding pockets that are critical in regulating trypsinogen activation and trypsin inactivation.[10] For example, trypsin has a built-in self-destruction (or autolysis) site at R122 that can only be accessed by another trypsin molecule when the site is not being protected by calcium occupying one of the binding sites. Biochemical studies have proven that eliminating the R122 autolysis site because of a mutation preserves trypsin survival in solutions with low calcium concentrations,[11,12] such as those that exist within acinar cells. Taken together, studies of the cationic trypsinogen mutations in humans with hereditary pancreatitis point to the importance of unregulated trypsin activity in initiating acute pancreatitis. They also indicate the significance of maintaining low calcium concentrations within the acinar cell, which facilitates trypsinogen activation and prevents trypsin inactivation and can therefore lead to trypsin related injury and acute pancreatitis.[13]

The importance of mutated trypsin in hereditary pancreatitis led researchers to investigate other molecules in humans that normally protect the pancreas from inappropriate trypsin activity. Indeed, mutations in the pancreatic secretory trypsin inhibitor (PSTI) gene (or serine protease inhibitor Kazal type 1, SPINK1) were found to be associated with chronic pancreatitis in children,[14] families,[15] tropical pancreatitis,[16] and to a lesser degree, in alcoholics.[17,18] Because SPINK1 is expressed as an acute phase protein, it likely becomes relevant only after inflammation has occurred,[19] and therefore protects against recurrent acute pancreatitis rather than an initial attack. Other factors within the acinar cell that can activate trypsinogen include cathepsin B,[20,21] a lysosomal enzyme that is normally segregated from trypsinogen, and is located in zymogen granules in a cell's cytoplasm. In experimental animals in which acute pancreatitis is being induced, colocalization of these vesicles appears to be associated with trypsin activation and worsens pancreatitis.[22] Recently, this potential mechanism has also been linked to humans because mutations in the cathepsin B gene alter the risk of tropical pancreatitis.[23] Finally, the pancreas must generate a bicarbonate-rich fluid to "flush" trypsinogen and the other zymogens out of the pancreatic duct following secretion from the acinar cell. This action depends on the cystic fibrosis transmembrane membrane conductance regulator (CFTR), a regulated anion channel in the pancreatic duct cells that is permeable to chloride, and to a lesser degree, bicarbonate. Mutations in the CFTR gene lead to cystic fibrosis,[24,25] a multisystem genetic disorder causing chronic pancreatitis beginning in utero. Severe CFTR mutations, as well as some more moderate ones, are predicted to limit CFTR-depended bicar-

bonate secretion in the pancreas.[26] Failure to produce sufficient bicarbonate-rich pancreatic juice because of CFTR mutations is linked with both recurrent acute and chronic pancreatitis.[27,28] Thus, multiple lines of evidence demonstrate that molecular defects that are predicted to increase the risk of unregulated trypsin activity within the pancreas lead to recurrent acute and chronic pancreatitis.

Acute and chronic pancreatitis are well-defined syndromes that are observed in patients throughout the world. The primary symptoms and signs of acute pancreatitis are the sudden onset of upper abdominal pain with elevation of blood amylase or lipase levels to 3 times the upper limits of normal.[2] Acute pancreatitis is often accompanied by fever, nausea, vomiting, and adynamic ileus. Chronic pancreatitis is a destructive inflammatory process characterized by chronic inflammation and fibrosis.[29] The main clinical features of chronic pancreatitis include recurrent or chronic abdominal pain, steatorrhea, and in the later stage of the disease, namely diabetes mellitus. The morphologic changes include early infiltration of the pancreas with inflammatory cells and the loss of acinar cells and at later stages reduced islet cell mass, glandular atrophy, acinar–ductal metaplasia, progressive fibrosis, and calcifications. These changes may be focal, segmental, or diffuse in nature. By convention, acute and chronic pancreatitis were regarded as 2 distinct entities.[30,31] However, the identification of PRSS1 mutations in chronic pancreatitis provided evidence that recurrent acute pancreatitis may lead to chronic pancreatitis,[32] thereby linking the 2 clinical entities. Indeed, it has recently been proposed in the sentinel acute pancreatitis event (SAPE) model[10,33] that acute pancreatitis is a prerequisite for chronic pancreatitis because it specifically activates the immune system within the pancreas. Although there is strong support for the SAPE model of acute and chronic pancreatitis in animal models of alcoholism with recurrent pancreatitis triggered by the CCK analogue cerulean,[34] a persistent concern remains that animal models do not reflect fully the human condition.

Developing animal models of acute or chronic pancreatitis that faithfully reflect and reproduce the human condition has been difficult. A variety of models of chronic pancreatitis have been developed based on the application of toxins or combinations of alcohol and stimulation of the exocrine pancreas with either physiologic or supraphysiologic doses of cerulein, because alcohol application alone fails to induce acute or chronic pancreatitis. Moreover, some genetically engineered mice have revealed an unexpected phenotype resembling components of human chronic pancreatitis. Mice deficient for protein kinase PERK (PKR-like ER kinase), involved in the coupling stress signals initiated by protein misfolding in the lumen of the endoplasmic reticulum, develop diabetes and pancreatic dysfunction.[35] E2F1/E2F2 double mutant mice exhibited diabetes and exocrine pancreatic insufficiency, suggesting that these key regulators of growth control are necessary for the maintenance of the differentiated pancreatic phenotype in adults.[36] Ectopic expression of transforming growth factor (TGF)-β1, a dominant-negative mutant TGF-β type II receptor of human keratin 8, resulted in tissue destruction, fibrosis, and atrophy of the pancreas.[37] Although these models share some histologic features of chronic pancreatitis, they are not based on genetic alterations that have been identified in the human disease.

In this issue of GASTROENTEROLOGY, Cano et al[38] describe that cilia deletion in pancreatic epithelial cells results in pancreatic pathology reminiscent of chronic pancreatitis in some aspects. Cre/lox technology was used to conditionally inactivate kif3a coding for a subunit of the kinesin-2 complex essential for cilia formation in pancreatic epithelia. The pancreas of these mutant mice displays loss of acinar cells shortly after birth, an acinar-to-ductal metaplasia and periductal fibrosis at 2 weeks after birth. At 12 weeks, the acinar cells are replaced by adipose tissue. At 6 months, the pancreas is composed of cysts that enlarge over time. Remarkably, cilia deletion results in ductal dilation during pancreatic organogenesis prior to acinar cell loss. Ductal dilation is a hallmark of late-stage chronic pancreatitis, although the pathophysiology underlying these changes is not completely understood. It will be interesting to investigate whether cilia formation and function is altered in chronic pancreatitis.

Inactivating or altering a number of genes in mice that are known to be associated with chronic pancreatitis in humans has failed to reproduce the human disease. For example, CFTR-deficient mice have no overt feature of chronic pancreatitis, although they do harbor mild histologic abnormalities that are hallmark features of early human cystic fibrosis.[39] Very old $cftr^{-/-}$ mice display more severe changes of acinar cell dropout and foci of inflammatory cells.[40] In the mouse, the ortholog of human SPINK1 is the Spink3 gene. Spink3-deficient mice die a few days after birth. The pancreas develops normally but involutes owing to autophagic degeneration of acinar cells and impaired regeneration.[41] Interestingly, transgenic overexpression of wild-type SPINK1 protects mice from secretagogue-induced pancreatitis, thereby reinforcing the premise that SPINK1 is protective.[42]

In the current issue of GASTROENTEROLOGY, Archer et al[43] report on a novel mouse model of chronic pancreatitis. A transgenic mouse line was generated in which expression of the most frequently observed trypsinogen mutation, R122H, was under the transcriptional control of the elastase promoter (R122H_mPRSS1). Expression of the transgene was restricted to the acinar cell compartment, but diminished after 12 months of age most likely owing to loss of acinar cell mass. Acinar cell damage was detectable starting at 7 weeks of age, interacinar inflammatory infiltrates were detected beginning at 12 weeks, and a fibrotic reaction was evident in animals $>$24 weeks old. At 1 year, 40% of the mice displayed fibrosis and signs of inflammation and infiltrating cells were composed T and B lymphocytes as well as macrophages. Acinar cells displayed increased proliferation, indicative of regeneration. Morphologic changes included the development of tubular complexes. Thus, the histologic appearance of the pancreas of 1-year-old R122H_mPRSS1 mice convincingly resembles human chronic pancreatitis. This represents the first genetically defined model of chronic pancreatitis in which the genetic alteration resulted in the predicted phenotype. In further experiments, mice were challenged with cerulein at supramaximal doses, which is known to induce a mild self-limiting form of acute pancreatitis. Although wild-type controls recovered completely, transgenic mice displayed extensive deposition of collagen in periacinar and interlobular areas. This specific experiment demonstrated an increased susceptibility of acinar cells harboring an R122H mutation to injurious agents.

The key question is whether the observed phenotype depends on persistent trypsin activity in the pancreas. If this were the case, one would expect that pretreatment of