
Leading article

Hereditary pancreatitis: new insights into acute and chronic pancreatitis

Hereditary pancreatitis has recently emerged as an important pancreatic disease. Although relatively rare, this genetic disorder has provided major breakthroughs in our understanding of acute and chronic pancreatitis and pancreatic cancer. Furthermore, study of this disorder promises additional insights into the pathophysiology of human pancreatitis, new strategies for developing animal models of disease, and new therapies to treat or prevent acute and chronic pancreatitis. A review of recent findings and mechanisms of disease should benefit scientists who study the pancreas and physicians who care for individuals with pancreatitis. This report will focus on acute and chronic pancreatitis.

Clinical features

The first family with hereditary pancreatitis was described by Comfort and Steinberg in 1952.¹ Careful evaluation of four definite and two probably affected family members revealed an autosomal dominant inheritance pattern, onset of symptoms between five and 23 years, but no other “...earmarks which distinguish it from non-hereditary chronic relapsing pancreatitis”. These observations remain accurate with the caveat that penetrance, the fraction of patients with the mutation that eventually express the disease, is 80%.²⁻⁶ Attacks of acute pancreatitis usually begin in childhood,⁷ but age of onset can range from infancy to the fifth or sixth decade of life. The acute attacks may vary from mild abdominal discomfort to severe life-threatening episodes with pancreatic necrosis, splenic vein thrombosis,⁸ pseudocysts,⁹ and death.³⁻¹⁰ Chronic pancreatitis follows recurrent attacks of acute pancreatitis with all of its common complications—unrelenting pain, parenchymal and ductal calcifications, duct distortion, fibrosis, maldigestion and diabetes mellitus.^{3-6 10 11} These features make hereditary pancreatitis indistinguishable from other causes of acute and chronic pancreatitis, save the relatively early age of onset, the autosomal dominant inheritance pattern and lack of other identifiable aetiologies.

Identification of mutations in the cationic trypsinogen gene

Many investigators pursued the aetiology of hereditary pancreatitis via access to hereditary pancreatitis kindreds. The cause remained obscure, however, until modern molecular genetic techniques were applied to the problem. Using genetic linkage studies, the hereditary pancreatitis locus was independently narrowed to the long arm of chromosome 7 by Le Bodic and colleagues¹² and Whitcomb and colleagues¹³ in 1996, and later confirmed by

Pandya *et al.*¹⁴ Within months, the disease gene was identified by Whitcomb and coworkers¹⁵ through mutational analysis of candidate genes within the newly mapped region. A single point mutation, a G to A transition, was identified in the third exon of cationic trypsinogen that resulted in an arginine (R) (CGC) to histidine (H) (CAC) substitution at the 105 amino acid of trypsinogen, which is numbered as residue 117, or R117H (fig 1). (Note: “cationic trypsinogen” is the same enzyme as trypsinogen 1,²¹ trypsinogen II^{22 23} or trypsinogen 3²⁴ and the amino acid numbering system follows that of the serine proteases as originally determined for chymotrypsinogen. These mutations have been classified as protein, serine, 1; (PRSS1) allelic variants 276000.0001 (PRSS1, ARG117HIS), and 276000.0002 (PRSS1, ASN211LE)). This cationic trypsinogen R117H mutation was observed in all individuals affected by hereditary pancreatitis and the obligate carriers from five kindreds, but not in individuals who married into the families nor in 140 unrelated individuals. A second mutation in cationic trypsinogen was also discovered in two families with hereditary pancreatitis without the R117H mutation. In these kindreds a point mutation in exon 2, an A to T transversion, was identified that resulted in an asparagine (N) (AAC) to isoleucine (I) (ATC) amino acid substitution at residue 14 (amino acid 21 using the common chymotrypsinogen numbering system (fig 1)), or N21I. The N21I mutation results in a clinical syndrome of hereditary pancreatitis similar to the R117H mutation, although the average age of onset seems to be slightly delayed and the clinical features less severe.^{10 25} These two, and only two cationic trypsinogen gene mutations were identified in other families from Italy,¹⁵ France,²⁶ Germany,^{27 28} UK,²⁹ the United States,^{14 30} and Japan.²⁵ Thus, cationic trypsinogen mutations seem to play a central role in the common, autosomal dominant form of hereditary pancreatitis.

Why do cationic trypsinogen mutations cause hereditary pancreatitis?

To date, the genetic studies on hereditary pancreatitis families reveal only two specific mutations in the cationic trypsinogen gene. No other disease associated mutations have been identified in any pancreatic digestive enzyme genes, including anionic trypsinogen or mesotrypsinogen. However, this should not be a great surprise because

Abbreviations used in this article: PSTI, pancreatic secretory trypsin inhibitor; TAP, trypsinogen activation peptide; SAPE, sentinel acute pancreatitis event; TGF, transforming growth factor.

Exon 1 *signal peptide*

CT *MNPLLILTFVAAAL*
 AT *MNLLLILTFVAAAV*
 MT *MNPFLLILAFVGAAV*
 Chy *MAFLWLLSCWALLGTTTF*

Exon 2	1	10	20	21	30	40	50	57
CT	A	<u>APFDDDDK</u>	IVGGY	<u>NCEENS</u>	VPPYQVSLN	SGYHFCGGSL	<u>LINEQ</u>	WVVSAGHCYKS
AT	A	<u>APFDDDDK</u>	IVGGY	<u>ICEENS</u>	VPPYQVSLN	SGYHFCGGSL	<u>LISEQ</u>	WVVSAGHCYKS
MT	A	<u>VPFDDDDK</u>	IVGGYT	<u>CEENS</u>	LPYQVSLN	SGSHFCGGSL	<u>LISEQ</u>	WVVSAAHCYKT
Chy	G	<u>CGVPAIHPVLSGLSR</u>	IVNGE	DAVPGS	WPVQVSLQDKTGFHFCGGSL	ISED	WVVTAAH	CGVR

Exon 3	62	70	80	90	100	102	110	117	120	130	140
CT	RIQVRL	GEHNIEVLEGNEQF	INAAKI	IIRHPQYDRKTL	N	D	IMLIKLS	SR	<u>AVINAR</u>	VSTISLPT	APPA TGTKCLISGWGNTASSGA
AT	RIQVRL	GEHNIEVLEGNEQF	INAAKI	IIRHPKYNSRTL	N	D	ILLIKLS	SPAVINS	RVSAISLPT	APPA AGTESLISGWGNTLSSGA	
MT	RIQVRL	GEHNIKVLEGNEQF	INAAKI	IIRHPKYNRD	N	D	IMLIKLS	SPAVINAR	VSTISLPT	APPA AGTECLISGWGNTLSFGA	
Chy	TSDVVVAGEFDQGS	DEENIQVLKIAK	VFKNPKFS	ILTVN	N	D	ITLLKLAT	PARFSQ	TVSAVCLPSADD	DFPAGTLCATTGWGKTKYAN	

Exon 4	150	160	170	180	190
CT	DYPDELQCLDAPVLSQAK	EASYPGKIT	SNMFCVGFLEGGK	DSCQ	
AT	DYPDELQCLDAPVLSQA	EACEASYPGKIT	NMFCVGFLEGGK	DSCQ	
MT	DYPDELKCLDAPVLTQA	ECKASYPGKIT	NSMFCVGFLEGGK	DSCQ	
Chy	KTPDKLQQAALPLLSNA	ECKKSWGRRITD	VMICAG	ASGV	SSCM

Exon 5	195	200	210	s	220	s	230	240
CT	GDSGGFPVVCNGQ	LQGVVSWG	GCAQKNKPGVYTKVYNYVKWIKNTIAANS					
AT	GDSGGFPVVSNGE	LQGVVSWG	GCAQKNRPGVYTKVYNYVDWIKDTIAANS					
MT	RDSGGFPVVCNGQ	LQGVVSWG	GCAWKNRPGVYTKVYNYVDWIKDTIAANS					
Chy	GDSGGPLVCQKDGAW	TLVGVVSWGSDTCS	TSSPGVYARVTKLIPWVQKILAA					

Figure 1 Cationic trypsinogen (CT) compared with anionic trypsinogen (AT), mesotrypsinogen (MT) and chymotrypsinogen (Chy). Cationic trypsinogen is divided according to the amino acids coded for in each of the five exons. The numbering system is based on alignment with chymotrypsinogen at serine 195.¹⁶ The sequences of cationic and anionic trypsinogen are from¹⁷; mesotrypsinogen from¹⁸ and¹⁹; and chymotrypsinogen from cDNA (introns unknown).²⁰ Signal peptides are in italics, activation peptides are underlined italics, the catalytic triad (H57, D102 and S195), N21, and R117 are bold. The amino acids determining specificity of the enzymes (D189, G216 and G226) are marked with "s". Amino acids in trypsinogen that occur between the chymotrypsinogen numbered residues are marked with an asterisk.

hereditary pancreatitis, as an autosomal dominant disorder, differs from autosomal recessive diseases like cystic fibrosis where hundreds of mutations have been reported in the cystic fibrosis associated CFTR gene.³¹⁻³² Autosomal dominant disorders, like hereditary pancreatitis, usually occur when the mutation involves a critical regulatory part of the gene product that results in gain-of-function through overexpression, constitutively active function, a new function (e.g. the α -1-antitrypsin Pittsburgh allelic variant³³) or, as in the case of hereditary pancreatitis, loss of inhibitory regulation.³⁴ Thus, the finding of a limited number of trypsinogen gene mutations resulting in gain-of-function is consistent with an autosomal dominant disease.

Several lines of evidence suggest that the mutations in cationic trypsinogen that result in hereditary pancreatitis do so by rendering prematurely activated trypsin resistant to inactivation through autolysis. We proposed a mechanistic hypothesis after considering the location of the mutation within the x-ray crystallographic three dimensional structure of the trypsinogen-pancreatic secretory trypsin inhibitor (PSTI) complex,^{15,35} and a literature review of the potential functions of R117. Trypsin contains two globular domains connected by a side chain, with an enzymatically active site at the interface between domains.

As figure 2A shows, the R117H mutation occurs on the external face of the trypsinogen molecule opposite the substrate binding site and catalytic site, opposite the PSTI binding site, and away from the trypsinogen activation peptide (TAP) side chain.¹⁵ Thus, it seemed unlikely the arginine to histidine substitution would affect trypsin function, PSTI inhibition, or trypsinogen activation. Conversely, trypsin recognises the arginine and lysine residues within peptide chains as sites for hydrolysis. Indeed, R117 functions as the initial site of hydrolysis of trypsin by trypsin itself, followed by further degradation of the molecule as internal hydrolysis sites are exposed. Substitution of histidine for arginine at residue 117 eliminates this initial hydrolysis site, thereby rendering trypsinogen and/or trypsin resistant to autolysis and permanent inactivation.³⁶ Therefore, if trypsinogen is activated within the pancreas in quantities that exceed the inhibitory capacity of PSTI (as PSTI is only able to inhibit 20% of potential trypsin activity),²⁴ and if the resulting trypsin remains active by escaping hydrolysis at the mutant H117 site, then trypsin could activate all of the other digestive proenzymes, initiate pancreatic autodigestion and cause pancreatitis. Thus, the cationic trypsinogen R117H mutation seems to cause this autosomal dominant disease through a trypsin gain-of-

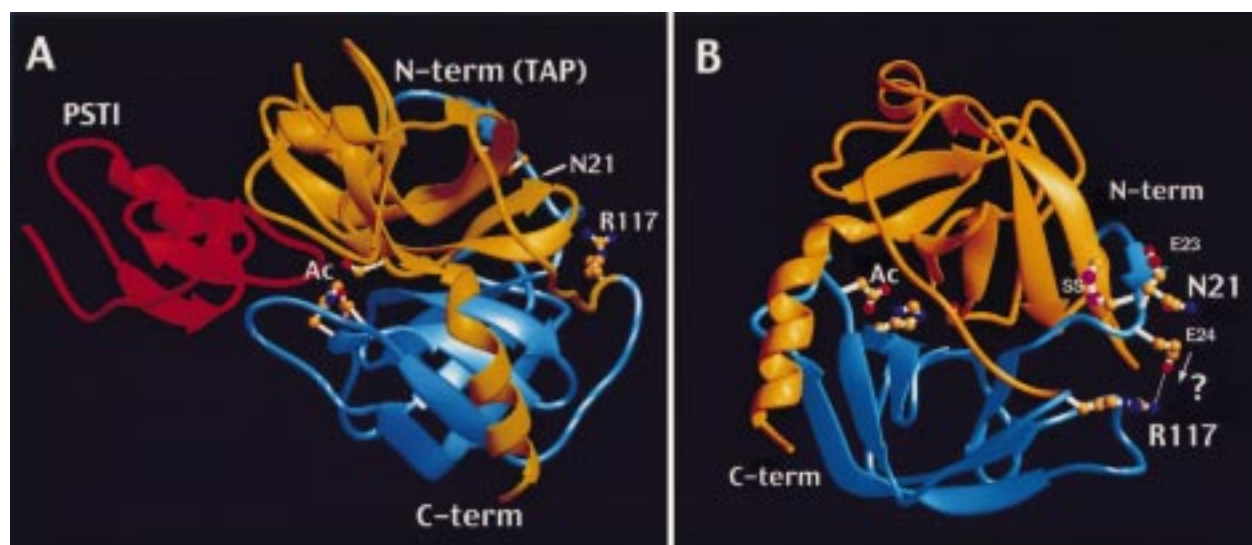


Figure 2 Crystallographic structure of trypsin. (A) The two domain structure of bovine trypsinogen–pancreatic secretory trypsin inhibitor (PSTI) complex.³⁵ Note the transition from the blue N-terminal (N-term) domain to the yellow C-terminal (C-term) domain at R117 in the flexible chain segment connecting the domains. The trypsinogen activation peptide (TAP) portion of the molecule resides in the N-term region but is not visualised crystallographically. PSTI (red) is shown interacting with the active site (Ac) of trypsin. The location of N21 is also noted. (B) The two domain structure of human cationic trypsin.²¹ Structural location of N21, E24, and R117. One possible effect of the N21I substitution would be to bring E24 closer to R117, forming a salt bridge between the oppositely charged side chains (arrow and “?”).

function mutation that eliminates a key inhibitory mechanism. When the inhibitory capacity of PSTI is overwhelmed by excessive trypsinogen activation in hereditary pancreatitis the control of trypsin activity within the pancreas is lost, resulting in pancreatitis.

The N21I mutation

The mechanisms causing pancreatitis with a cationic trypsinogen R117H mutation seem clear, whereas the pancreatitis causing mechanism of the cationic trypsinogen N21I mutation is still speculative. The mutation is especially interesting because this amino acid substitution of isoleucine for asparagine renders the amino acid sequences of anionic and cationic trypsinogen identical through residue 47 (fig 1). Although several theories have been put forward,^{10, 25, 37} one likely explanation was proposed by Professor William Furey from the Biocrystallography Laboratory of the Pittsburgh Veterans Affairs Medical Centre.^{10, 38} Human trypsin differs significantly from trypsin of other animals at the loop containing

residues E23–S26,²¹ and the N21I substitution would place a hydrophobic isoleucine on the surface of cationic trypsin adjacent to this loop. The predicted conformational changes,²⁵ even if small, may bring E24 close enough to R117 to form a salt bridge (fig 2B).

The importance of the hypothetical E24–R117 electrostatic bond becomes apparent after considering the modelling studies of the R117 containing 12 residue connecting side chain (A112–L123) required for another trypsin to lyse the peptide bond at R117.³⁹ Figure 3 is a stereo view of the backbone of trypsinogen (black) with the side chain shown in native conformation (blue) and the modelled conformation (pink) that may be necessary for R117 (shown with its P1 side chain in light blue and pink respectively) to be attacked by another trypsin molecule (green). Note: R117 only fits into the specificity pocket of the second trypsin when the A112–L123 side chain rotates outward as shown in red (an animated three dimensional model is available on the world wide web—URL: <http://prosci.org/Kinemage/>; filename: Hubbard.kin 3.5.).⁴⁰ If the flexible A112–L123

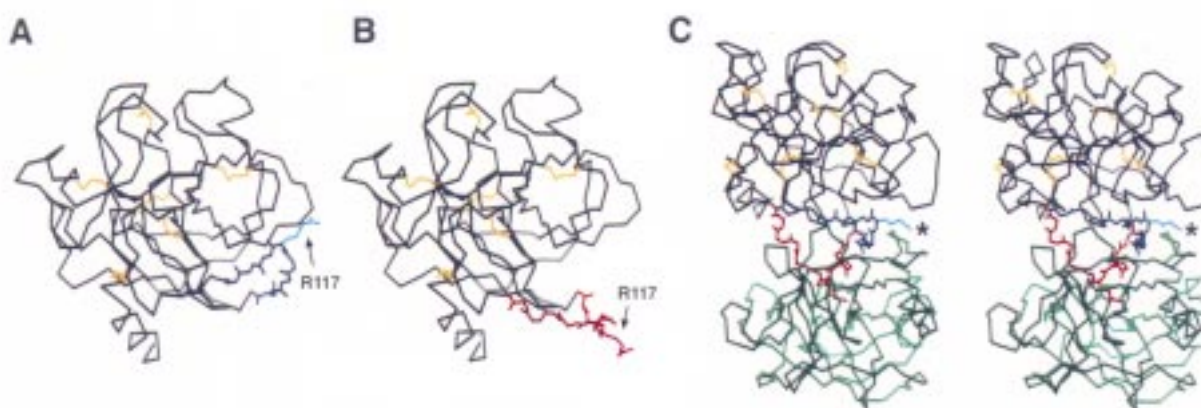


Figure 3 Limited proteolysis model of trypsin by trypsin. The backbone of the primary trypsinogen (black) is illustrated with the A112–L123 side chain shown in native conformation (A; blue) and the modelled conformation (B; red) that may be necessary for limited proteolysis of trypsin at R117 (arrow) by another trypsin molecule. (C) Stereo view of the trypsin–trypsin autolysis limited proteolysis model with a second trypsin (green) in the attack position. The two trypsin molecules are oriented with the active site facing upward. The A112–L123 side chain from A and B are both illustrated, with R117 in the native position marked with an asterisk. When the side chain is in the modelled (red) conformation the R117 of the primary (black) trypsin molecule easily fits within the active site of the second trypsin molecule. (To see the molecule in three dimensions, position the figure approximately 30 cm (12 inches) from the face, relaxing and crossing the eyes to allow the images to merge. One should see three images, with the one in the middle appearing in three dimensions.)

side chain must indeed be in this modelled conformation it would also explain why trypsin preferentially activates trypsinogen (by attacking the lysine at residue 15 thereby cleaving TAP) rather than inhibiting trypsin/trypsinogen (by attacking R117). Furthermore, this model explains why an electrostatic bond at E24–R117, formed by the yet to be proved conformational change caused by an N21I substitution, would result in a phenotype similar to R117H. In both types of hereditary pancreatitis, the critical autolysis site at position 117 cannot be attacked by trypsin-like enzymes.

Unique features of human cationic trypsin

Why do humans seem to be more susceptible to pancreatitis than animals, and why is cationic trypsin, rather than anionic trypsin, associated with hereditary pancreatitis? We believe that there are several unique features of human cationic trypsinogen that may contribute to these observations.⁴¹ In rats, trypsin activation seems to require the lysosomal enzyme cathepsin B,^{42–43} which is normally kept separate from the zymogens, including trypsinogen. Human trypsinogen does not have this requirement as it has the propensity to autoactivate.^{43–44} Thus, for animals to develop pancreatitis through the trypsin activation cascade, conditions must exist that allow trypsinogen to interact with cathepsin B (e.g. by lysosome–zymogen co-localisation⁴⁵), whereas humans may not have this requirement.⁴⁶

There are several differences between human cationic and anionic trypsinogen that may also be important in human acute pancreatitis. Firstly, in humans the cationic trypsinogen to anionic trypsinogen ratio is high (2:1)²³ compared with animals (less than 1:20 in rats).⁴⁷ Secondly, cationic trypsinogen autoactivates more easily than anionic trypsin.⁴⁴ Thirdly, cationic trypsin, in the presence of raised calcium concentrations, is more resistant to autolysis than anionic trypsinogen.⁴⁸ Thus, cationic trypsinogen is present at higher concentrations, activates more readily, and is more resistant to autolysis than anionic trypsinogen. Because of these characteristics, other pancreatic defence mechanisms (see review by Rinderknecht²⁴) may be sufficient to handle mutant anionic trypsin, but not mutant cationic trypsin, and partially explain why cationic trypsin, rather than anionic trypsin, seems to be associated with hereditary pancreatitis.

Insights into acute pancreatitis

Identification of cationic trypsinogen as the key molecule in hereditary pancreatitis provides broad implications for understanding acute pancreatitis. The critical finding is that initiation of acute pancreatitis in humans involves intrapancreatic trypsinogen activation. Thus, it is not surprising that the trypsin inhibitor gabexate, which acts inside the acinar cell, is effective in noticeably reducing endoscopic retrograde cholangiopancreatography induced acute pancreatitis, even at suboptimal doses.⁴⁹ Furthermore, insights from human hereditary pancreatitis help to clarify the use of some animal models of acute pancreatitis. For example, although early trypsinogen activation was noted in various animal models of acute pancreatitis,^{50–54} the extreme conditions necessary to initiate acute pancreatitis, and the failure to translate successful therapeutic interventions in these models into equally successful trials in humans⁵⁵ raised serious questions as to their relevance. It now seems that early trypsinogen activation is relevant, and the greater difficulty in inducing acute pancreatitis in animals than humans may be a function of the quantity and properties of human cationic trypsin.⁴⁶ Finally, the importance of autolysis resistant human cationic trypsin R117H in acute pancreatitis may be mimicked in wild type human cationic trypsin in a high calcium environment.

Under these conditions wild type cationic trypsin remains active and resistant to autolysis.^{44–56} Thus, we hypothesised that during hyperstimulation, when intracellular calcium concentrations are notably raised,^{57–59} trypsinogen autoactivates and remains active, driving the digestive enzyme activation cascade leading to pancreatic acinar cell autodigestion and pancreatitis.⁴¹ The pivotal point between trypsinogen activation and pancreatic autodigestion may therefore depend on the acinar cell defence mechanisms, including the ability to handle oxidative stress, prevent intracellular hypercalcaemia, inhibit trypsin, and repair damage.

Insights into chronic pancreatitis

The relation between acute pancreatitis and chronic pancreatitis, especially in the alcoholic patient, has been the subject of great debate. For the past 30 years the primary lesion responsible for chronic pancreatitis was believed to reside within the pancreatic duct, based on the conclusions of the 1963 Marseilles conference.^{60–62} Furthermore, acute alcoholic pancreatitis was thought to occur primarily in the context of preexisting alcoholic chronic pancreatitis^{55–56} (see also the review by Hank and Singer⁶³).

An alternate hypothesis was originally proposed by Comfort and colleagues⁶⁴ and is now referred to as the necrosis–fibrosis sequence hypothesis. This hypothesis suggests that chronic pancreatitis is the result of repeated episodes of acute pancreatitis. The data supporting this hypothesis come primarily from pathological studies,^{65–66} and a recent long term, prospective clinicomorphological study by Ammann *et al.*⁶⁷

The discovery of a autolysis resistant trypsinogen molecule in hereditary pancreatitis associated with acute, and later chronic pancreatitis in a minority of affected family members, offers the strongest support yet in favour of the necrosis–fibrosis hypothesis.^{10–15} Although some authors are critical of this conclusion,⁶⁸ most experts now agree that, in the case of hereditary pancreatitis, a necrosis–fibrosis type sequence leading to chronic pancreatitis is likely.^{62–69} This observation is important because it notably alters our understanding of the pathophysiology of chronic pancreatitis and suggests different targets for therapeutic intervention.

The SAPE hypothesis

Although the necrosis–fibrosis sequence hypothesis has many attractive features, this theory fails to explain why some patients with hereditary pancreatitis, or alcoholic patients, seem to progress to chronic pancreatitis with little evidence of significant pancreatic necrosis. Is there a critical event that triggers the development of chronic pancreatitis in some patients with hereditary pancreatitis or alcoholic pancreatitis? After reviewing the current evidence from our laboratory and others, I would propose a new perspective.

Current evidence suggests that pancreatic fibrosis, the hallmark of chronic pancreatitis, requires differentiation and stimulation of pancreatic stellate cells,^{70–71} and that there is a tight link between injury, chronic inflammation, and fibrosis.⁷² Furthermore, the driving force for fibrosis seems to be transforming growth factor (TGF) β .^{73–74} Although all of the sources of TGF- β in the pancreas are yet to be defined, a significant amount originates from monocytes and/or resident macrophages.⁷⁵ Furthermore, preliminary results from our laboratory suggest the balance of resident macrophages are anti-inflammatory,⁷⁶ possibly to suppress the cytotoxic effects of leucocytes,⁷⁷ during recurrent pancreas stressing conditions.

Infiltration of these macrophages requires an episode of moderately severe acute pancreatitis.^{76–78–79} Thus, we

propose that a sentinel acute pancreatitis event (SAPE) must occur as the a priori step toward development of chronic pancreatitis. To be the sentinel event, an episode of acute pancreatitis must be of sufficient severity to attract monocytes (which become resident macrophages), and to cause infiltration, differentiation, and/or proliferation of pancreatic stellate cells. Finally, for fibrosis to occur we hypothesise that there must be recurrent acinar cell injury, either through oxidative stress (e.g. alcohol, ischaemia) or recurrent pancreatitis (e.g. hereditary pancreatitis, hyperlipidaemia), resulting in cytokine and chemokine release from acinar cells which stimulates resident macrophages. The macrophages, in turn, suppress acute inflammation by releasing TGF- β (and other cytokines), which stimulate pancreatic stellate cells to produce collagen, and therefore drive fibrosis. As fibrosis occurs, it limits the ability of the injured pancreatic acinar cells, eventually lost through apoptosis or necrosis, to be replaced, resulting in a fibrotic, agonal acinus. Thus, macrophages, pancreatic stellate cells and ongoing acinar cell stress and/or injury must all be present for chronic pancreatitis to develop in hereditary pancreatitis and perhaps other types of pancreatitis.

Conclusion

The study of hereditary pancreatitis has provided a number of crucial insights into pancreatic physiology, acute and chronic pancreatitis and pancreatic cancer that are applicable to human disease. The key molecule in human acute pancreatitis seems to be cationic trypsin with unique properties promoting autoactivation and stabilisation under conditions associated with acute pancreatitis. The process leading to chronic pancreatitis involves, in some way, episodes of acute pancreatitis. Continued work in this area promises to reveal new insights as new disease genes are discovered, and questions about why some individuals with the mutant trypsinogen genes escape pancreatitis are answered.

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