Pathophysiology of acute pancreatitis : a multistep disease*

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Introduction

Acute pancreatitis is an inflammatory disease diagnosed mainly in presence of acute abdominal pain associated with a concomitant rise of serum amylase and lipase levels (1-3). In western countries, gallstone migration into the common bile duct and alcohol abuse account for most of the aetiologies of the disease (4-7). The injury is usually mild in 70 to 80% of cases, but 10-20% of the patients have a severe injury and, among them, 15 to 25% will die (4,8).

The pathophysiology of acute pancreatitis includes the activation and release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas, and a multiple organ dysfunction following their release into the systemic circulation (9-12). Evidences accumulated that synthesis and release of pro-inflammatory cytokines and chemokines are responsible for the local injury and the systemic dispersion of the inflammation (13,14). Inflammatory mediators produced within the gland increase the pancreatic injury, and spread out in distant organs, transforming a local inflammation into a severe systemic disease. The effects of these mediators and/or their receptors in the pancreas and remote organs have been evaluated using genetically modified mice or blocking experiments (15). However, the factors that regulate the ultimate severity of the attack are still unknown (15-18) (19,20). Whatever the initial cause (alcohol, gallstone migration), the severity of acute pancreatitis is related to the injury of acinar cells and to the activation of the immune system including activation of various cells such as neutrophils, monocytes, lymphocytes and endothelial cells (21-25). This article will review the emerging concepts in the pathophysiology of acute pancreatitis.

1. Classification of pancreatitis

During the past decades, several attempts to give a clear definition of acute and chronic pancreatitis have been made. Major attempts are the first classification of Marseilles, the second classification of Marseilles and the classification of Marseilles-Rome 1988 (26-28) (Fig. 1). The first classification of Marseilles in 1963 defined acute and chronic pancreatitis on the basis of morphologic criteria (28). The definition differentiates between acute pancreatitis, acute relapsing pancreatitis, chronic relapsing pancreatitis and chronic pancreatitis. The classification states that it is unusual for acute pancreatitis to develop into chronic pancreatitis. The second Marseilles classification of 1984 again stated that acute pancreatitis rarely leads to chronic pancreatitis (29). The 1988 Marseilles-Rome classification states that the morphologic changes of acute pancreatitis are reversible (26). In 1996, the discovery that hereditary human pancreatitis results from mutation of the trypsinogen gene pointed out the possibility for certain cases of acute pancreatitis to evolve into chronic pancreatitis (30,31). Since then, a continuum may exist between acute and chronic pancreatitis particularly in alcoholics.

CLASSIFICATION

1963: MARSEILLES	ACUTE CHRONIC
1984: AMSTERDAM	ACUTE CHRONIC CHRONIC OBSTRUCTIVE
1988: ROME	CONFIRMS 1963 and 1984
1992: ATLANTA	DEFINITION OF ACUTE FORM AND ITS COMPLICATION
1997: ZURICH	ACUTE ---> CHRONIC ?
2000: BERN	CONTROVERSES...

Fig. 1. — Classification of acute pancreatitis

2. Emerging concept in the pathophysiology of acute pancreatitis : early and late events

The initial phase (i.e. the early events) of the disease originates from the activation of trypsinogen into active trypsin within acinar cells, which in turn activates various enzymes such as elastase and phospholipase A2, and the complement and kinin systems. The trypsinogen activation peptide or TAP (a-5 amino-acid), which is cleaved when trypsinogen is activated into trypsin, is found

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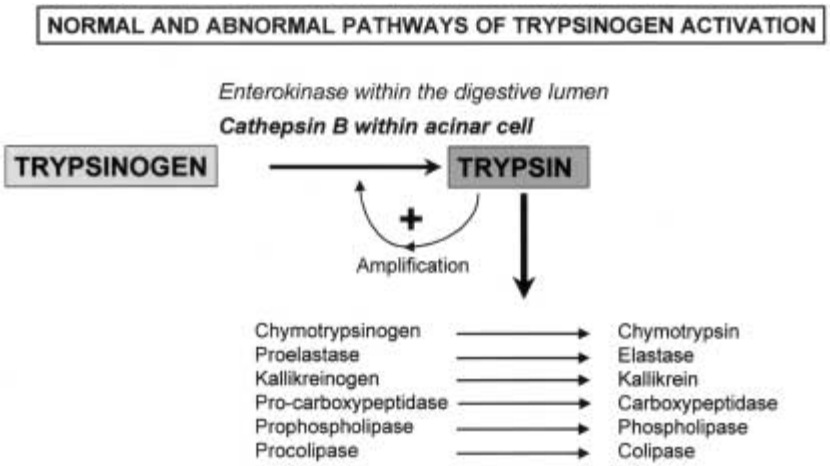

Fig. 2. — Pathway of trypsinogen activation in the digestive lumen and within acinar cells under pathological conditions. In the digestive lumen, trypsinogen is normally activated by the brush border enzyme enterokinase. Then, the activated trypsin is responsible for the activation of all other pro-enzymes into their active form.
Trypsinogen activation within acinar cells is a result of the colocalization of trypsinogen and the lysosomal enzyme cathepsin B within the same subcellular compartment (reproduced with permission from reference 17).

in pancreatic tissue during both experimental and human pancreatitis (16-18).

To prevent a premature activation, the potential harmful digestive enzymes are synthesized in acinar cells and released as inactive precursors (Fig. 2). Moreover, when passing through the Golgi complex, these digestive enzymes are separated from other lysosomal enzymes which may activate trypsin from trypsinogen. Intra-acinar co-localization of digestive and lysosomal enzymes is one of the important feature of experimental pancreatic injury but the full relevance of this co-localization in the pathology of human acute pancreatitis remains unclear (32,33). Following trypsinogen activation, a local inflammation is initiated which results in the local production of inflammatory mediators, a step corresponding to the late events in acute pancreatitis (16,34). Experimental studies show that pancreatic injury and injury to remote organs are mediated by the release of pro-inflammatory mediators such as interleukin-1 (IL-1), IL-6, IL-8, as well as by the activation of inflammatory cells such as neutrophils, macrophages, lymphocytes and endothelial cells. Tumor necrosis factor-a (TNF-a) released by both acinar cells and macrophages within pancreatic tissue correlates with the severity of experimental disease (35,36). Synthesis and release of pro-inflammatory cytokines within the pancreas amplifies a local inflammation into a systemic one. The balance between pro and anti-inflammatory mediators are thought to regulate the ultimate severity of the attack (16,37).

2.1. *Early events in acute pancreatitis*

Several studies have evaluated the location of the early events by examining pancreatic tissue removed from opossum at very short intervals after duct obstruction (38). Changes within acinar cells and evidence of acinar necrosis could be detected within 3-6 hours after the duct obstruction and the magnitude of these changes increased over the subsequent periods. Patchy acinar cell necrosis was observed 12 hours after the duct obstruction, but perilobular and periductal changes were not observed until 24 hours after the obstruction. These observations have led the investigators to conclude that the earliest events in acute pancreatitis occur in acinar cells (38). More than 90% of the proteins synthesized by the acinar cells consists of digestive enzyme proteins which are exported from the apical surface of acinar cells to the lumen of the small intestine by passing in ductal space. Most of these enzymes are synthesized and secreted as inactive zymogen that get activated only under the influence of trypsin which is activated by the intestinal brush border enzyme enterokinase (11) (Fig. 2).

The acinar cell is protected from premature activation of zymogens within the cell because these proteins are contained within small membrane-bound organelles from their site of synthesis to their site of secretion. Additionally, the presence of protease inhibitor such as PSTI (Protease Trypsinogen Inhibitor) synthesized along with the zymogens prevent any premature activation in response to minor pancreatic insult (17). It is now believed that the fact these changes occur in each of these experimental models suggests that they may also occur in the human disease.

2.2. *Co-localization theory*

Digestive enzyme secretion from the pancreas is abruptly halted during the early stages of several models of pancreatitis. Synthesis and the early phases of intracellular transport are not altered, but the usual process of digestive enzyme segregation is perturbed (10,34). Digestive enzyme zymogens, which are destined for

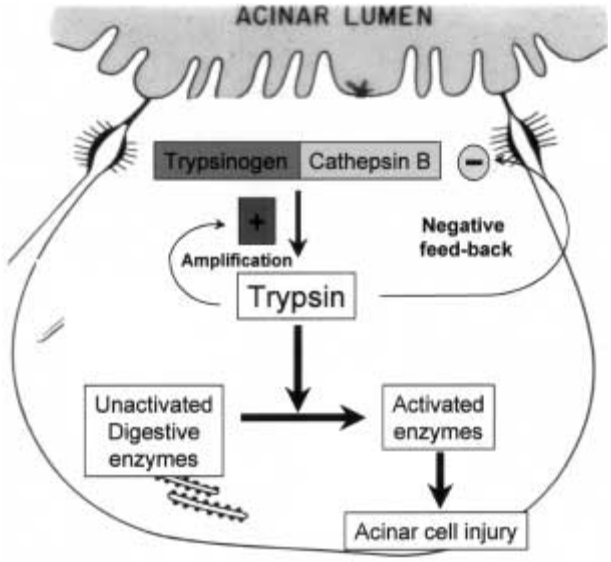

Fig. 3. — The colocalisation theory. After a pancreatotoxic stimulus, experimental data suggest that trypsinogen is abnormally colocalised with the lysosomal enzyme cathepsin B in the same subcellular organel. This colocalisation within acinar cells is responsible for a premature activation of trypsinogen into active trypsin which in turn activates all other proenzymes leading to the autodigestion of the gland (reproduced with permission from reference 17).

export, and lysosomal hydrolases, which are destined for transport intracellularly to lysosomes, normally are separated from each other while they pass through the Golgi complex (39,40). Lysosomal hydrolases are glycosylated, 6-mannose phosphorylated, bound to mannose 6-phosphate receptors, and conveyed to lysosomes. In contrast, digestive zymogens are not 6-mannose phosphorylated, pass through the Golgi complex, and are packaged into condensing vacuoles, which in turn mature into zymogen granules as they migrate from Golgi to the apical membrane of acinar cell. During each of these experimental models of pancreatitis, separation and segregation of digestive zymogens from lysosomal hydrolases is defective and, as a result, both types of enzymes become colocalized within fragile intracellular vacuoles (39). This colocalization phenomenon may result in premature activation of digestive enzyme zymogens, because the lysosomal hydrolase cathepsin B can activate trypsinogen into active trypsin, and trypsin can activate the remaining zymogens (41,42) (Fig. 3). Subsequent rupture of these fragile vacuoles might result in liberation of activated digestive enzymes within the cytoplasmic space of acinar cell and begin the cascade of events that finally results in acinar cell injury and acute pancreatitis.

Interestingly, cerulein-induced in vitro activation of trypsinogen was completely prevented when cathepsin B activity was inhibited. Recently, Halangk *et al.* developed cathepsin B deficient mice by targeted disruption (43). After induction of pancreatitis, the trypsin activity in the pancreas of cathepsin B deficient mice was more than 80% lower than in cathepsin sufficient mice. Pancreatic injury was also 50% lower in cathepsin B deficient mice. Interestingly, the prevention of trypsinogen activation by genetic deletion of cathepsin B was incomplete suggesting that additional mechanisms such as trypsinogen activation by other lysosomal enzyme must be considered as potential alternatives.

2.3. *Human hereditary pancreatitis is caused by a mutation in the trypsinogen gene*

The hypothesis of an intraacinar premature activation of trypsinogen is also supported by recent human studies demonstrating that a mutation of the trypsinogen gene (now more than 16 are known) is responsible for the human hereditary pancreatitis (30,31). Individuals affected with this genetic disorder usually suffer from recurrent acute pancreatitis, progression to chronic pancreatitis and finally they are exposed to a high probability of developing a pancreatic cancer reaching 40% by age of 70 years (44). This mutation eliminates the site of hydrolysis of trypsin that makes trypsin, once activated, resistant to inactivation and therefore permitting autodigestion of the gland by premature intraacinar activation of trypsinogen into active trypsin, resulting in pancreatitis (45).

2.4. *Role of free radicals in acute pancreatitis :*

Reactive oxygen species are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation (46-50). Peroxidation products have been shown to increase already after 30 minutes of cerulein infusion suggesting that this increase may play an important role in signal transduction and can cause alterations in cytoskeleton function (51). When scavenger enzymes were administered in a model of ischemic pancreatitis to the perfusate prior to exposure to the deleterious injury, less pancreatic edema and serum amylase elevation were noted (52). Although the results of experimental studies are interesting and provide new insights in the pathogenesis of acute pancreatitis, results of well-designed clinical studies have not yet proven any benefit of enzymes scavengers to patients with acute pancreatitis (46).

2.5. *Trouble of microvascular circulation in pancreatitis*

Pancreatitis has been reported in association with vascular abnormalities such as vasculitis, atherosclerotic embolization or during low flow state (5,53). Complete ischemia of the pancreas followed by reperfusion corresponds to the situation encountered with pancreas transplantation, a condition in which the rate of posttransplant pancreatitis is as high as 17 to 87% (54,55). Using the bile salt-induced pancreatitis, Kusterer *et al.* have demonstrated that pancreatitis is associated with early arteriolar vasodilatation with cessation of capillary blood perfusion, followed by arteriolar vasodilatation with re-

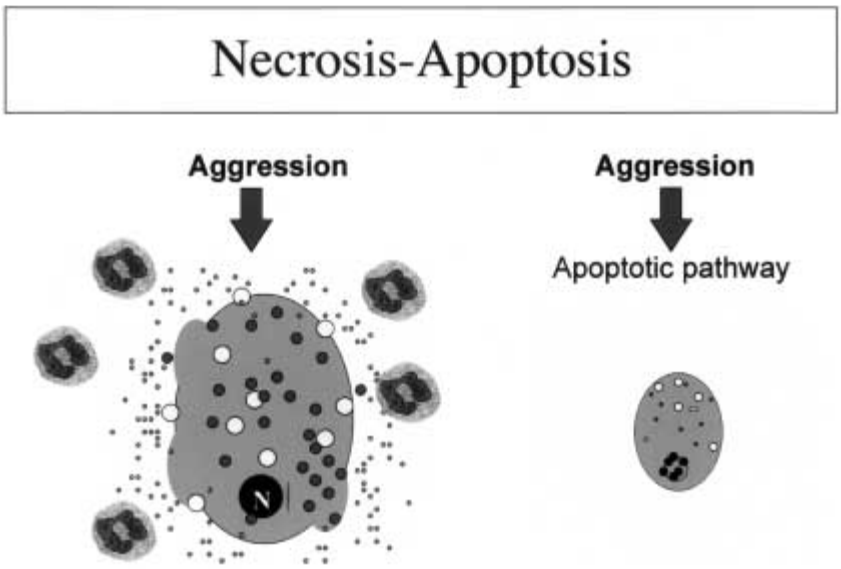

Fig. 4. — Necrosis-apoptosis. While necrotic cells release their cytosolic content in the interstitium and participate to increase the local inflammation, apoptotic cells do not spill their cytosolic content and are rapidly phagocytosed by macrophages or neighbouring cells. Interestingly, mild experimental pancreatitis is associated with extensive apoptosis while more severe forms of pancreatitis involve more necrosis than apoptosis.

establishment of blood flow (56). In these experiments, increased leukocyte-endothelial cell interactions in post-capillary venules were not observed during vasoconstriction, but were particularly important during vasodilatation. Finally, reinforcing the role of vascular abnormalities was the observation that perfusion of phenylephrine in animals treated with cerulein causes conversion of edematous pancreatitis to hemorrhagic pancreatitis with formation of parenchymal necrosis by both ischemia-reperfusion (57).

2.6. *Necrosis versus apoptosis : a subtle balance regulating the disease severity*

The acinar cell response to pancreatotoxic injury may be an important determinant of the disease severity (16,58). Indeed, in many tissues, cells are programmed to kill themselves if they do not receive specific signals for their survival. Too little cell death can be as dangerous to the health of the organism as too much proliferation, and mutation that inhibit cell death by causing overexpression of antiapoptotic proteins have been implicated in the development of cancer (59,60). Interestingly, cells that die accidentally usually swell and burst (necrosis). Whereas cells that die by necrosis spill their cytosolic contents into the extracellular space and elicit an inflammatory response, cells that die by apoptosis disappear in a more efficient way for the organism (Fig. 4). Indeed, they are so rapidly phagocytosed by macrophages that there is no leakage of cytosolic content and no subsequent inflammatory response (59,61). Mild experimental pancreatitis is associated with extensive apoptotic acinar cell death while severe acute pancreatitis was noted to involve extensive acinar cell necrosis but very little acinar cell apoptosis (62,63). All these observations led to the hypothesis that apoptosis might be a favourable response to acinar cell injury suggesting to certain that interventions which favour induction of apoptosis might reduce the severity of an attack of pancreatitis (64). Indeed, prior administration of crambene (a compound known to induce apoptosis in vivo) to mice receiving cerulein reduced the severity of pancreatitis (63,65). This observation indicates that induction of apoptosis can effectively reduce the severity of the disease and may be beneficial in the clinical management of acute pancreatitis.

3. Late events in acute pancreatitis

Edema and inflammation are the basic lesions of acute pancreatitis. A number of investigators have suggested that the lesion may progress from this relatively mild form to a more severe form characterised by necrosis and haemorrhage. Changes in the pancreatic microcirculation may lead to pancreatic ischemia, which favours progression from mild to severe form of the disease (57). Alternatively, the severity of the injury may be established at its very beginning, i.e. mild or severe. In this regard, studies by Ranson *et al.* may be of great importance (66). It seems that the severity of pancreatitis and the potential for subsequent mortality and/or development of complications, can be determined by evaluating a carefully selected group of clinical and biochemical parameters that are available within a short period of time after onset of the disease (66,67). These observations therefore suggest that the severity of the lesion is established very early in its natural history and

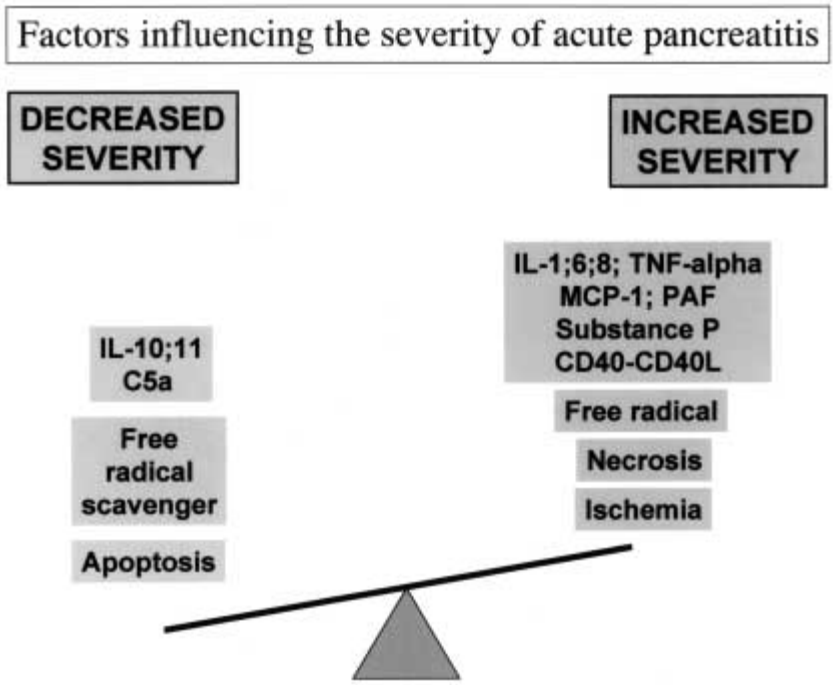

Fig. 5. — Many biological mediators are involved during the course of acute pancreatitis. Although it is impossible to predict the severity of the disease, several observers believe that the ultimate severity of pancreatitis is dictated by a balance between pro and anti inflammatory mediators, free radicals and free radical scavengers and between the apoptosis-necrosis ratio.

argue against the concept that mild pancreatitis may evolve into the more severe form. Thus, a better understanding of the pathophysiology of acute pancreatitis should help us to find an early and specific mediator for the rapid scoring of the disease severity. So far numerous biological factors occurring during the course of the disease have been studied, but none could be considered as a valuable therapeutic target. The problem is that we don't know which factor, among several already studied, will predominantly dictate the ultimate severity of the disease (Fig. 5).

3.1. *Role of inflammatory cells and cytokines during acute pancreatitis*

After supramaximal cerulein stimulation, one of the earliest morphological change is the formation of pancreatic edema. Then, granulocytes appear following the first biochemical evidence of pancreatitis (< 1 hour) and peak in number within 4-12 hours. Sequestration of inflammatory cells within areas of inflammation is a multistep process that begins with leukocyte activation, involves the adhesion of circulating activated inflammatory cells to activated microvascular endothelial surfaces, and culminates in the transmigration of those cells across the endothelial barrier and into the involved tissue. Considerable attention has been directed at identifying the chemoattractant substances responsible for leukocyte sequestration within inflamed tissues and at determining the factors released from these inflammatory cells that contribute to the progression of a local inflammation into a systemic one. Intercellular adhesion molecule-1 (ICAM-1) has thus been studied since this molecule plays a central role in the firm adhesion of activated leukocytes on endothelial surfaces. Macrophages are the major resident cells in normal pancreas. The role of this cell during pancreatitis is to amplify a local inflammation into a systemic one. Indeed, studies blocking macrophage activity by gadolinium showed that the severity of pancreatitis was not modified, whereas the severity of the pancreatitis-associated lung injury was reduced (68). Persisting alteration in lymphocyte function in patients with acute pancreatitis have been reported even one month after the clinical event (69). In experimental studies, lymphocytes have been recently shown to play a central role in the development of acute pancreatitis (22). In control mice, CD4 cells are present in the pancreas and are recruited during cerulein-induced acute pancreatitis. In nude mice, histological lesions and serum amylase levels are significantly decreased (22). T-lymphocyte transfer into nude mice partially restores the severity of acute pancreatitis. Furthermore, the severity of acute pancreatitis is also reduced by in vivo CD4 T-cell depletion. Taken together, these data confirm the pivotal role of lymphocytes in the development of pancreatitis in mice.

Acute pancreatitis exhibits many of the features of the systemic inflammatory response syndrome (SIRS) which is responsible for multiple organ failure in conditions such as multiple trauma or burns (70). Consequently, the

role of cytokines has been extensively evaluated using either blocking experiments or transgenic animals which permit to define the role of a specific protein in a determined condition (15,24,25,71-74) (Fig. 5). Thus, the genetic deletion of genes encoding for pro-inflammatory cytokines resulted in decreased severity of acute pancreatitis whereas the genetic deficiency in anti-inflammatory cytokines resulted in increased severity of experimental pancreatitis (22,25,37,63,75). However, the utility of such experimental models might have limitations, and a full extrapolation of experimental data from genetically modified mice to humans must be done with caution.

2.3. *PAF in acute pancreatitis : a new therapeutic target ?*

The locally activated neutrophils release phospholipase A2 which can in turn catalyse the release of platelet activating factor (PAF) from membrane phospholipid. Furthermore, pancreatic acinar cells also contain phospholipase A2 and when the cells are disrupted, activated enzyme is able to release PAF locally (76,77). PAF is a mediator of multiple organ failure and is involved in local complications (78,79). In experimental models, PAF is released in the pancreas and is also found in serum and in remote organs such as the lungs. PAF antagonism diminishes the severity of experimental pancreatitis (80, 81). Consequently, clinical studies with PAF antagonists have been undertaken in the mids 1990. Initially, the results of such studies were promising. PAF antagonism (Lexipafant) was associated with a significant reduction in the number of patients with complications after three days of lexipafant (82) and in their severity after 7 days of treatment (83). However, the last European multicenter study failed to prove any benefit to patients with acute pancreatitis (84) and the production of lexipafant has ended.

Conclusion

The emerging concept of the pathophysiology of acute pancreatitis includes the early and premature activation of pancreatic enzymes within acinar cells, the release of these activated enzymes in the interstitium and the autodigestion of the gland. The late events of acute experimental pancreatitis include the synthesis and release of pro-inflammatory mediators which may amplify a local inflammation into a systemic one. The development of a multiple organ failure may then occur due to the release of activated pancreatic enzymes and other factors into the circulation. Although the factors that dictate the ultimate severity of the disease remain unknown, several observers believe that the severity is in part dictated by a subtle balance between anti and pro-inflammatory cyto/chemokines, a favourable ratio between necrosis and apoptosis as well as between reactive oxygen species and free radical scavengers.

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