Subsequent Loss of the Proliferated Pancreatic Ductulus in Experimental Pancreatitis: Is it Caused by Apoptosis?

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Abstract: Using a guinea pig model, we examined the mechanism of cell loss in experimental pancreatitis by immunohistochemistry and electron microscopy. Acinar cells in pancreatitis foci were completely deleted by one week, and proliferation of pancreatic ductuli appeared at three days, increased by two weeks, and then decreased by six months. Approximately 30% of acinar cells in the pancreatitis foci were positive with TUNEL immunohistochemistry at three days. By one week, numbers of positive cells had decreased. TUNEL-positive ductular cells appeared at three days, increased by two weeks, and then gradually decreased by three months. At three days, most acinar cells showed necrotic change by electron microscopy, with some cells undergoing apoptosis. Proliferated ductular cells did not show necrotic change, but were undergoing apoptosis continuously from one week to six months, therefore apoptosis does participate in the course of ductular deletion. As apoptosis is a mechanism of silent cell death carrying no inflammatory aggregates, it should be favorable for pancreatic ductuli to be deleted by apoptosis in the healing state of pancreatitis. It is concluded that apoptosis, rather than acinar cell loss, plays a major role in subsequent loss of proliferated pancreatic ductuli in experimental pancreatitis.

Key words: experimental pancreatitis, pancreatic ductulus, apoptosis

Introduction

The participation of apoptosis in acinar cell loss is well known in experimental pancreatitis¹⁴), but the mechanism in loss of proliferated pancreatic ductuli, which appear as a reaction to acinar cell loss²), is still uncertain. We undertook immunohistochemical and electron microscopic studies to determine the mechanism of loss of proliferated pancreatic ductuli, using animal models of pancreatitis.

Materials and Methods

As previously reported³), we induced proliferated pancreatic ductulus pancreatitis in 70 guinea pigs. These experimental models were generated by injection of fluid vegetable gelatin into the pancreatic ducts of each guinea pig. Ten guinea pigs were autopsied at each

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of seven post-injection intervals: at three days; at one, two and three weeks; and at one, three and six months. After fixation in 20% buffered formalin, pancreatic tissues were embedded in paraffin for preparation of hematoxylin-eosin sections. Both acinar cell loss and ductular proliferation were estimated morphometrically, and the ratios of acinar cells and ductuli per unit were calculated in blocks of ten. Immunohistochemistry was performed using a TdT-mediated dUTP-biotin nick end-labeling method (TUNEL; S7101 Intergen Company, USA), and sections were stained with benzidine and lightly counterstained with hematoxylin for light microscopy. The TUNEL labeling index, the percentage of positive nuclei in greater than five hundred tumor cells, was calculated in blocks of ten. For electron microscopy, tissues were fixed with 2.5% glutaraldehyde in 0.05 mol/L phosphate buffer, post-fixed in 2% osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin. The sections were then stained with uranyl acetate and lead citrate, and examined with an electron microscope.

Results

Histological examination

Scattered but not diffuse pancreatitis foci were observed at each time interval by light microscopy. The morphometric results of acinar cell loss and ductular proliferation are shown in Figure 1. In the pancreatitis foci, 50% of acinar cells (range: 40-60%) were undergoing necrotic change at three days post-injection, and were completely deleted after one week. They did not regenerate until six months later (Fig. 2A). Pancreatic ductuli appeared in the foci at three days post-injection and occupied 30% per unit (range: 20-40%). After one week they occupied 80% per unit (range: 70-90%) and by six months they had gradually decreased to 10% per unit (range: 0-20%).

Apoptosis was recognized in the pancreatic ductular epithelia from one to three weeks by the presence of acidophilic bodies with condensed nuclear chromatin (Fig. 2B). The degree of inflammatory aggregation was severe at three days and one week, but was reduced after three weeks. Pancreatic stroma was granulomatous by three weeks, fibrotic by three months, and was replaced by adipose tissues by six months.

Immunohistochemical examination

The TUNEL labeling index of acinar cells and proliferated ductuli in the time course is shown in Figure 3. Thirty percent of acinar cells (range: 20-40%) in the pancreatitis foci were positive with TUNEL at three days, and positive acinar cells were decreased by one week. Ten percent of ductular cells (range: 0-20%) were positive with TUNEL at three days, 90% (range: 80-100%) by two weeks (Fig. 4A) with a gradual decrease to 10% (range: 0-20%) by six months (Fig. 4B).

Electron microscopic examination

At three days, most acinar cells showed necrotic change such as swelling of mitochondria and intra-cellular vacuolation (Fig. 5A), but a few cells were undergoing apoptosis (Fig. 5B). Proliferated ductular cells did not show necrotic change, but underwent apoptosis continuously from one week to six months (Fig. 3C). Evidence of apoptosis included condensation of chromatin against the nuclear membrane, the presence of rounded clumps of electron-dense granules, convolution of the nuclear outline, and a sharp line of
Apoptosis of Pancreatic Ductulus

Fig. 1. Morphometrical finding in pancreatic tissue
□ represents the ratio of acinar cells, and ○ represents the ratio of proliferated ductulus.

Fig. 2. Light microscopic findings
(A) Acinar cells were completely deleted, and proliferated ductulus was diffusely seen.
(2 week case, hematoxyline and eosin staining, original magnification ×25)
(B) Apoptosis was recognized in acidophilic bodies with condensed nuclear chromatin among the proliferated ductular epithelia (arrow). (1 week case, hematoxylin and eosin staining, original magnification ×200)
Fig. 3. The TUNEL labeling index

□ represents the index of acinar cells, and ○ represents the index of proliferated ductulus.

Fig. 4. TUNEL staining of the proliferated ductulus

(A) TUNEL positive findings were diffusely seen in the proliferated ductular epithelia.
   (1 week case, original magnification ×50)

(B) TUNEL positive findings were focally seen in the proliferated ductular epithelia.
   (arrow heads, 1 month case, original magnification ×50)
Discussion

In our experimental pancreatitis model, scattered pancreatitis foci can be observed and we can generate a chronic pancreatitis model with our long time course. We examined the mechanisms of cell loss in the acute and subacute phases of our pancreatitis model.
Participation of apoptosis in acinar cell loss is well known in experimental acute pancreatitis\(^1\)\(^-\)\(^4\). In pancreatic duct ligation models generated by Watanabe et al.\(^6\), the pancreatic tissues show severe atrophic change and their volume decreases to less than 10% compared to the normal pancreas at seven days after treatment. We also found the same histologic results as Watanabe et al.\(^6\) in acute pancreatitis foci, but with a different estimation of acinar cell loss. They reported that most of the acinar cell loss was caused by apoptosis, however our results suggest that the loss was mainly caused by necrosis. Sakagami et al.\(^8\) examined the occurrence of apoptosis in arginine-induced pancreatitis, and reported that necrosis rather than apoptosis plays a central role in acinar cell loss. Thus, we conclude that acinar cell loss in the acute phase of experimental pancreatitis is predominantly caused by necrosis.

In the subacute phase of pancreatitis, proliferation of the pancreatic ductulus as a reaction to acinar cell loss can be seen in many models\(^2\),\(^6\)\(^-\)\(^8\) and this continues until complete acinar cell loss in the pancreatitis foci occurs. Pancreatic ductuli are derived from centroacinar cells in an attempt to depress the increased pancreatic duct pressure in pancreatic duct ligation models\(^2\),\(^6\),\(^7\). In addition, they play a role in reconstruction of pancreatic structure in arginine-induced models\(^8\). However they are also known to decrease in number during the course of pancreatitis\(^6\),\(^8\). In the ligation model this is explained by the fact that the proliferated ductulus is of no use after complete acinar cell loss, as there is no secretion of pancreatic juice and therefore no more increase in pancreatic duct pressure in the foci. The excessive ductuli in arginine-induced models are also of no use after the regeneration of pancreatic structure. As several authors have reported\(^6\),\(^8\), we have found that apoptosis participates in the course of ductular deletion. As apoptosis is a mechanism of silent cell death carrying no inflammatory aggregates, it should be favorable for pancreatic ductuli to be deleted by apoptosis in the healing state of pancreatitis.

Participation of apoptosis was reported not only in acinar cell loss but also in ductular deletion in human chronic pancreatitis\(^9\), although the significance is still uncertain. Apoptosis in ductular cells was also found after six months in our experimental pancreatitis model, and this suggests that our model may be useful to study the roles of apoptosis in chronic pancreatitis.

It is concluded that apoptosis plays a significant role in subsequent loss of proliferated pancreatic ductuli rather than in acinar cell loss in the acute and subacute phases of experimental pancreatitis.

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References


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