RAPID COMMUNICATION

Dynamic changes of IL-2/IL-10, sFas and expression of Fas in intestinal mucosa in rats with acute necrotizing pancreatitis

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Abstract

AIM: To investigate dynamic changes of serum IL-2, IL-10, IL-2/IL-10 and sFas in rats with acute necrotizing pancreatitis. To explore the expression of Fas in intestinal mucosa of rats with acute necrotizing pancreatitis (ANP).

METHODS: A total of 64 Sprague-Dawley (SD) rats were randomly divided into two groups: normal control group (C group), ANP group (P group). An ANP model was induced by injection of 50 g/L sodium taurocholate under the pancreatic membrane. Normal control group received isovolumetric injection of 9 g/L physiological saline solution using the same method. The blood samples of the rats in each group were obtained via superior mesenteric vein to measure levels of IL-2, IL-10, sFas and calculate the value of IL-2/IL-10. The levels of IL-2, IL-10 and sFas were determined by ELISA. The severity of intestinal mucosal injury was evaluated by pathologic score. The expression of Fas in intestinal mucosal tissue was determined by immunohistochemistry staining.

RESULTS: Levels of serum IL-2 were significantly higher in P group than those of C group (2.79 ± 0.51 *vs* 3.53 ± 0.62, 2.93 ± 0.89 *vs* 4.35 ± 1.11, 4.81 ± 1.23 *vs* 6.94 ± 1.55 and 3.41 ± 0.72 *vs* 4.80 ± 1.10, respectively, *P* < 0.01, for all) and its reached peak at 6 h. Levels of serum IL-10 were significantly higher in P group than those of C group at 6 h and 12 h (54.61 ± 15.81 *vs* 47.34 ± 14.62, 141.15 ± 40.21 *vs* 156.12 ± 43.10, 89.18 ± 32.52 *vs* 494.98 ± 11.23 and 77.15 ± 22.60 *vs* 93.28 ± 25.81, respectively, *P* < 0.01, for all). The values of IL-2/IL-10 were higher significantly in P group than those of C group at 0.5 h and 2 h (0.05 ± 0.01 *vs* 0.07 ± 0.02 and 0.02 ± 0.01 *vs* 0.03 ± 0.01, respectively, *P* < 0.01, for all), and it were significantly lower than those of C group at 6 h (0.05 ± 0.02 *vs* 0.01 ± 0.01, *P* < 0.01) and returned to the control level at 12 h (0.04 ± 0.01 *vs* 0.05 ± 0.02, *P* > 0.05). In sFas assay, there was no significant difference between P group and C group (3.16 ± 0.75 *vs* 3.31 ± 0.80, 4.05 ± 1.08 *vs* 4.32 ± 1.11, 5.93 ± 1.52 *vs* 5.41 ± 1.47 and 4.62 ± 1.23 *vs* 4.44 ± 1.16, respectively, *P* > 0.05, for all). Comparison of P group and C group, the pathological changes were aggravated significantly in P group. Immunohistochemistry staining show the expression of Fas was absent in normal intestinal tissues, however, it gradually increased after induction of pancreatitis in intestinal tissue, then reached their peaks at 12 h.

CONCLUSION: Fas were involved in the pathogenesis of pancreatitis associated intestinal injury. The mechanisms of Fas may be associated to Fas mediated T helper cell apoptosis.

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INTRODUCTION

Acute pancreatitis (AP) is sudden inflammation of the pancreas that may be mild or life threatening but that usually subsides. Although usually self-limiting, 10% to 20% of afflicted patients will progress to acute necrotizing pancreatitis (ANP)[1,2]. The mortality rate among patients with ANP may approach 30% when they progress to multiple organ failure (MOF)[3]. It is generally accepted that AP is often complicated by intestinal injury. Failure of intestinal barrier function often occurs in this condition, resulting in the increased intestinal permeability. It is clear that increased intestinal permeability and bacteria with

or without endotoxin translocation plays a key role in the development of severe complications such as systemic inflammatory response syndrome (SIRS), sepsis, multiple organ dysfunction syndrome (MODS) and MOF[4-7]. However, its pathogenesis remains unclear.

The Fas system was originally characterized as a key mechanism for inducing apoptosis in immune cells, but later it transpired to be very common in various tissues such as liver, ovary, kidney, and testis, especially under I/R conditions. Apoptosis is a teleologically beneficial form of cell death in AP. However, little is known about how the induction of apoptosis reduces the severity of AP[8]. Recent research has demonstrated that a Th1 to Th2 immune deviation is beneficial to ANP. The antigen-induced deletion of Th is often accompanied by an imbalance in Th1 and Th2. The two most polarized patterns of cytokine production, Th1 (characterized by production of IL-2, IFN-γ, TNF-α, and TNF-β) and Th2 (characterized by production of IL-4, 5, 6, 10, and -13) were reported[9].

In the present study, we performed immunohisto-chemistry staining of apoptosis-related protein Fas and investigated dynamic changes of serum IL-2, IL-10, IL-2/IL-10 and sFas in rats with ANP.

MATERIALS AND METHODS

Animals
Adult Sprague-Dawley rats of both sexes weighing 250-300 g were provided by the Laboratory Animal Center of Jiangsu University. The animals were fed with standard rat chow and water *ad libitum*. The rats were allowed to acclimatize to our laboratory conditions for 1 wk and then subjected to mesh stainless-steel cages at a constant temperature (21 ± 1°) in a 12 h day/night cycle. The animals were fasted for 12 h before the experiments but had free access to water. Animal care and experimental procedure were performed in accordance with the guidelines for Animal Experimentation of Jiangsu University with the approval of the Institutional Animal Care and Use Committee.

Experimental design
The animals were randomly divided into: control group (C group), ANP group (P group) with 32 rats in each group. Each group was further divided into 0.5, 2, 6 and 12 h subgroups, respectively. The mortality in the present series was not calculated. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The rats were infused with sodium taurocholate (4 mL/kg, Na-Tc, Sigma) the pancreatic membrane to induce ANP model as previously described[10]. After 30 min, pancreatic edema and dotted bleeding occurred. Normal control group received isovolumetric injection of 9 g/L physiological saline solution using the same method. Animals in each group were sacrificed at 0.5, 2, 6 and 12 h after infusion for further examination. Part of distal ileum and pancreas were removed immediately and fixed in paraformaldehyde solution for 12-24 h and paraffin-embedded for routine histopathologic analysis. The histopathologists were blinded to routine histopathologic analysis.

Analysis of Th1/Th2 cytokines and sFas
The blood of rats in each group was obtained via superior mesenteric vein for determination of serum IL-2, L-10 and sFas levels at 0.5, 2, 6 and 12 h after infusion. Serum levels of IL-2, L-10 and sFas were measured by double antibody sandwich ELISA according to the manufacturer's protocol (Shanghai Senxiong Technology Enterprise Co., Ltd.). The optical density of each well was determined within 30 min using a microplate reader (492 nm).

Pathological examination
The whole pancreas and parts of distal ileum were obtained and promptly fixed in 40 g/L phosphate-buffered formaldehyde for further studies. Paraffin-embedded tissue sections (5 μm thick) were stained with hematoxylin and eosin. Mucosal damage was assessed according to the standard scale of Chiu *et al*[11]. Grading was performed and classified as 0 = normal mucosa; 1 = development of subepithelial space at the tip of the villus; 2 = extension of the space with epithelial lifting; 3 = massive epithelial lifting; 4 = denuded villi; 5 = disintegration of the lamina propria.

Immunohistochemistry
After embedding in paraffin, sections 5 μm in thickness were immersed twice into xylene for 5 min each, followed by immersion twice for 3 min each in 100% ethanol and then 95% ethanol. Slides were rinsed for 30 sec using deionized water and then immersed twice in deionized water for 5 min. To detect Fas expression, heat-induced Ag retrieval was performed using 0.01 mol/L citrate buffer (pH 6.2) and 10 min slide immersion into 95℃ waterbath. Immunoenzyme double staining of intestinal tissue was performed using DAKO EnVision Doublestain System. The sections were then counterstained using hematoxylin before study.

Statistical analysis
All data were analyzed with the SPSS 11.0 software. The results were expressed in mean ± SD except for date on the grading of intestinal mucosal lesions. Differences of grading of intestinal mucosal lesions were determined using the non-parametric Mann-Whitney test. Statistical analysis was performed with post-hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Serum IL-2 level
At 0.5 h after injection of 50 g/L sodium taurocholate, serum IL-2 level in the samples from mesentery vein in P group were higher than those in C group. From 0.5 h, there was a significant difference between P group and C group ($P < 0.01$, Table 1).

Serum IL-10 level
Upon stimulation, serum IL-10 level was significantly increased in the P group as compared with that of C group at 6 and 12 h ($P < 0.01$, Table 1). There was no significant difference between P group and C group at 0.5 and 2 h.

Table 1 IL-2 and IL-10 level in each group (mean ± SD, $n = 8$) (pg/mL)

Group		0.5 h	2 h	6 h	12 h
IL-2	C	2.79 ± 0.51	2.93 ± 0.89	4.81 ± 1.23	3.41 ± 0.72
	P	3.53 ± 0.62[b]	4.35 ± 1.11[b]	6.94 ± 1.55[b]	4.80 ± 1.10[b]
IL-10	C	54.61 ± 15.81	141.15 ± 40.21	89.18 ± 32.52	77.15 ± 22.60
	P	47.34 ± 14.62	156.12 ± 43.10	494.98 ± 11.23[b]	93.28 ± 25.81[b]

[b]$P < 0.01$ *vs* control group.

Table 2 Serum IL-2/IL-10 AND Serum sFas in each group (mean ± SD, $n = 8$)

Group		0.5 h	2 h	6 h	12 h
IL-2/IL-10	C	0.05 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
	P	0.07 ± 0.02[b]	0.03 ± 0.01[b]	0.01 ± 0.01[b]	0.05 ± 0.02
sFas (pg/mL)	C	3.16 ± 0.75	4.05 ± 1.08	5.93 ± 1.52	4.62 ± 1.23
	P	3.31 ± 0.80[a]	4.32 ± 1.11[a]	5.41 ± 1.47[a]	4.44 ± 1.16[a]

[a]$P > 0.05$ *vs* control group, [b]$P < 0.01$ *vs* control group.

Table 3 Intestinal mucosal injury in each group ($n = 8$)

Group	0.5 h						2 h						6 h						12 h					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
C	7	1	0	0	0	0	6	2	0	0	0	0	7	1	0	0	0	0	6	2	0	0	0	0
P	0	2	5	1	0	0[b]	0	0	2	4	2	0[b]	0	0	0	1	4	3[b]	0	0	0	0	3	5[b]

[b]$P < 0.01$ *vs* C group.

Serum IL-2/IL-10

As shown in Table 2, the values of IL-2/IL-10 were higher significantly in P group than those of C group at 0.5 h and 2 h, and it were significantly lower than those of C group at 6 h ($P < 0.01$) and returned to the control level at 12 h ($P > 0.05$) (Table 2) .

Serum sFas level

In serum sFas assay as illustrated Table 2, there was no significant difference between P group and C group. sFas levels were moderate at 0.5 h, peaked at 6 h and decreased at 12 h.

Pathologic examination of pancreas and intestinal mucosa

After induction of ANP model, pancreas showed mild edema and congestion. 2 h after introduction of the model, typical pathologic changes were found in P group, such as a large number of inflammatory cells, necrosis of adjacent fat tissues, interstitial edema, parenchyma hemorrhage and necrosis, large amount of ascites. The degree of intestinal pathological injury is shown in Table 3. The grades of P group were significantly higher those of control group ($P < 0.01$).

Immunohistochemistry

We detected the expression of Fas on intestinal tissue with immunocytochemical technique. Immunohistochemistry staining showed Fas expression in intestinal tissue was absent in normal intestinal tissue, Fas expression in intestinal tissue gradually increased 0.5 h after induction of pancreatitis, and then reached a peak at 12 h (Figure 1).

DISCUSSION

The Fas system is a widely recognized apoptotic signal transduction pathway in which a ligand-receptor interaction triggers the cell death pathway[12]. The Fas system has been implicated in the control of the immune response and inflammation, the response to infection and death of parenchymal cells in several organs[13-15], which is involved in maintaining homeostasis in various systems, including maintenance of peripheral T cell[16]. Recent research has demonstrated that a Th1 to Th2 immune shift is beneficial to mucosal immunity[17,18]. A key component of the mucosal immune defense against pathogens is mediated by CD4+ T lymphocytes that can differentiate into functionally distinct subsets[19]. Whereas T-helper 1 (Th1) cells secrete the cytokines IL-2, IFN-γ, TNF-α, and TNF-β, Th2 cells secrete IL-4, 5, 6, 10, and 13. In the current study, we used IL-2 levels as a marker of Th1 response and IL-10 as a marker of Th2 response. In this study, the role of the Fas-mediated cell death pathway in intestinal mucosal injury models was assessed and dynamic changes of serum IL-2, IL-10, IL-2/IL-10 and sFas in rats with ANP were also investigated.

According to the result of immunohistochemistry, Fas had a lower expression in intestinal tissue in the P group at 0.5 h and higher expression after 2, 6 and 12 h. In the C groups, Fas was not detected in any part of intestinal tissue. Previous study has shown that IL-10 is a kind of important anti-inflammatory cytokine and plays a role of self-defense mechanism, limiting the intensity of inflammatory process[20-23]. However, the effect of IL-10 level in the course of acute pancreatitis is still not clear[24-26]. IL-10 is a powerful Th2 cell cytokine produced by lymphoid cells. A marked activation of immune system may be observed in patients with AP, being balanced between pro- and anti-inflammatory cytokines in patients with mild but not severe AP. A reduced functional reserve for the synthesis of IL-10 may be observed in patients with severe AP, which might lead to a worst prognosis[27-30].

From the results of this study, it was found that the release of IL-10 was significantly increased in the P group as compared with that of C group at 6 and 12 h. Serum IL-2 level in the P group were higher than those in C group. From 0.5 h, there was a significant difference between P group and C group. The IL-2/IL-10 ratio was significantly increased in the P group as compared with that of C group
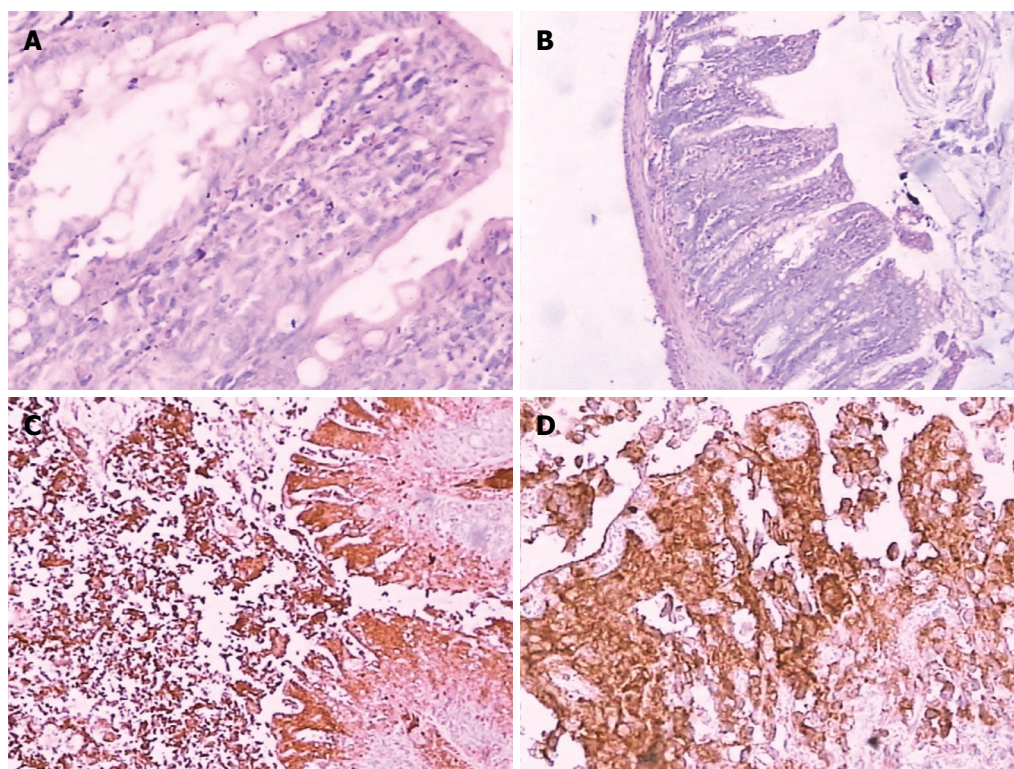
Figure 1 Morphological changes intestinal mucosa after induction of ANP. **A**: Intestinal section with normal mucosa (ANP 0.5 h); **B**: Disintegration of the lamina propria (ANP 6 h); **C**: Fas positive immunohistochemical staining was present in the intestinal mucosa (ANP 0.5 h); **D**: Fas positive immunohistochemical staining was present in the intestinal mucosa (ANP 12 h).

at 0.5 and 2 h, suggesting that a pro-inflammatory response was predominant in these rats and significantly lower in P group at 6 h suggesting that an anti-inflammatory response was predominant. In the sFas assay, there was no significant difference between P group and C group. Interestingly, IL-2, IL-10 and sFas levels were moderate at 0.5 h, peaked at 6 h and decreased at 12 h. In our model, the intestinal tissue injury which was assessed according to the standard scale of pathological examination was closely paralleled by Fas expression and dynamic changes of IL-2/IL-10.

In conclusion, the abnormal apoptosis of Fas can significantly affect the cytokine. Fas were involved in the pathogenesis of intestinal injury in ANP. The mechanisms of Fas may have been related to Fas mediated T helper cell apoptosis.

COMMENTS

Background
Acute pancreatitis (AP) is sudden inflammation of the pancreas that may be mild or life threatening but that usually subsides. It is generally accepted that AP is often complicated by intestinal injury. The Fas system was originally characterized as a key mechanism for inducing apoptosis in immune cells. Apoptosis is a teleologically beneficial form of cell death in AP. However, little is known about how the induction of apoptosis reduces the severity of AP. Recent research has demonstrated that a Th1 to Th2 immune deviation is beneficial to mucosal immunity.

Research frontiers
Recent research has demonstrated that a Th1 to Th2 immune deviation is beneficial to mucosal immunity. The antigen-induced deletion of Th is often accompanied by an imbalance in Th1 and Th2.

Innovations and breakthroughs
In the present study, we performed immunohistochemistry staining of apoptosis-related protein Fas and investigated dynamic changes of serum IL-2, IL-10, IL-2/IL-10 and sFas in rats with ANP.

Applications
To provide the experimental basis by expression of Fas in intestinal mucosa in rats with ANP and provide experimental evidence for the immune treatment of patients with ANP.

Terminology
Th1 and Th2 response: Th1-type cytokines tend to produce the proinflammatory responses responsible for killing intracellular parasites and for perpetuating autoimmune responses. Interferon gamma is the main Th1 cytokine. Excessive proinflammatory responses can lead to uncontrolled tissue damage, so there needs to be a mechanism to counteract this. The Th2-type cytokines include interleukins 4, 5, 13 and interleukin-10, which has more of an anti-inflammatory response.

Peer review
This is a potentially interesting study to understand the dynamic changes of several cytokines and sFas in rats with ANP and provide experimental evidence for the immune treatment of ANP patients.

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