

Effect of IL-4 on altered expression of complement activation regulators in rat pancreatic cells during severe acute pancreatitis

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Abstract

AIM: To investigate the effect of IL-4 on the altered expression of complement activation regulators in pancreas and pancreatic necrosis during experimental severe acute pancreatitis (SAP).

METHODS: SAP model of rats was established by retrograde injection of 5% sodium taurocholate (1 mL/kg) into the pancreatic duct. We immunohistochemically assayed the expression of three complement activation regulators: decay accelerating factor (DAF; CD55), 20 ku homologous restriction factor (HRF20; CD59) and membrane cofactor protein (MCP; CD46), in the pancreatic acinar cells of rats at 0, 3, 6, 12, and 24 h after the induction of SAP model. Meanwhile the levels of amylase and lipase were determined, and morphological examination was performed. Then, 61 rats were randomly divided into three groups. Group A ($n = 21$) received no treatment after the SAP model was established; group B ($n = 20$) was given IL-4 (8 μ g/animal) intraperitoneally 0.5 h before the SAP model was established; group C ($n = 20$) was given IL-4 (8 μ g/animal) intraperitoneally 0.5 h after the SAP model was established. Plasma amylase and lipase, extent of pancreatic necrosis and expression of complement activation regulators were investigated 6 h after the induction of SAP model.

RESULTS: Three complement activation regulators were all expressed in pancreatic acinar cells. MCP was not found on the basolateral surface as reported. Contrary to the gradually increasing plasma level of amylase and lipase, expression of complement activation regulators decreased after SAP model was set up. At the same time, the severity of pancreatic necrosis was enhanced. A strong negative correlation was found between the ex-

pression of MCP, DAF, CD59 in pancreatic acinar cells and the severity of pancreatic necrosis ($r = -0.748, -0.827, -0.723; P < 0.01$). In the second series of experiments, no matter when the treatment of IL-4 was given (before or after the induction of SAP model), the serum level of amylase or lipase was decreased and the extent of pancreatic necrosis was ameliorated significantly. Compared to SAP control group, the expression of DAF and CD59 in pancreas was reinforced when IL-4 was given before the induction of SAP model ($P < 0.01, P < 0.05$), but the expression of MCP was not influenced ($P > 0.05$). The expression of DAF was enhanced, when IL-4 was given after the induction of SAP model ($P < 0.05$), but the expression of CD59 and MCP did not change ($P > 0.05$).

CONCLUSION: Complement activation regulators may participate in the pathogenesis of pancreatic inflammation. Downregulation of complement activation regulators expression may be one of the causes of pancreatic necrosis. IL-4 treatment may control SAP aggravation by enhancing expression of DAF and CD59 in pancreas and decreasing pancreatic necrosis. Moreover, DAF and CD59 may play an important role in the regulation of complement activation regulators during SAP.

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Key words: Severe acute pancreatitis; Complement activation regulators; Interleukin-4

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INTRODUCTION

During activation of complement, fragments of complement proteins may be deposited on non-target cells^[1], thereby causing tissue injury. In order to prevent this, several proteins on cell membranes inhibit the activation of the complement cascade^[2]. These proteins include decay accelerating factor (DAF; CD55) which inhibits the formation of C3 and C5 convertases and promotes their catabolism^[3,4], 20 ku homologous restriction factor (HRF20; CD59) which inhibits the formation

of terminal complement complexes by preventing the polymerization of C9 on C5b-8^[5,6], and membrane cofactor protein (MCP; CD46) which functions as a cofactor in the factor I-mediated inactivation of C3b and C4b^[7]. These proteins, which are widely distributed in normal tissues^[8-10], are thought to protect host tissues from autologous complement-mediated damage.

Expression of these three proteins has been described in the gastrointestinal tract of human beings^[11-14]. In normal colonic and gastric mucosa, DAF and CD59 have been found only on the apical surface of epithelial cells, whereas MCP is localized on the basolateral surface of these cells^[11,13-15]. In patients with ulcerative colitis (UC) and chronic gastritis, the expression of DAF in colonic and gastric epithelial cells was markedly enhanced and was proportional to the severity of mucosal inflammation^[14,15]. Expression of these complement activation regulators has not been assayed in more severe types of inflammation. Being one of the main causes of pancreatic necrosis, activation of complement in the process of SAP has been proved by other authors^[16,17]. We therefore examined the distribution of DAF, CD59, and MCP in pancreatic acinar cells and the relationship between the expression of these proteins and the severity of pancreatic necrosis during SAP. In addition, we investigated the therapeutic effects of IL-4 on the expression of complement activation regulators and pancreatic necrosis in order to clarify the role of IL-4 and three membrane inhibitors of complement during SAP.

MATERIALS AND METHODS

Animals and model of severe acute pancreatitis

Male Wistar rats (250-300 g) were used in this study. Animals were allowed to have free access to water before the experiment. Anesthesia was induced with an intraperitoneal injection of 6% sodium pentobarbital (Northeast Drug Manufactory, China) (0.1 mL/100 g body weight). After midline laparotomy and transduodenal cannulation of the pancreatic duct, the hepatic duct was closed by a small bulldog clamp, and then 5% sodium taurocholate (Sigma, USA) in saline was infused at a rate of 0.1 mL/min. Control animals received an infusion of 0.9% saline solution.

Experimental design

In the first series of experiments, animals were killed at 3, 6, 12, and 24 h after the induction of pancreatitis ($n = 10$ per group). Pancreatic tissues were removed and processed as indicated below.

In the second series of experiments, to test the effect of IL-4 on complement activation regulators, 61 rats were randomly divided into three groups. Group A ($n = 21$) received no treatment after the SAP model was established, group B ($n = 20$) was given IL-4 (Peprotech, UK) (8 μ g/animal)^[18-20] intraperitoneally 0.5 h before the SAP model was established, group C ($n = 20$) was given IL-4 (8 μ g/animal) intraperitoneally 0.5 h after the SAP model was established. Animals were killed at 6 h after the

induction of pancreatitis model.

Pancreatic tissue was immediately frozen in liquid N₂ and embedded in OCT embedding medium. All blood samples were centrifuged at 3 000 r/min for 5 min. Supernatant was stored at -70 °C.

The serum levels of amylase and lipase were determined by a colorimetric kinetic method and nephelometry method respectively.

Tissue immunohistochemistry staining

Cryostat sections were serially cut at 6 μ m, and fixed in acetone for 10 min. After being washed with phosphate-buffered saline (PBS), the sections were treated with 30 mL/L H₂O₂ for 15 min at room temperature to inactivate endogenous peroxidase, and then with a 1:10 dilution of non-immune goat serum for 20 min to block non-specific immunoglobulin binding sites. After the excess serum was blotted, the sections were incubated with each of the primary antibodies (dilution 1:150 in PBS) [MCP (sc-7056), DAF (sc-9156), CD59 (sc-9157), Santa Cruz, USA] for 2 h at room temperature, control sections were incubated with PBS. Unbound antibody was washed from the tissue thrice in PBS for 5 min, incubated with the secondary biotin-labeled goat anti-mouse IgG antibody for 20 min, and then washed thrice for 5 min with PBS. Subsequently, the sections were incubated with peroxidase-labeled streptavidin-biotin for 20 min (ABC kit; Boster, Wuhan, China). After being washed with PBS for four times, the sections were stained with 0.02% (w/v) 3,3'-diaminobenzidine tetrahydrochloride (Boster, Wuhan, China). The sections were counterstained with hematoxylin, washed with PBS, dehydrated in graded concentrations of ethanol, and mounted.

Vascular endothelial cells were used as a positive control for DAF, MCP, and CD59. Expression of complement activation regulators in pancreatic acinar cells was scored on a scale of (-) to (+3)^[15], where (-) denotes faint or no staining; (1+) specific staining in 10-50% of cells; (2+) specific staining of 50-90% of the cells; (3+) specific staining of 90-100% of cells.

Evaluation of pancreatitis severity

The extent of pancreatic acinar cell necrosis was quantitated morphometrically by an observer who was not aware of the sample identity, as described by Kusske *et al.*^[21]. For these studies, cryostat sections were stained with hematoxylin and eosin. Ten randomly chosen microscopic fields ($\times 200$) were examined for each tissue sample, and the extent of acinar cell injury/necrosis was expressed as a percentage of total acinar tissue.

Statistical analysis

Data were presented as mean \pm SE. Differences between groups were compared using analysis of variance followed by Student's *t*-test. Correlation between the expression of complement activation regulators and pancreatic necrosis was analyzed using Spearman's rank correlation test. Correlation between IL-4 intervention and the expression of complement membrane inhibitors was analyzed using

Table 1 Serum amylase and lipase activities (u/L) and histological scores of pancreatic necrosis in the first series of experiment (mean±SE)

| | Control group | Pancreatitis group | | | |
|-------------------------------|---------------|-------------------------|-------------------------|--------------------------|--------------------------|
| | | 3 h | 6 h | 12 h | 24 h |
| Serum amylase | 1360±259 | 4715±1 120 ^b | 7527±1 595 ^b | 13321±3 848 ^b | 13686±4 175 ^b |
| Serum lipase | 2.52±0.35 | 11.09±2.93 ^b | 31.17±5.66 ^b | 40.39±9.04 ^b | 38.06±9.67 ^b |
| Scores of pancreatic necrosis | 0.00±0.00 | 0.30±0.48 ^b | 1.40±0.70 ^b | 2.70±0.48 ^b | 2.80±0.79 ^b |

^bP<0.01 vs control.

Table 3 Serum amylase and lipase activities (u/L) and histological scores of pancreatic necrosis in the second series of experiment (mean±SE)

| Group | n | Serum amylase | Serum lipase | Scores of pancreatic necrosis |
|-------|----|--------------------------|-------------------------|-------------------------------|
| A | 21 | 7 393±1 433 | 32.15±5.42 | 1.52±0.75 |
| B | 20 | 4 482±1 224 ^a | 11.75±4.42 ^a | 0.45±0.69 ^a |
| C | 20 | 5 947±1 503 ^a | 22.28±7.10 ^a | 0.95±0.76 ^a |

^aP<0.05 vs group A.

the χ^2 -test. P<0.05 was considered statistically significant.

RESULTS

Serum levels of amylase and lipase

Tables 1 and 3 summarize the changes of serum amylase and lipase activities in experiments 1 and 2.

Evaluation of morphology

In the first series of experiments, the morphological changes varied with the time elapsed after sodium taurocholate injection. By gross examination, the ductal tree became hemorrhagic immediately after injection, and the whole pancreas became edematous and brownish red in color within the next 5-10 min. As time elapsed, edema, hemorrhages, acinar cell necrosis (Table 1), amount of ascitic fluid and inflammatory cell infiltration aggravated gradually. At 24 h, the color of pancreas was pale and slightly yellowish. Many animals had marked distension of the stomach.

In the second series of experiments, pancreatic necrosis was significantly reduced, when IL-4 was administered either prophylactically or therapeutically (P<0.01), (Table 3).

Expression of complement activation regulators

At the microscopic level, we observed the expression of DAF, CD59, and MCP on the luminal surface of pancreatic acinar cells. We did not find positive staining of MCP on the basolateral surface of pancreatic acinar cells, though staining for MCP was positive on the basolateral surface of gastric and colonic epithelial cells^[14,15]. Although

Table 2 Correlation between expression of DAF, CD59, MCP, and pancreatic necrosis

| | | Histological scores of pancreatic necrosis | | | | |
|------|-----|--|---|---|----|---|
| | | 0 | 1 | 2 | 3 | 4 |
| MCP | - | 1 | 5 | 6 | 12 | 2 |
| | + | 8 | 3 | 3 | 0 | 0 |
| | ++ | 6 | 2 | 0 | 0 | 0 |
| DAF | +++ | 2 | 0 | 0 | 0 | 0 |
| | - | 0 | 6 | 7 | 12 | 2 |
| | + | 6 | 4 | 2 | 0 | 0 |
| | ++ | 10 | 0 | 0 | 0 | 0 |
| | +++ | 1 | 0 | 0 | 0 | 0 |
| CD59 | - | 2 | 6 | 6 | 12 | 2 |
| | + | 5 | 3 | 3 | 0 | 0 |
| | ++ | 7 | 1 | 0 | 0 | 0 |
| | +++ | 3 | 0 | 0 | 0 | 0 |

Table 4 Effects of IL-4 on expression of MCP, DAF, and CD59 in pancreas during SAP

| Group | n | Expression intensity of DAF | | | Expression intensity of CD59 | | | Expression intensity of MCP | | |
|-------|----|-----------------------------|----|-----------------|------------------------------|---|-----------------|-----------------------------|---|----------------|
| | | - | + | ++ | - | + | ++ | - | + | ++ |
| | | A | 21 | 12 | 7 | 2 | 12 | 6 | 3 | 11 |
| B | 20 | 2 | 7 | 11 ^b | 3 | 7 | 10 ^a | 5 | 8 | 7 ^c |
| C | 20 | 4 | 8 | 8 ^a | 9 | 6 | 5 ^c | 7 | 7 | 6 ^c |

^aP<0.05, ^bP<0.01, ^cP>0.05 vs group A.

DAF, CD59, and MCP were well recognized as membrane binding proteins, immunostaining of these proteins was also detected in the cytoplasm^[15]. No staining was observed when samples were treated with control PBS. Expression of complement activation regulators decreased gradually after SAP model was set up. We also examined the relationship between the expression of three complement inhibitors and the pancreatic necrosis. We found a strong negative correlation between the expression of MCP, DAF, and CD59 in pancreatic acinar cells and the severity of pancreatic necrosis ($r = -0.748, -0.827, \text{ and } -0.723; P<0.01$), (Table 2). In the second series of experiments, compared to SAP control group, the expression of DAF and CD59 in pancreas was reinforced when IL-4 was given before the induction of SAP model (P<0.01, P<0.05), but the expression of MCP was not influenced (P>0.05). The expression of DAF was enhanced when IL-4 was given after the induction of SAP model (P<0.05), but the expression of CD59 and MCP did not change (P>0.05) (Table 4).

DISCUSSION

Severe acute pancreatitis is a common acute abdominal disorder. Since angiorrhhexis and hemorrhage in pancreas are frequent during severe acute pancreatitis, and the complement system is activated by trypsin^[16,17], it is possible that inflammation may result in constant exposure of pancreatic acinar cells to activated complement

fragments in the blood. Then, severe pancreatic necrosis is inevitable. Being the membrane inhibitors, complement activation regulators in the acinar cells may be important for local defense system during SAP. We investigated the role of RCA in the process of SAP and then attempted to attenuate inflammatory reaction by regulating the expression of complement activation regulators.

It has been reported that expression of DAF on gastric epithelial cells and colonic epithelial cells is strongly enhanced during gastritis and ulcerative colitis, and a strong positive correlation between DAF expression and degree of inflammation (extent of inflammatory cell infiltration) has been found^[14,15]. At the same time, other researches revealed that expression of MCP and CD59 in alveolar epithelial cells is conspicuously enhanced during acute lung injury, but is greatly decreased during acute respiratory distress syndrome^[22]. These results suggest that complement activation regulators play a different role in these inflammatory diseases.

We utilized immunohistochemical methods to assay the expression of DAF, CD59, and MCP in normal and pathological pancreatic specimens. We found that MCP, DAF, and CD59, which are glycosyl phosphatidylinositol (GPI) anchored membrane proteins, were present on the apical side of pancreatic acinar cells^[23], whereas MCP, a transmembrane protein, was not distributed in these cells as in epithelial cells of the colonic and gastric mucosa^[14,15]. While complement activation regulators were slightly or moderately expressed in the acinar cells of normal pancreatic tissue, their expression in pancreatic acinar cells decreased gradually after the model of SAP was induced. These phenomena are similar to those in ARDS^[22]. Although there are differences in the inflammatory process between pancreatic and pulmonary epithelial cells, the lower expression of complement activation regulators detected in the inflammatory cells suggests that complement activation regulators play an important role in the regulation of complement activation in epithelia during severe inflammation. In this study, a strong negative correlation was observed between the expression of complement activation regulators and inflammation (pancreatic necrosis). It is not clear, which of MCP, DAF, and CD59 is the most important factor in the regulation of complement activation during SAP. Although the precise mechanism by which the repression of complement activation regulators is regulated remains to be elucidated, it is clear that these proteins are important in the regulation of the inflammatory process of the pancreas.

In general, the expression of membrane inhibitors of complement is mediated by various cytokines^[24-27]. Being an anti-inflammatory mediator, IL-4 has been proven to enhance the expression of DAF in small intestine epithelial cells and vascular endothelial cells *in vitro*^[24-28]. We applied IL-4 to rats with severe acute pancreatitis to testify its therapeutic effects on the expression of complement activation regulators and pancreatic necrosis *in vivo*. We found that the expression of DAF and CD59 was upregulated when IL-4 was administered prophylactically (given before the model was established),

but the expression of MCP did not change significantly. When IL-4 was administered therapeutically (given after the model was established), only DAF was upregulated. Compared to control group, the serum amylase and lipase activities and the extent of pancreatic necrosis were significantly reduced when IL-4 was administered either prophylactically or therapeutically. These findings suggest that activation of complement during severe acute pancreatitis may be mediated primarily by DAF and CD59, and IL-4 treatment may be a valuable strategy for severe acute pancreatitis. Considering the therapeutic value of IL-4, some other biological activities of IL-4 should be mentioned. It has been reported that IL-4 displays its anti-inflammatory feature by inhibiting the activation and accumulation of macrophages^[29], preventing the production of TNF- α and IL-1 β ^[30], upregulating the expression of IL-1 receptor antagonist^[31], stimulating 15-lipoxygenase activity of macrophages^[32]. These functions are important to control systemic inflammation reaction that is common during SAP.

In conclusion, complement activation regulators may play an important role in the regulation of complement activation, and downregulation of complement activation regulators may be one of the causes of pancreatic necrosis. IL-4 treatment provides a new therapeutic strategy for controlling systemic inflammatory reaction during severe acute pancreatitis.

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