8

Role of Peritoneal Macrophages on Local and Systemic Inflammatory Response in Acute Pancreatitis

Marcel Cerqueira Cesar Machado and Ana Maria Mendonça Coelho
University of São Paulo,
Brazil

1. Introduction

Severe acute pancreatitis is a serious disease with high morbidity and mortality.[1,2] Despite many experimental and clinical studies its pathophysiology is yet not completely understood. It is well accepted that activation of enzymes within the pancreatic tissue is the initial event in acute pancreatitis followed by a cascade of events that modify not only the local process but also affect distant organs and systems.[3] Indeed, previous studies have demonstrated that acute pancreatitis is associated with an increase in inflammatory mediators that induce the systemic inflammatory response syndrome (SIRS) and are associated with distant organ dysfunction being responsible for the morbidity and mortality related to disease.[4,5] Acute pancreatitis is characterized by release of proteolytic enzymes from the pancreas and activation of several signaling pathway in macrophages resulting in the releasing of TNF-α.[6,7]

2. Macrophages

Macrophages are released from bone marrow migrate to many tissues and undergo to final differentiation into specific type of resident macrophages. Macrophages are concentrated in lungs, spleen, liver (Kupffer cells), lymphnodes, and in the serosal membrane of pleural and peritoneal cavity. Activation of macrophages by different insults may result in the production of many substances that interfere in the immune response. Inflammation lymphocytes activation and secretion of various cytokines are some of the many function of macrophages.

The degree of macrophages activation seems to be an important factor determining the severity of acute pancreatitis.[8-10] In the pancreatic tissue, besides enzymatic activation, and acinar cells production of TNF-α, macrophages and monocytes are the main inflammatory cells involved in the pathogenesis of local and systemic inflammation. The production of proinflammatory substances by these cells results in amplification of the inflammation to distant organs as liver, lungs kidneys intestines and might result in multi organs failure.[11-13]

Following pancreatic inflammation several organs like liver, spleen and lungs with a large population of macrophages function as important source of cytokines production further amplifying the inflammatory response.[14] In spite the production of cytokines macrophages also induce the activation of enzymes as inducible nitric oxide synthase (iNOS) or

cyclooxygenase 2 (COX-2) involved in the production of nitric oxide and arachdonic acid.[15] The severity of acute pancreatitis seems to be related to the levels of proinflammatory cytokines as IL-1, IL-6, IL-8, and TNF-α, PAF, and C-reactive protein.[3,16] Administration of agents that antagonize or diminish the production of TNF-α is associated with decreasing of the systemic effects of acute pancreatitis.[17,18]

In a previous study we have showed that treatment of rats with acute pancreatitis by administration of hypertonic saline solution reduces the production of inflammation markers (inducible nitric oxide synthase, cyclooxygenase 2, TNF-α, and IL-6) in pancreatic tissue. The volume of ascitic fluid and level of cytokines in ascitic fluid were also reduced, decreasing the systemic inflammatory response in experimental acute pancreatitis.[19] In this study pancreatic enzymes activation was not reduced in animals receiving hypertonic saline, therefore ascitic fluid volume and TNF-α reduction were probably caused by decrease of peritoneal macrophages proinflammatory response.[20,21] Administration of hypertonic saline solution reduces the production of cytokines by these cells and reduces the systemic inflammatory response in experimental acute pancreatitis.[19]

	Pancreatic TNF-α	Pancreatic IL-6	Pancreatic IL-10	Pancreatic IL-10/TNF- α ratio
Control (without AP)	12,6± 0,9	9,3 ±1,9	2,7 ± 0,27 *	0.22
AP Non-treated	45,0 ± 6,9 *	44,1 ± 5,6 *	14,6 ± 2,1	0,32
AP hypertonic saline-treated	23,1 ± 4,2 #	18,7 ± 1,2 #	16,4 ± 2,8	0,88*

Pancreatic levels of TNF- α, IL-6; and IL-10 were measured in homogenates of pancreas (pg/mg protein). Date are expressed of mean ± SEM *, #p<0.05

Table 1. Effect of hypertonic saline solution on pancreatic inflammation in acute pancreatitis (AP).

	COX-2	iNOS
Control (without AP)	100 ± 0	100± 0
AP Non-treated	329 ± 16 *	505 ± 105 *
AP hypertonic saline-treated	206 ± 38 #	213 ± 48#

Date are expressed of mean ± SEM *, #p<0.05

Table 2. Effect of hypertonic saline solution on the expression of COX-2 and iNOS (Western blot analysis) in the pancreas in acute pancreatitis (AP)

	Ascitic Fluid Volume (ml)	Ascitic Fluid TNF-α (pg/ml)
Control (without AP)	0	0
AP Non-treated	1.8 ± 0.3*	192 ± 23 *
AP hypertonic saline-treated	0.8 ± 0.2 #	64 ± 10#

Date are expressed of mean ± SEM *, #p<0.05

Table 3. Effect of hypertonic saline solution on peritoneal inflammation in acute pancreatitis (AP).

In the early stage of inflammatory response there is activation of macrophages (M1 macrophages) that release proinflammatory cytokines as TNF-α IL-1β and IL-6.[15] Besides M1 macrophages another population of macrophages was observed (M2a) called alternative related to wound healing. This population of macrophages is unable to produce NO and to present antigen to T cells. This population of macrophages up regulates mannose receptor expression and arginase II [22,23] and seems to act in the production of extracellular matrix. There is yet another population of macrophages (M2b) found in the later stages of inflammation which are related to the production of IL-10 and TGF beta with inhibition of the production of proinflammatory mediators.[24]

2.1 Peritoneal macrophages

Peritoneal macrophages in spite to be a small fraction of the total population seem to performing an important role in the defense against infection in the peritoneal cavity and in the severity of acute pancreatitis.

Previous study demonstrated that trypsin stimulates the production of cytokines from peritoneal macrophages in vitro and in vivo.[25] In acute pancreatitis there is activation and leakage of enzymes from the intracellular compartment to pancreatic interstitium, peripancreatic tissues and peritoneal cavity[26], therefore activating peritoneal macrophages and increasing the production of TNF-α[25] Peritoneal macrophages from animals with acute pancreatitis are more efficient in the production of TNF-α than controls when challenged with LPS.[27] Indeed some investigators in 1970s proposed that most of the mediators of inflammatory process in acute pancreatitis could be found in the peritoneal cavity and should be removed in order to attenuate the systemic inflammatory syndrome associated with acute pancreatitis.[28] Endotoxin can also increase the production of TNF-α by peritoneal macrophages that makes infected pancreatic necrosis extremely dangerous. Many investigators demonstrated that pancreatitis associated ascitic fluid has noxious effects on mitochondria[29], kidney and lungs besides an apoptosis-inducing-factor.[30] The key point in these studies is to understand the importance of peritoneal macrophages on the systemic inflammatory response in acute pancreatitis.[31,32] Peritoneal macrophages represent a small fraction of the total macrophage population however they are strategically located lining the serosal peritoneal membrane. Activated peritoneal macrophages have many activities such

as lymphocyte activation, tissue damage and microbiocidal activity through the production of several cytokines as TNF-α, IL-1, IL-8 TGF-β1, superoxide and nitric oxide.[30]
Overproduction of these substances however, may be dangerous inducing systemic inflammatory response. Reducing the production or removal of these substances may have beneficial effects on the inflammatory response in acute pancreatitis. Indeed, previous study has shown the beneficial effect of peritoneal lavage in acute pancreatitis. [28]
Recently, we have demonstrated that peritoneal lavage in an experimental model of acute pancreatitis not only leads to a decrease in serum levels of TNF-α and IL-6 but also results in an increase in serum levels of IL-10.[33] (Table 4). We have also demonstrated in the pancreatic tissue a reduction in cycloxygenase-2 and inducible nitric oxide synthase expression.[33] (Table 5)

	Serum TNF-α (pg/ml)	Serum IL-6 (pg/ml)	Serum IL-10 (pg/ml)
AP Non-treated	32 ± 10	258 ± 46	39 ± 8
AP Peritoneal lavage-treated	7 ± 5*	141 ± 26*	149 ± 46*

Date are expressed of mean ± SEM * p<0.05

Table 4. Effect of peritoneal lavage on systemic inflammation in acute pancreatitis (AP).

	COX-2	iNOS
Sham	100 ± 0	100± 0
AP Non-treated	454 ± 38 *	609 ± 104 *
AP Peritoneal lavage-treated	304 ± 25 #	410 ± 63 #

Data are expressed of mean ± SEM *,# p<0.05

Table 5. Effect of peritoneal lavage on the expression of COX-2 and iNOS (Western blot analysis) in the pancreas in acute pancreatitis (AP).

We concluded that peritoneal lavage has an anti-inflammatory effect in acute pancreatitis. It is possible that a special subset of peritoneal macrophages with anti-inflammatory properties is preserved or activated during peritoneal lavage.[24]
It has been demonstrated that CO_2 pneumoperitoneum decreases TNF-α and interleukin IL-6 production while increasing the production of IL-10.[34]
Indeed peritoneal macrophages exposed to CO2 in vitro have a significant decrease of TNF-α production when stimulated with LPS.[35] CO2 pneumoperitoneum pretreatment alters acute-phase response[36] and increases survival in an experimental model of lipopolysaccharide (LPS)-contaminated laparotomy.[37]

It has been suggested that the local tissue acidification due to CO2 pneumoperitoneum decreases the production of cytokines by peritoneal macrophages.[36] Recently, we have demonstrated that CO_2 abdominal insufflation decreases pancreatic inflammation and systemic inflammatory response in an acute pancreatitis model.[38] (Tables 6-8).

	Ascitic Fluid (ml)	TNF-α (pg/ml ascitic fluid)	Peritoneal cells (x10^6)
AP Non-treated	4.2 ± 0.4	534 ± 129	21 ± 2
AP CO_2-treated	2.4 ± 0.4*	188 ± 47*	14 ± 2*

Date are expressed of mean ± SEM * p<0.05

Table 6. Effect of CO_2 insufflation on the peritoneal inflammation induced by acute pancreatitis (AP).

	Serum TNF-α (pg/ml)	Serum IL-6 (pg/ml)	Serum IL-10 (pg/ml)	Serum IL-10/TNF- α ratio
AP Non-treated	228 ±92	92 ±19	40 ±5	0.18
AP CO2-treated	34 ±16*	42 ±12*	37 ± 9	1.09*

Date are expressed of mean ± SEM * p<0.05

Table 7. Effect of CO_2 insufflation on the systemic inflammation induced by acute pancreatitis (AP).

	COX-2	iNOS
Sham	100 ± 0	100± 0
AP Non-treated	545 ±159 *	446 ±85*
AP CO_2-treated	198 ±31	132 ±5

Data are expressed of mean ± SEM * p<0.05

Table 8. Effect of CO_2 insufflation on the expression of COX-2 and iNOS (Western blot analysis) in the pancreas in acute pancreatitis (AP).

In this study we also demonstrated that CO_2 abdominal insufflation not only reduces the serum levels of proinflammatory cytokines but did not changed the serum levels of IL-10. The ratio of IL-10 over TNF-α demonstrates a clear anti inflammatory effect of CO_2 pneumoperitoneum.[38] The CO_2 pneumoperitoneum also decreases the inflammation in the peritoneal cavity reducing the volume of ascitic fluid and the total content of TNF- α.[38] It is conceivable that if an endoscopic procedure for stone retrieval has to be performed it should be done during the laparoscopic cholecystectomy to decrease the inflammatory response secondary to acute pancreatitis. These results suggested that peritoneal macrophages play an important role on the outcome of acute pancreatitis and should be considered an important target for therapeutic management in acute pancreatitis. However, peritoneal macrophages are also important in the defense against infection in the peritoneal cavity. In a large experience in the surgical treatment of acute pancreatitis we have observed that even draining pancreatic abscess through the abdominal cavity we rarely observed bacterial peritonitis. It seems that peritoneal macrophages priming by pancreatic enzymes are more effective to protect peritoneal cavity from bacterial infection.[27]

3. Conclusion

In spite to be a small fraction of body population peritoneal macrophages have an important role in the pathophysiology of acute pancreatitis and should be object of future clinical trials and probably a target for the modulation of systemic inflammatory response in acute severe pancreatitis.

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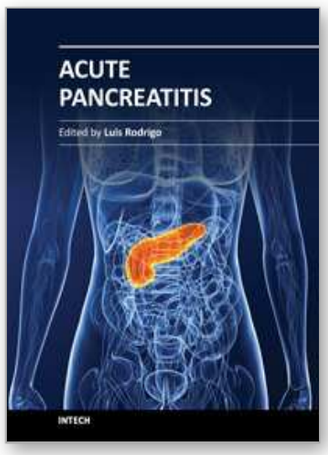

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Acute Pancreatitis (AP) in approximately 80% of cases, occurs as a secondary complication related to gallstone disease and alcohol misuse. However there are several other different causes that produce it such as metabolism, genetics, autoimmunity, post-ERCP, and trauma for example... This disease is commonly associated with the sudden onset of upper abdominal pain that is usually severe enough to warrant the patient seeking urgent medical attention. Overall, 10-25% of AP episodes are classified as severe. This leads to an associated mortality rate of 7-30% that has not changed in recent years. Treatment is conservative and generally performed by experienced teams often in ICUs. Although most cases of acute pancreatitis are uncomplicated and resolve spontaneously, the presence of complications has a significant prognostic importance. Necrosis, hemorrhage, and infection convey up to 25%, 50%, and 80% mortality, respectively. Other complications such as pseudocyst formation, pseudo-aneurysm formation, or venous thrombosis, increase morbidity and mortality to a lesser degree. The presence of pancreatic infection must be avoided.

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