

## Acute pancreatitis as a model of SIRS

Madhav Bhatia

Department of Pharmacology, National University of Singapore, Yong Loo Lin School of Medicine, Centre for life Sciences, 28 Medical Drive, Singapore 117456

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## 1. ABSTRACT

Acute pancreatitis is a common clinical condition. Excessive systemic inflammatory response syndrome (SIRS) in acute pancreatitis leads to distant organ damage and multiple organ dysfunction syndrome (MODS), which is the primary cause of morbidity and mortality in this condition. Development of *in vivo* experimental models of acute pancreatitis and associated systemic organ damage has enabled us to study the role played by inflammatory mediators in the pathogenesis of acute pancreatitis and associated systemic organ damage. Using these models, recent studies by us and other investigators have established the critical role played by inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, PAF, IL-10, CD40L, C5a, ICAM-1, chemokines, substance P and hydrogen sulfide in acute pancreatitis and the resultant MODS. This chapter intends to present an overview of different experimental animal models of acute pancreatitis and associated MODS and the role of inflammatory mediators in the pathogenesis of this condition.

## 2. INTRODUCTION

Acute pancreatitis (AP) is a common clinical condition with potentially devastating consequences. It is one of the most frequent causes of acute inflammatory states in the abdomen. About 40 cases of AP per 100,000 adults are reported every year. At present, there is no treatment against severe acute pancreatitis, other than supportive critical care (2). The main etiological factors of AP are biliary disease leading to ductal obstruction and excessive alcohol consumption. Other minor factors include hyperlipidemia, viral infection, drugs and hypercalcemia (3). The incidence of the disease differs geographically; however, the death rate of this disease has remained high at 8-13 % over the past 20 years. The severity of AP can vary from mild to severe in different cases. Majority of the patients (80 %) suffer mild pancreatitis, which is self-limiting and recover in a few days. The other 20 % may require intensive care treatment for haemorrhagic and necrotic lesions of the pancreas with a mortality rate of 40 %. High incidence of death is due to

**Table 1.** Comparisons of animal models of AP and associated SIRS

Model	Advantages	Disadvantages
Secretagogue-Induced Pancreatitis	Noninvasive Rapid induction Ease of controlling the degree of severity Reproducible Applicable to different animals Possibility of mechanistic <i>in vitro</i> studies	Lack of clinical relevance (secretagogue-induced AP is not known in the clinic)
Choline-Deficient Ethionine Supplement Diet-Induced Pancreatitis	Noninvasive Convenient Very severe with high mortality rate (severity can be controlled by limiting the days mice are fed on the diet)	Nonspecific toxic effect to pancreas Restricted to female juvenile mice
Duct Obstruction-Induced Pancreatitis	Clinical relevance: similar to gallstone-induced AP	Invasive, surgical model Availability of American opossum a limiting factor
Duct Infusion-Induced Pancreatitis	Flexibility of studying the etiology of AP and control of severity Similarity to clinical AP in some models Applicable in many species	Involves hydrostatic pressure, which itself may induce inflammation/edema in the pancreas Invasive, surgical model
Arginine-Induced Pancreatitis	Noninvasive Rapid induction Easily control the degree of severity Toxic effect selectively in pancreas	Lack of clinical relevance

the systemic inflammatory response syndrome (SIRS) leading to multiple organ failure (1-5).

Development of *in vivo* experimental models of acute pancreatitis and associated systemic organ damage has enabled us to study the role played by inflammatory mediators in the pathogenesis of acute pancreatitis and associated systemic organ damage. Furthermore, specific therapeutic treatment for AP and its systemic complications has yet to be developed. As randomized studies of AP in the clinical setting have their limitations, *in vivo* animal studies are of particular importance. Moreover, targeted treatments need to be tested carefully before going to clinical trials to ensure that there are no harmful side effects. In this regard, reliable AP animal models are of paramount importance. An ideal AP animal model should reproduce the disease in terms of etiology, symptomatology, effectiveness in treatment and pathophysiology. Thus, the AP animal models developed ought to mimic the clinical AP such as increased serum pancreatic enzymes, histological changes, and pancreatitis-associated complications. To that end, a variety of well-developed AP animal models have been used by us and other investigators. The methods of induction can be non-invasive or invasive, ranging from simple diet to exogenous chemical administration or even surgical manipulation-induced pancreatitis. Some of the models are simple and repeatable, whereas the others are relatively complex. Some focus on etiology, while others are designed to be clinically relevant (2, 6). In this chapter, the different animal models of AP are discussed and compared (Table 1).

An important characteristic feature of AP is the pancreatic inflammation with excessive recruitment of leukocytes. Inflammatory mediators appear to play a critical role in pancreatitis and more so in the subsequent inflammatory response (1-5). Animal models of AP and associated SIRS have enabled us to investigate the role of inflammatory mediators in this condition. Inflammatory mediators believed to participate in the pathophysiology of this condition include: tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), platelet

activating factor (PAF), intercellular adhesion molecule-1 (ICAM-1), interleukin 10 (IL-10), CD40L, complement component C5a, chemokines, substance P and hydrogen sulfide (H<sub>2</sub>S) (1-5).

## 3. ANIMAL MODELS OF ACUTE PANCREATITIS AND ASSOCIATED SIRS

### 3.1. Secretagogue-induced pancreatitis

Physiological concentrations of secretagogues trigger normal secretion from the pancreas. When excess of an exogenous secretagogue is given to the animals, the excess stimulation leads to abnormally high digestive enzyme secretion, resulting in AP. The cholecystokinin (CCK) analogue caerulein has been used to induce AP in rodents (7, 8). Rapid induction, noninvasiveness, high repeatability, high applicability, and AP-like pancreatic histological changes have made caerulein the most favored AP animal model. As such, the pathophysiology of caerulein-induced pancreatitis has been evaluated extensively. Caerulein, acting through the CCK receptors, yields exaggerated stimulation of acinar cells, which leads to prematuration of trypsinogen. Caerulein, like other secretagogues, can be administered via intraperitoneal injection (50  $\mu$ g/kg body weight) or intravenous infusion (5  $\mu$ g/kg body weight). Variation in the amount of caerulein, by adjusting the number of injections, results in differences in degree of severity. We have shown earlier that when multiple injections of caerulein are given, quite severe AP can be achieved in mice and rats as evidenced by AP-associated pulmonary injury (8, 9). This model of AP is particularly useful because AP-associated pulmonary injury is a common complication, contributing significantly to the high mortality rate of AP. Another advantage of the secretagogue-induced model is the availability of parallel *in vitro* approach, using isolated pancreatic acini. Unlike other animal models of AP, secretagogues can be administered to isolated acini or exocrine cells *in vitro* to mimic hyperstimulation-induced pancreatitis

### 3.2. Choline-deficient ethionine supplement diet-induced pancreatitis

In 1975, Lombardi *et al* reported that female juvenile mice developed acute hemorrhagic pancreatitis with massive fat necrosis when fed a choline-deficient diet

supplemented with 0.5% dl-ethionine [(the ethyl analog of methionine) (CDE diet)] (10). The mortality rate was 100 % after 4 days on the regimen and the animals consistently developed fat necrosis in the abdominal cavity. The precise mechanism of CDE diet-induced pancreatitis remains unclear; it has been hypothesized that ethionine may be toxic to the pancreas by interfering with RNA protein, and phospholipid metabolism (10). Choline deficiency may act synergistically with ethionine, increasing the severity of the condition.

CDE diet-induced pancreatitis is the most non-invasive method of all of the experimental models of AP. It requires no injections or surgical manipulation; thus, exerts minimal exogenous shock. Moreover, it takes a rather long time, approximately 2 to 3 days, to produce pancreatitis. The slow progression of pancreatitis by CDE diet makes it suitable to observe the propagation of the disease. The limitation of this model, however, is that it is difficult to control the amount of the diet each animal consumes, and therefore variation between animals can be high, making the use of a large number of animals in each group necessary so as to get meaningful results. Another limitation of this model is that it is restricted to young female mice. Also, such a small animal can sometimes be difficult to manipulate, especially surgically or in therapeutic interventions involving intravenous administration. In our studies, we were able to establish evidence of lung injury associated with CDE diet-induced acute pancreatitis as well (11), which enabled us to investigate the role of neutrophils in AP and associated SIRS.

### 3.3. Duct obstruction-induced pancreatitis

The duct obstruction model mimics gallstone obstruction-induced AP in the clinical setting. The surgical manipulation is simple, requiring either ligation of the common biliopancreatic duct (CBPD) or obstruction of the pancreatic duct by vertical cannulation or insertion of a balloon-tipped catheter (12). It is postulated that bile reflux, triggering intrapancreatic digestive enzyme activation, accounts for the major pathological factor of this model. This model is most effective in the American opossum, leading to severe necrotizing AP.

The duct obstruction model has high clinical relevance in that it simulates gallstone obstruction-induced AP. This major advantage has made this model a favorite for investigating the pathophysiology, as well as the therapeutic treatment of gallstone obstruction-induced pancreatitis. In our studies, we were able to establish evidence of lung injury associated with acute pancreatitis induced by biliopancreatic duct ligation in the opossum (13).

### 3.4. Duct infusion-induced pancreatitis

Cannulation of the pancreatic duct provides another way of inducing an experimental model of AP. Once the cannula has been implanted, an exogenous substance can be infused into the pancreas via the pancreatic ductal system. Duct perfusion-induced pancreatitis is a well-established, reliable and highly

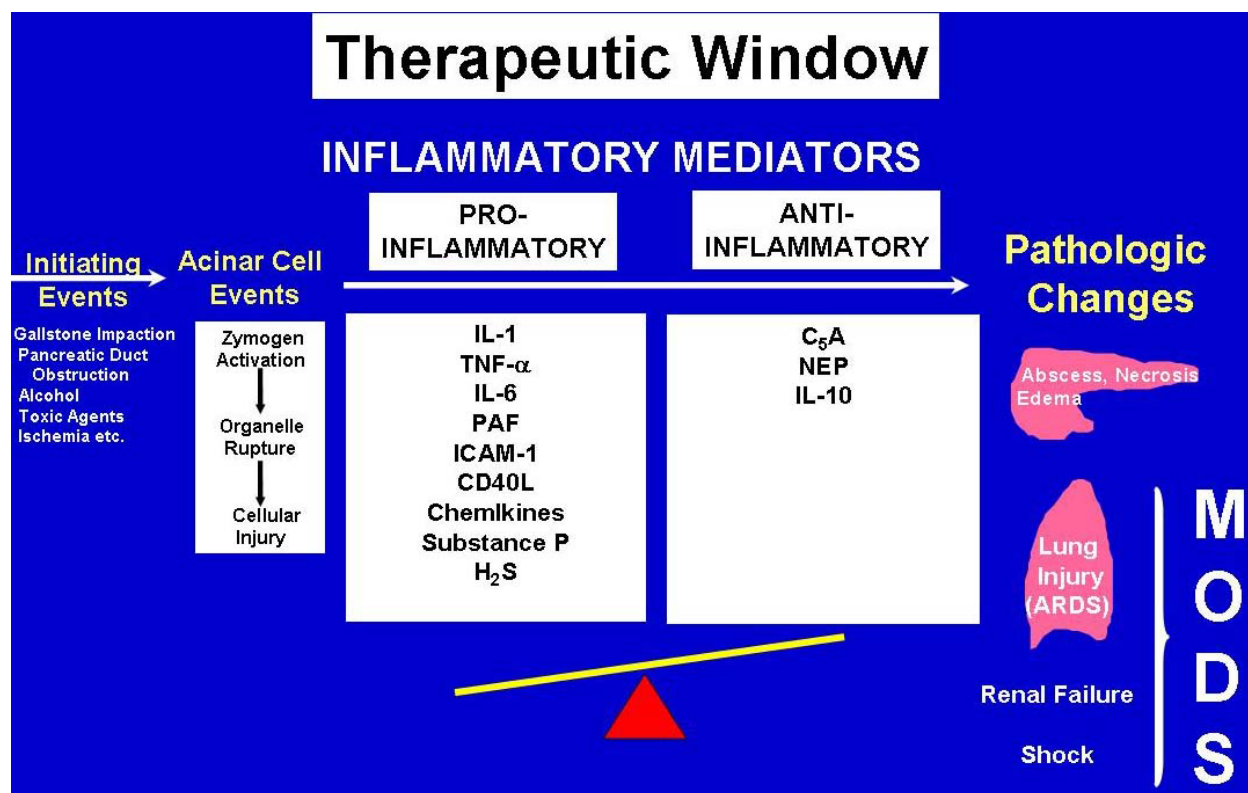
repeatable model of AP with clinical relevance. It requires cannulation of the pancreatic duct (usually from the duodenum wall via the sphincter of Oddi), temporary closure of the biliary duct near the liver hilum, and slow retrograde infusion of a foreign solution into the pancreatic duct. The infusion rate is controlled by a pump that keeps the pressure from becoming too high throughout the experiment. Several substances have been used as inducers of AP, the most common being bile acids [taurocholate or glycodeoxycholic acid (GDOC)]. A distinct advantage of the duct perfusion model is the flexibility in studying different etiologic factors by simply varying the concentration of the substance infused into the pancreatic duct. A biliary edematous model of AP can be established by the infusion of a low concentration of GDOC, which can be converted to a necrotizing hemorrhagic form when given at a high concentration (34 mM). Animals subjected to this highly concentrated GDOC or taurocholate treatment exhibit very severe pancreatitis that is accompanied by systemic inflammatory response syndrome (SIRS), manifested by pulmonary injury (14, 15).

The flexibility (in terms of etiology and severity of AP), repeatability, and clinical relevance associated with the duct perfusion-induced pancreatitis make it an excellent experimental model for AP studies. However, it requires careful monitoring of perfusion pressure and an invasive surgery. Infact, intraductal infusion of saline alone, which is neither a toxin nor a stimulant, has been reported to induce mild pancreatitis. Despite this drawback, the duct perfusion model remains a common model of AP for studies examining pathophysiology of AP and potential therapies for AP because of its similarity to clinical pancreatitis.

### 3.5. Arginine-induced pancreatitis

Treatment with arginine can induce acute necrotizing pancreatitis in rats (16) and mice (17). AP is induced by a single high-dose intraperitoneal injection of arginine. The most effective dose of arginine for inducing AP lies between 250 and 500 mg/100 g body weight. The mechanism of arginine-induced AP remains to be fully understood. However, its toxicity in the pancreas is probably due to inhibition of protein synthesis, excessive nitric oxide production or lipid peroxidation. Excessive arginine, as well as other basic amino acids, could suppress ornithine decarboxylase, which is the rate-determining enzyme in polyamine synthesis. The resultant reduction in polyamine levels retards nucleic acid synthesis which may interfere with protein synthesis. Pancreatic tissue is particularly vulnerable to this form of toxicity because of its very active protein metabolism.

Arginine-induced pancreatitis is a relatively non-invasive AP animal model. A single injection of arginine is usually strong enough to induce acute necrotizing pancreatitis, although sometimes multiple injections may be required. The degree of severity of this animal model depends upon the dose of arginine and time of exposure. Usually, mild histological deterioration, including interstitial edema, inflammatory cell infiltration, and acinar cell degranulation, is observed when arginine is



**Figure 1.** Schematic diagram of inflammatory mediators in the pathogenesis of acute pancreatitis. Activation of various digestive enzymes in acinar cells leads to autodigestion of the pancreas and release of inflammatory mediators. When the inflammatory reaction is severe, it leads to pathological damages in various organs such as pancreas, lung and kidney and eventually death. The severity of acute pancreatitis is determined by an interplay of these pro- and anti-inflammatory mediators. The time between symptom onset in acute pancreatitis and the development of distant organ dysfunction provides an ideal therapeutic window in this condition.

administered in low doses. However, higher doses of arginine can cause SIRS leading to lung injury in severe AP (18). Its specificity, efficiency and flexibility in controlling the degree of severity have made it a common non-invasive model of AP in recent years.

One of the disadvantages of arginine-induced pancreatitis is its lack of clinical relevance. Because of this reason, the popularity of the arginine-induced model of AP has faded in recent years.

Table 1 summarizes the most prevalent animal models of AP discussed in this section.

#### 4. MEDIATORS OF ACUTE PANCREATITIS-ASSOCIATED SIRS IDENTIFIED USING ANIMAL MODELS

##### 4.1. TNF-α and IL-1β

Both tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are derived predominantly from activated macrophages and act via specific cell membrane bound receptors. Levels of both these pro-inflammatory mediators are elevated upon the onset and during the progress of acute pancreatitis (19, 20). Naturally occurring

soluble TNF receptors (sTNFR) and interleukin-1 receptor antagonist (IL-1ra), by neutralizing the activity of TNF-α and IL-1β respectively, act as anti-inflammatory mediators (19). Intrapaneatic TNF-α and IL-1β are detectable one hour following induction of acute pancreatitis and levels increase rapidly over the following six hours (19, 20). Both TNF-α and IL-1β are thought to play an important role in acute pancreatitis.

Combined infusions of TNF-α and IL-1β have synergistic pro-inflammatory effects. Using knockout mice deficient in IL-1 type 1 receptors, TNF type 1 receptors or both, it has been shown that IL-1β and TNF-α make an equivalent contribution to the severity of an attack. Preventing the activity of both cytokines concurrently has no additional effect on the degree of pancreatitis but does attenuate the systemic stress response and is associated with an additional but modest decrease in mortality (20). Numerous investigators have employed specific antagonists to TNF-α or IL-1β in experimental models of acute pancreatitis. Examples of this approach include the use of an anti-TNF-α antibody, soluble type 1 TNF receptor and IL-1ra (20, 21). Blockade of the IL-1 receptor before or soon after induction of pancreatitis is associated with decreased severity of pancreatitis and reduced intrinsic

pancreatic damage. Also, neutralization of TNF- $\alpha$  with a polyclonal antibody significantly reduces the severity of acute pancreatitis in rats (21).

### 4.2. IL-6

Interleukin 6 (IL-6) is produced by a wide range of cells including monocytes/macrophages, endothelial cells, fibroblasts and smooth muscle cells in response to stimulation by endotoxin, IL-1 $\beta$  and TNF- $\alpha$  (1-3). Transgenic mice overexpressing IL-6 are more susceptible to acute pancreatitis and in these mice monoclonal anti-IL-6 antibody has a protective effect (22).

### 4.3. IL-10

Interleukin-10 (IL-10) is an anti-inflammatory cytokine. Its plasma levels are elevated in animal models of endotoxemia and inhibit the release of pro-inflammatory cytokines (i.e. IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ) from monocytes/macrophages thus preventing subsequent tissue damage. IL-10 also stimulates production of naturally occurring IL-1 receptor antagonist (IL-1ra) and release of soluble p75 TNF receptor (23). IL-10 is believed to have a protective role in acute pancreatitis. Administration of IL-10 in experimental acute pancreatitis reduces the local inflammatory response and subsequent mortality (23).

### 4.4. PAF

Platelet activating factor (PAF) is a low molecular weight phospholipid which acts via specific cell surface receptors that have been identified on numerous cells and tissues including platelets, leukocytes and endothelial cells (1-5). Isolated pancreatic acini have been reported to synthesize PAF and pancreatic tissue concentrations rise during the course of an attack. In animal models, intra-peritoneal or intravascular injection can bring about or increase the severity of acute pancreatitis (24). Blood and pulmonary tissue levels also rise co-ordinately indicating that PAF is a key mediator of the systemic inflammatory response (14).

Specific PAF antagonists have been evaluated in experimental models with varying success. Prophylactic treatment with these antagonists causes a reduction in local inflammation and acinar cell necrosis, in several experimental models of acute pancreatitis (25, 26). In a recent study using a model of severe acute pancreatitis induced by infusion of bile salts into the pancreatic duct in combination with caerulein administered intravascularly, treatment with lexipafant, a PAF antagonist, had no effect either on survival or on local inflammation (26).

PAF is inactivated by the enzyme platelet activating factor acetylhydrolase (PAF-AH). In severe acute pancreatitis induced by the ligation of combined biliopancreatic duct in the opossum, there was significant protection against acute pancreatitis and associated lung injury by treatment with PAF-AH (13).

### 4.5. CD40L

CD40, a member of the tumor necrosis factor (TNF) receptor family, is expressed on the membrane of a variety of cells, including B lymphocytes, monocytes,

dendritic cells, and biliary epithelial cells. CD40 binds to its ligand CD40L (also referred to as CD154) to mediate major immunoregulatory signals (1-5). Although CD40L expression was previously thought to be restricted to activated T lymphocytes, this cell surface protein has been detected in various cell types, including macrophages, smooth muscle and endothelial cells. In pancreatic tissue from control mice and caerulein-treated mice, the expression of both CD40 and CD40L was detected on the acinar cell surface. Interestingly, pancreatitis and pancreatitis-associated lung injury were markedly decreased in mice deficient in CD40L compared with wild-type mice, suggesting an important pro-inflammatory role of CD40L in the pathogenesis of acute pancreatitis and associated lung injury (27).

### 4.6. ICAM-1

Intercellular adhesion molecule-1 (ICAM-1; CD54) is an inducible protein expressed on the surface of endothelial cells. Under physiological conditions, ICAM-1 is not constitutively expressed or is expressed at low levels in most tissues; during inflammation its levels are upregulated (1-5). ICAM-1 knockout mice are protected against acute pancreatitis and associated lung injury, pointing to an important role for ICAM-1 in the development of pancreatitis and subsequent organ damage (28). The protective effect of ICAM-1 gene deletion does not differ from that seen following neutrophil depletion in the choline-deficient, ethionine supplemented (CDE) diet model of acute pancreatitis (11). Indeed neutrophil depletion in ICAM-1 knockout mice affords no additional protection (28). Blocking ICAM 1 has been shown to have a protective effect against local and systemic organ damage in different experimental models of acute pancreatitis (29). These results suggest that ICAM-1 deficiency interferes with neutrophil recruitment and supports the concept of a therapeutic strategy directed against neutrophil migration and activation.

### 4.7. C5a

C5a is a potent anaphylatoxin and chemoattractant that is generated from C5 as part of both the classic and alternate pathways of complement activation. C5a, acting via C5aR on target cells, is generally believed to serve as a "complete" proinflammatory mediator. We evaluated the role of C5a in a model of pancreatitis and systemic injury after pancreatitis using two independent but complementary approaches. In the first, mice that do not express C5aR were used, whereas in the second set of experiments, mice that do not express C5 were employed. The results of both studies were similar, i.e., interruption of C5a action either by deletion of its receptor or by deletion of its parent protein resulted in worsening of pancreatitis. The severity of pancreatitis-associated lung injury was also increased when C5a action was rendered inoperative (30).

### 4.8. Chemokines

Leukocyte chemotaxis in acute pancreatitis is a well orchestrated process that involves a number of proteins, including pro-inflammatory cytokines, adhesion molecules, matrix metalloproteinases and the large

cytokine subfamily of chemotactic cytokines - the chemokines (1-5). Numerous chemokines have now been identified as inflammatory mediators with potent leukocyte activating properties and many of them have been shown to be involved in the patho-physiological process of experimental acute pancreatitis.

Chemokines have been divided into four major sub-groups: C, CC, CXC and CX<sub>3</sub>C, on the basis of the position and spacing of N-terminal cysteine residues (1-5). Historically, CC chemokines (such as MCP-1, MIP-1 $\alpha$ , RANTES) have been believed to act principally upon monocytes and CXC chemokines which contain a three amino acid ELR motif at the amino terminal end (such as IL-8, GRO- $\alpha$ , ENA-78) are believed to act upon neutrophils. Recent work has, however, shown that these narrow definitions are no longer valid (31-35).

We have shown that pancreatic acinar cells produce the CC chemokine MCP-1 and that treatment with supramaximally stimulating doses of caerulein causes an upregulation of MCP-1 production. Caerulein-induced stimulation of chemokine production is regulated via NF- $\kappa$ B and Ca<sup>2+</sup> (31).

The chemokines are an ideal target for anti-inflammatory therapy. Of the rat CXC chemokines, the best characterised is CINC (cytokine-induced neutrophil chemoattractant). Circulating levels of CINC are raised in experimental acute pancreatitis (15) and treatment with neutralizing antibody against CINC protects rats against acute pancreatitis-associated lung injury (9). Furthermore, treatment with antileukinate, a hexapeptide antagonist of the CXCR2 chemokine receptor, protects mice against acute pancreatitis and associated lung injury (32). We have also shown that in knockout mice, the deletion of the MIP-1 $\alpha$ /RANTES receptor CCR1 decreased the pulmonary damage seen in severe acute pancreatitis. There was little protection against pancreatic damage (33). Similarly, treatment with Met-RANTES, a CCR1 antagonist, protected mice against acute pancreatitis-associated lung injury, with little protection against pancreatic damage (34). In a recent study, we have shown that treatment with a small molecule CCR1 antagonist BX 471 protects mice against acute pancreatitis and associated lung injury (35). These studies show the critical role of chemokines in the pathogenesis of acute pancreatitis and associated lung injury.

There are over 50 different chemokines and over 20 different receptors with overlapping functions. Despite the complexity and apparent redundancy of this system, it is reasonable to believe that specific chemokine receptor antagonists, that interfere with leukocyte migration and activation, could be useful in acute pancreatitis.

### 4.9. Substance P

Substance P is an 11 amino acid neuropeptide that is released from nerve endings. Subsequent to its release, substance P binds to neurokinin-1 (NK1) receptors on the surface of effector cells and in addition to being a mediator of pain it plays an important role in inflammation.

In the first study on the role of substance P as a mediator of inflammation in AP and associated SIRS, we have shown the presence of substance P in the pancreas and of NK1 receptors on pancreatic acinar cells in mice (8). On induction of pancreatitis, there is a several fold upregulation of pancreatic substance P levels and of NK1 receptors on pancreatic acinar cells (8). Moreover, knockout mice deficient in NK1 receptors are protected against pancreatitis. Interestingly, these mice are almost completely protected against pancreatitis-associated lung injury (8). In a subsequent paper, we have highlighted the role of preprotachykinin-A (PPT-A) gene products (e.g. substance P and neurokinin-A) in the pathogenesis of acute pancreatitis and associated lung injury (36). NK1 receptors bind other peptides in addition to substance P, not all of which are derived from the PPT-A gene. We have also found that knockout mice deficient in the PPT-A gene were protected against acute pancreatitis and associated lung injury (36). Furthermore, both prophylactic and therapeutic treatments with CP-96,345, an antagonist of the NK1 receptor, protected mice against acute pancreatitis and associated lung injury (37). These three papers (8, 36, 37) clearly show that PPT-A gene products, acting via NK1 receptors, are critical pro-inflammatory mediators in acute pancreatitis and the associated lung injury. In a more recent study, we have shown a differential regulation of tachykinins and tachykinin receptors in the pancreas and lungs in acute pancreatitis (38). In another recent study, we have shown a differential regulation of adhesion molecules in acute pancreatitis and associated lung injury (39). These early results point to a differential regulation of inflammation in the pancreas and lungs, an interesting concept that merits further study.

More recent studies have also shown that substance P can induce the synthesis of chemokines. In the first study on the interaction of substance P and chemokines in acute pancreatitis, we have recently shown an interaction of SP with chemokines in pancreatic acinar cells (40). SP was found to stimulate chemokine synthesis in pancreatic acinar cells (40). This is the first direct evidence of the role of substance P, acting via NK-1R present on mouse pancreatic acini, in inflammation and points to the mechanism by which SP contributes to inflammation in AP.

We have also shown that there are temporally and spatially selective chemokine responses in CCK secretagogue caerulein-induced acute necrotizing pancreatitis in mice. CC chemokines MCP-1 and MIP-1 $\alpha$  and CXC chemokine MIP-2 are elevated after induction of AP. They are early mediators in AP, mediating both local as well as systemic inflammatory responses. In contrast, another CC chemokine RANTES is only involved in local pancreatic inflammation at a later stage of the disease. Either prophylactic or therapeutic treatment with a potent selective NK-1R antagonist CP-96,345 significantly suppressed caerulein-induced increase in MCP-1, MIP-1 $\alpha$  and MIP-2 expression but had no apparent effect on RANTES expression (41). Our data suggests SP, probably by acting via NK-1R upon various chemokine-secreting cells in the pancreas and lungs, stimulates the release of

chemokines that aggravate local AP and the development of its systemic sequelae.

These results demonstrate the critical role played by substance P in the pathogenesis of acute pancreatitis and point to a potential therapeutic approach against this clinical condition.

### 4.10. H<sub>2</sub>S

The toxic effect of H<sub>2</sub>S on living organisms has been recognized for nearly three hundred years. However, growing evidence has accumulated in recent years which suggests that H<sub>2</sub>S is formed naturally in mammalian tissues and exhibits a range of biological and physiological functions. Cystathionine-γ-lyase (CSE), which utilizes L-cysteine as substrate, is the enzyme responsible for the production of H<sub>2</sub>S in mammalian vascular tissues. In a recent paper, we have shown the presence of H<sub>2</sub>S synthesizing enzyme activity and CSE (as determined by mRNA signal) in the pancreas. Also, prophylactic as well as therapeutic treatment with the CSE inhibitor, DL-propargylglycine (PAG), significantly reduced the severity of caerulein-induced pancreatitis and associated lung injury (42). This study has shown H<sub>2</sub>S as a novel inflammatory mediator that plays a key role in acute pancreatitis and associated lung injury.

Recently, we have investigated the involvement of substance P and neurogenic inflammation in H<sub>2</sub>S-induced lung inflammation. Intraperitoneal administration of NaHS, an H<sub>2</sub>S donor, to mice caused a significant increase in circulating levels of substance P in a dose-dependant manner. H<sub>2</sub>S alone could also cause lung inflammation, as evidenced by a significant increase in lung myeloperoxidase activity and histological evidence of lung injury. Maximum effects of H<sub>2</sub>S on substance P levels and on lung inflammation were observed 1 h after NaHS administration. At this time, a significant increase in lung levels of TNF-α and IL-1β was also observed. In substance P deficient mice, the preprotachykinin-A (PPT-A) knockout mice, H<sub>2</sub>S did not cause any lung inflammation. Furthermore, pre-treatment of mice with the NK1 receptor antagonist CP-96,345 protected mice against lung inflammation caused by H<sub>2</sub>S. Depleting neuropeptide from sensory neurons by capsaicin significantly reduced the lung inflammation caused by H<sub>2</sub>S. In addition, pre-treatment of mice with capsazepine, an antagonist of the transient receptor potential vanilloid (TRPV)-1, protected mice against H<sub>2</sub>S-induced lung inflammation. These results demonstrate a key role of substance P and neurogenic inflammation in H<sub>2</sub>S-induced lung injury in mice (43). Treatment of mouse acinar cells with H<sub>2</sub>S donor drug, sodium hydrosulfide (NaHS) showed a significant increase in SP concentration and expression of PPT-A and NK1-R genes [44]. These results suggest that the pro-inflammatory effect of H<sub>2</sub>S may be mediated by SP-NK1-R related pathway in pancreatic acinar cells. Recent evidence also points to a dual pro- and anti-inflammatory role of H<sub>2</sub>S in AP, depending on the rate of release (45). A possible interaction between H<sub>2</sub>S and substance P in AP will be the subject of future studies.

## 5. SUMMARY AND PERSPECTIVE

Significant progress has been made in recent years in our awareness on the role of inflammatory mediators in the pathogenesis of acute pancreatitis and associated SIRS. In most of the studies, experimental animal models were used, and these models have been invaluable in assessing the role of inflammatory mediators in AP and investigating novel therapeutic targets. Early studies have shown clinical relevance of these findings, which proves the usefulness of studies in employing appropriate experimental models. An understanding of the elucidation of the key mediators of inflammation in acute pancreatitis and associated MODS coupled with the discovery of specific inhibitors is likely to make it possible to develop clinically effective anti-inflammatory therapy for this, as yet incurable, clinical condition.

## 6. ACKNOWLEDGEMENT

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**Send correspondence to:** Madhav Bhatia, Department of Pharmacology, National University of Singapore, Yong Loo Lin School of Medicine, Centre for life Sciences, 28 Medical Drive, Singapore 117456, Tel: 65-6516-8256, Fax: 65-6775-7674, E-mail: mbhatia@nus.edu.sg

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