Immune modulation in acute and chronic pancreatitis

Aida Habtezion¹ and Hana Algül²

¹Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA 94305, USA
²II. Medizinische Klinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Version 1.0, October 13, 2016 [DOI: pending]

1. Introduction

Acute pancreatitis (AP)
Acute pancreatitis (AP) is one of the most common gastrointestinal causes for hospital admission. AP can develop in response to various factors e.g., gallstones, excessive alcohol consumption, viral infections, as well as strong reactions to certain medications. Initiating events for the acinar cell injury take place locally. Premature enzyme activation and abnormal exocytosis of zymogens are potent triggers of inflammation, edema, and tissue damage. In the beginning, inflammation, edema, and tissue damage are localized. However, these localized events can progress to systemic complications e.g., multiple organ dysfunction (MOD) primarily affecting lung, liver and kidney.

Based on physiological findings and laboratory values, AP can be classified as mild, moderate and severe. In most cases, patients are suffering from mild AP, which is reflected in upper abdominal pain that can radiate into the back, often accompanied by swollen and tender abdomen, and in 85% of cases, followed by nausea and vomiting. In contrast to this, less than 25% of patients develop moderate to severe pancreatitis (52, 94). Severe pancreatitis is characterized by pancreatic dysfunction, local and systemic complications e.g., MOD, followed by difficult and long recovery, and in some cases, death.

Immune cells are crucial mediators, which determine the complex pathophysiology of this disease. The balance between pro- and anti-inflammatory events in AP is key to the severity of disease (39).

Chronic pancreatitis (CP)
Recurrent acute pancreatitis can result in chronic pancreatitis, which is a progressive inflammatory and fibrotic disease that can lead to exocrine and endocrine insufficiency (56). Although less common than AP, CP is associated with significant morbidity and health care cost. CP is commonly associated with excessive alcohol consumption and remains an important risk factor for developing pancreatic cancer (99). Other factors such as the genetic mutations causing hereditary pancreatitis also contribute to acquiring this chronic debilitating disease. Immune responses associated with CP are more and more appreciated, although the role of immune cells is not as well studied as in AP. More recently, manipulations of immune pathways have challenged the notion of the “irreversible” nature of this disease.

In this review, we focus on the role of immune cells, pathways, and immune mediators
associated with acute and chronic pancreatitis. Autoimmune pancreatitis is covered elsewhere.

2. Immune cells in pancreatitis

Neutrophils

In normal, healthy pancreas neutrophils are not typically present (75). However, in AP release of inflammatory mediators by acinar cells, in response to the damage, triggers innate immune mechanisms that recruit immune cells to the site of inflammation. Initially, neutrophils and monocytes are recruited, and dendritic cells, mast cells, T-cells and platelets follow. Migration of immune cells is a multistep process that engages diverse adhesion molecules (73). A prominent protein required for neutrophil adhesion to endothelium and epithelium is intercellular adhesion molecule-1 (ICAM-1) (31). ICAM-1 is constitutively expressed at low level on endothelium, and some epithelium. Nonetheless, at the sites of inflammation, e.g. damaged acinar cells, ICAM-1 is produced in higher amount, thus leading to increased neutrophil adhesion. Furthermore, it was demonstrated that the CCK analog, cerulein up regulates ICAM-1 mRNA and protein expression in cerulein-induced pancreatitis (30, 102). Interestingly, ICAM-1 knockout mice were protected compared to control mice (25). Furthermore, in another study, using the same mouse model, it was demonstrated that serum, pancreatic and lung levels of ICAM-1 were increased during AP (25).

Oxidative stress is one of the mechanisms by which infiltrated neutrophils induce damage in AP. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an important oxidative stress protein (92). In AP, NADPH oxidase expression and activity are increased in pancreas (12). Through oxidative stress, infiltrating neutrophils contribute substantially to trypsin activation in acinar cells during AP (34, 79) In a model of cerulein-induced pancreatitis in rats, it was demonstrated that trypsin activation was supported through a mechanism involving NADPH oxidase (12). Furthermore, immunohistochemical analysis and measurement of reactive oxygen species (ROS) production indicated that NADPH oxidase was present in infiltrated neutrophils, but not in pancreatic acinar cells. Consistent with this finding, it has been demonstrated that neutrophil depletion as well as NADPH oxidase deficiency, inhibited trypsin activation and cerulein-induced damage in pancreas (34).

Monocytes/Macrophages

Along with neutrophils, monocytes are one of the major mediators of AP (66). Similarly to neutrophils, recruiting mechanisms involve signals deriving from damaged pancreatic acinar cells (33). It is believed that activation of primary monocytes is influenced by chemokine (C-C motif) ligand 2 (CCL2), CCL3, and CCL5 (75). Signals, originally sent by acinar cells, are multiplied by activated monocytes (5). As a result TNF-α, interleukin-1 (IL-1), IL-6 and ICAM-1 are produced in higher extent, which assists progression of disease. This signal amplification specifically affects lung, liver and kidney tissues, leading to systemic inflammation (57). Interestingly, a recent study addressing the role of myeloid RelA/p65 in IL-6 regulation in cerulein induced AP, unequivocally demonstrated that myeloid cells, namely macrophages, play the central role and are the major source of IL-6 (103). Macrophages and IL-6, through IL-6 trans-signaling were responsible for AP associated acute lung injury.

Interestingly, several macrophage populations are responsible for systemic organ inflammation. In severe pancreatitis, peritoneal macrophages are rapidly activated, due to excessive production of pancreatic enzymes and cytokines. Consequently, this leads to release of mediators, e.g. pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and enzymes such as nitric oxide synthase (iNOS) that easily reach the bloodstream, thus contributing to the inflammatory responses in severe pancreatitis (18, 26). Association of these macrophages with the complications of severe pancreatitis was clearly demonstrated in several
studies (90). Peritoneal lavage in rats suffering from acute pancreatitis, led to significant reduction of the cytotoxic effect of ascitic fluid. Then investigators concluded that this was a consequence of reduced number of peritoneal macrophages, thus milder response to activating mediators from ascitic fluid.

A second population of macrophages that significantly contribute to the secondary complications in AP are alveolar macrophages. Upon activation, these cells have great capability to produce cytokines, nitric oxide (NO), and to attract large number of leukocytes in the lungs. Lung injury, as a secondary effect of AP, is largely related to high activity of iNOS and high levels of NO (15). Another population of macrophages involved in AP are Kupffer cells. Normally, Kupffer cells respond to toxic substances in the blood, thus participating in the acute response of the liver. However, during the AP, inflammatory mediators released into the bloodstream by a damaged pancreas can activate Kupffer cells, hence inducing systemic inflammation (53). In support of this, in vitro analysis of Kupffer cell activity demonstrated that pancreatic enzymes could activate these cells (23). Nonetheless, hepatic damage is evident only in late stages of pancreatitis. Interestingly, another study raised doubt regarding the possibility that acute liver responses in AP could be induced by inflammatory mediators released by the pancreas (27). This study provided evidence of endotoxin contamination of porcine pancreatic elastase responsible for activating Kupffer cells in AP. Pancreatic elastase, free of contamination, failed to activate murine macrophages to release TNF-α and to cause pro-inflammatory effect in vivo. Nonetheless, the authors could not exclude that other fragments might activate Kupffer cells.

Dendritic cells

As active participants of inflammation, through mentoring T-cell response, dendritic cells (DCs) emerge as both potent promoters or suppressors of inflammation (97). Numerous publications single out their importance in number of organ-specific inflammatory diseases. It was demonstrated that depletion of DCs in mice, in a model of cerulein induced acute pancreatitis, resulted in a massive increase of pancreatic damage, pancreatic exocrine cell death, followed by consequent mortality (4). Interestingly, it seems that DCs emerge as a dual role cells in AP. Namely, DCs at the same time galvanize the inflammatory response to damage via production of different inflammatory mediators e.g., interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and CCL2, but on the other hand protect the pancreas upon cellular stress. The same group demonstrated that DCs are required for pancreatic viability in acute pancreatitis, as the major cell type clearing byproducts of injury.
Dendritic cells also increase in cerulein-induced chronic pancreatitis (62). Moreover, adoptive transfer of bone marrow derived dendritic cells and in vivo expansion of dendritic cells, using Fms-like tyrosine kinase-3 ligand (FLT3L), resulted in PSC activation and worsened chronic pancreatitis. These effects seem to be mediated via dendritic cell-CDC4+ T cell activation, since CD4+ T cell-deficient mice were protected from the effects of the dendritic cells. TLR4 can signal in MyD88-dependent and -independent pathways; interestingly, in this study MyD88-deficient dendritic cell transfer further worsened chronic pancreatitis suggesting opposing roles of TLR4 downstream signalling in dendritic cells during pancreatic inflammation. More studies are likely to reveal the role of different subsets of dendritic cells in pancreatitis.

**T cells**

Initial studies that demonstrated the involvement of CD4+ T-cells were performed in nude mice and in vivo CD4(+) or CD8(+) T cell-depleted mice. These experiments revealed a pivotal role in the development of tissue injury during acute experimental pancreatitis in mice. Indeed, the reduction of peripheral blood CD4+ T lymphocytes is associated with persistent organ failure during acute pancreatitis (100). In contrast in chronic pancreatitis, an increase in immune cell infiltration, mainly T cells and macrophages, is observed (21, 51, 98). In the dibutyltin dichloride (DBTC) rat model of chronic pancreatitis, increase in both CD4+ and CD8+ T cells was observed and over time characterized by a decrease in CD4+/CD8+ T cell ratio due to a continuous rise in infiltrating CD8+ T cells (85). Moreover, increase in IFNγ, IL-2, and IL-2 receptor transcripts during the chronic phase in this study suggested importance of lymphocyte activation in disease pathogenesis.

Increases in mononuclear cell (lymphocytes and macrophages) infiltrates in pancreatic tissues are observed in patients with chronic pancreatitis as compared to the normal pancreas (21, 47). Among the infiltrating lymphocytes, T cells were predominant with higher CD8+ relative to CD4+ T cells and were localized between the parenchyma and areas of fibrosis. Interestingly, significant increase in perforin mRNA-expressing CD8+ and CD56+ cells were observed in pancreatic tissue sections from patients with alcoholic chronic pancreatitis, suggesting a possible role for cytotoxic CD8+ and/or NK T cells in this disease (45). In contrast, amongst circulating leukocytes CD8+ and CD56+ were reported to be lower in a handful number of chronic pancreatitis patients as compared to healthy controls (63). In addition, unlike the decreased CD4+/CD8+ T cell ratio reported by Hunger et al in pancreatic tissue, an increased CD4+/CD8+ T cell ratio was found in the circulation of patients with chronic pancreatitis (63) suggesting differences not only in T cell activation but also recruitment. However, another study found that CD4+ but not CD8+ as the predominant tissue infiltrating T cells in chronic pancreatitis patients (19). This study found the CD4+ T cell to be localized in the fibrous area whereas CD8+ T cells expressing the αE integrin (CD103), implicated in mediating T cell adhesion to intestine epithelium E-cadherin, were scattered in between ductal cells suggesting possible microenvironment dependent functional differences among the infiltrating T cell subsets.

An interesting study comparing bone marrow and blood mononuclear cells from healthy, chronic pancreatitis, and pancreatic cancer patients, as well as infiltrating lymphocytes from chronic pancreatitis lesions found that only the chronic pancreatitis patients had a strong IL-10 producing Foxp3+ regulatory T cell responses against pancreatitis-associated antigens (78). Moreover, increased circulating memory T cells and persistence of dysregulated immune responses even long after the removal of chronic pancreatitis lesions, (32) support the hypothesis of ongoing chronic pancreatitis-specific T cell responses.

3. **Mediators for immune cell recruitment:**
Examples of cytokines/chemokines involved
Under inflammatory and non-inflammatory conditions, cytokines and in particular chemokines play an important role in leukocyte recruitment (37, 61, 89). Distinct and differential expression of cytokines and chemokines have been observed in acute and chronic pancreatitis (38, 76, 82). Based on overexpression of chemokines and chemokine receptors observed, several investigators employed blockade approaches to modulate and show protective effects against experimental pancreatitis.

Protective effects of inhibiting the chemokine CCL2/MCP-1 (CCR2 ligand) have been shown by multiple investigators using different rodent models of acute pancreatitis (8, 24, 46, 104). Results form CCR2 deficient mice were also consistent with these findings (59). CCR5 deficient mice on the other hand had exacerbated cerulein-induced acute pancreatitis, and may have been due to the fact that CCR5 knock out mice had increased CCL2/MCP-1 and other monocyte/macrophage chemoattractant production that may have accounted for the pronounced pancreatic inflammation (58). Inhibition of other chemokines or chemokine receptors such as cytokine induced neutrophil chemoattractant (CINC), CX3CL1/fractalkine, CXCR2, and CCR1 were also shown to ameliorate pancreatic and/or its associated lung inflammation (6, 7, 28, 41, 44, 86). Leukocyte migration is a multistep process involving trafficking receptors and adhesion molecules (9, 11). Consistent with the significance of leukocyte migration in pancreatitis, a pathogenic role was also demonstrated for the intercellular adhesion molecule 1 (ICAM-1) in various models of experimental acute pancreatitis (55, 70, 71, 88, 93).

CCR2 ligands are also elevated in experimental chronic pancreatitis and competitive bone marrow studies show a role for CCR2 in monocyte/macrophage accumulation in the chronically inflamed pancreas (98). However, a worse disease outcome was reported in cerulein-induced chronic pancreatitis in CCR2 knockout mice as compared to their wild type counterparts (59). Thus further studies are needed to determine the role for CCR2 in chronic pancreatitis. Similar to acute pancreatitis, CXCR2 inhibition had a protective effect in experimental chronic pancreatitis (86). Significant increase in CCR5 and its chemokine ligands CCL5 and CCL3 mRNA in the pancreas was evident in patients with chronic pancreatitis (29). Moreover, the majority of the CCR5-positive cells by immunostaining were CD68-positive, suggesting a role for CCR5 in monocyte/macrophage recruitment in chronic pancreatitis. Overall, there are fewer experimental studies on chemokine and chemokine receptor blockade in chronic pancreatitis compared to acute pancreatitis models. Nevertheless, modulations of chemokine and chemokine receptors appear to impact immune cell infiltration and disease outcomes at least in experimental models of both acute and chronic pancreatitis.

Transcription factors in Pancreatitis
Nuclear factor kappa B (NF-κB) is one of the central transcription factors, and main mediator responsible for pathophysiology of pancreatitis. The two most prominent functions of NF-κB are regulation of inflammatory responses and regulation of cell proliferation and apoptosis NF-κB is formed by different homo- and heterodimers of members of NF-κB/Rel family and can be activated by different stimuli e.g., cytokines, LPS, oxidative stress, activators of protein kinase C (17, 40, 65, 67).

The earliest study to demonstrate early activation of NF-κB in acute pancreatitis was presented by Stephen Pandol’s group (36) and confirmed by another group one year later (87). However, both studies presented different conclusions with respect to the role of NF-kB in acute pancreatitis. Since then, NF-κB activation has been studied in numerous publications (35, 64, 69).

In most studies, pharmacologic NF-κB inhibition ameliorated the inflammatory response, necrosis,
and other parameters of pancreatitis severity. However, the pharmacologic agents were largely nonspecific, such as antioxidants and proteasomal inhibitors. Thus, it was expected that the new tool of genetically engineered mouse models would help to clarify the role of NF-κB in acute pancreatitis. But surprisingly, the controversies were perpetuated with evidence that the IKK/NF-κB/RelA pathway leads to both aggravation (1, 60, 87, 91) and amelioration (2, 3, 14, 36, 43) of pancreatitis. However, this paradox can be resolved, at least in part, by realizing that acinar cell NF-κB activation, indeed, triggers both pro- and anti-inflammatory pathways. Even more, this system is strikingly “context-dependent” and its’ fine-tuning will require exhaustive characterization of the underlying mechanisms. A recent study demonstrated indeed that fine-tuning of this pathway via the IκB protein Bcl-3 determines severity of acute pancreatitis (84). All these studies underscore both the complexity and the gaps in our understanding of this pathway.

Although, intra-acinar NF-κB activation was shown to be highly present in human chronic pancreatitis (74), its pathophysiological role is less understood. While loss of IKKβ has no impact on pancreatic integrity (54), deletion of IKKα in the pancreas induces spontaneous chronic inflammation. IKKα was shown to potentially control autophagic protein degradation and maintain pancreatic acinar cell homeostasis. Further, other studies revealed the critical role for NF-κB in myeloid cells in inducing fibrosis during chronic pancreatitis (91).

Signal transducer and activator of transcription 3, STAT3

Signal transducer and activator of transcription 3 (STAT3) is among the most promising new targets for cancer therapy. It is generally considered to be a direct transcription factor and IL-6 is a well known traditional activator of STAT3 (42). STAT3 is remarkably involved in several pathological process in the pancreas e.g., acinar-to-ductal metaplasia and acute/chronic pancreatitis progression. It is highly associated with cell survival, proliferation and differentiation, and tissue inflammation (101). Using conditional STAT3 knock out mice, in a model of cerulein induced AP, it was demonstrated, that STAT3 knock out mice wshowed more damage with higher level of serum amylase and lipase, as well as significantly higher infiltration of inflammatory cells in the pancreas (83).

Interestingly, STAT3 activation in the pancreas emerged as highly responsible for the secondary effect of severe acute pancreatitis, acute lung injury (103). Furthermore, the authors demonstrated different STAT3 phosphorylation sites, namely STAT3S727 and STAT3Y705. Additionally, genetic inhibition of IL-6 signaling (in IL-6−/− mice) where STAT3S727 emerged, unlike blocking IL-6 trans-signaling (in opt_sgp130Fc mice) where STAT3S727 did not emerge, eliminates protective mechanisms during inflammation. Nonetheless, the authors could not explain whether these differences in STAT3 phosphorylation account for the differences in local tissue damage or not.

4. Therapeutic approaches targeting inflammation in the pancreas

Acute pancreatitis

Given the profound and increasing understanding of pathology and regulatory mechanisms of AP, numerous studies have evaluated mediators and different pathways with the aim to provide evidence for the development of pharmaceutical therapies. Unfortunately, several facts have contributed to treatment limitations: AP can be the result of multiple causes, which are often unidentified; it is accepted belief that disease initiation is followed by the common inflammatory mechanisms, but this might not be the case in all patients; disease initiation and duration is
individual, so the time for the treatment start is not uniform; it is still unpredictable at the beginning of disease to foresee the final outcome, as to whether a patient develops mild or severe pancreatitis (80). Conservative management, such as antibiotics and bowel rest have been insufficient in treatment of AP. As well, many agents beneficial for AP treatment in animal studies failed to repeat the same success in early clinical trials.

One of the first attempts to influence immune system mediators was with Lexipafant (48). This drug is one of the most potent platelet-activating factor (PAF) receptor antagonists. Disappointingly, a clinical study in patients with severe acute pancreatitis failed to demonstrate an effect on new organ failure during treatment (49). Thus, this study demonstrated that an antagonist of PAF activity on its own is not sufficient to ameliorate SIRS in severe acute pancreatitis. In the latest reviews, several authors summarized recent therapeutics and experimental approaches that target immune responses in AP (50, 80). Several therapeutic agents, depleting or regulating immune cells via various mechanisms of action, demonstrated high protective role against AP in animal models:

- **Glycyrrhizine** is a therapeutic agent which demonstrated reduction in serum levels of CCL2, amylase and lipase activity through inhibition of the recruitment of inflammatory cells into pancreas (22)

- **Sivelestat** demonstrated strong anti-inflammatory potential; it interfered with regulatory mechanisms of immune cells, and demonstrating its action through reduced expression of lipase, amylase, IL-1β, TNF-α and NF-κB; its administration increased antioxidant power and IL-4 serum level (10)

- **Flavocoxid** reduced levels of TNF-α, serum levels of prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), and reduced histological damage. It influenced neutrophil and macrophage action via cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) blockade (68)

- **Rofecoxib** and Lisinopril demonstrated reduced levels of CCL2, CCL3, TNF-α, IL-6, influencing macrophage infiltration via COX-2 pathway inhibition (72)

It is essential to mention IL-6 inhibitors, as highly promising drug targets for the treatment of acute pancreatitis. Tocilizumab is one of the drugs that has emerged as remarkably efficient in the treatment of several inflammatory diseases. Recent reports demonstrated positive effects of tocilizumab on experimental severe acute pancreatitis and associated acute lung injury in rats (13). Severe acute pancreatitis was induced by retrograde injection of sodium taurocholate into the biliopancreatic duct. Following the administration of tocilizumab, pancreatic and lung histopathological scores were reduced, serum amylase, C-reactive protein and lung surfactant protein level were decreased, and myeloperoxidase activity was attenuated. In line with these findings, pancreatic NF-κB and STAT3 were decreased, and the serum chemokine (C-X-C motif) ligand 1 (CXCL1) was down regulated in rats after tocilizumab administration.

In an interesting and novel approach proposed only recently, treatment with CO-releasing molecule-2 (CORM-2) decreased mortality, pancreatic damage, and lung injury in a mouse model of AP (96). This treatment decreased systemic inflammatory cytokines and suppressed systemic and pancreatic macrophage activation. Such cellular therapeutic approaches, therefore, offers an alternative treatment route.

**Chronic pancreatitis**

As mentioned previously increases in mononuclear cell (particularly T cells and macrophages) infiltrates in pancreatic tissues are observed in patients with chronic pancreatitis as compared to the normal pancreas (21). Alternatively activated macrophages are abundant in chronic pancreatitis especially in the vicinity of...
the fibrosis and activated PSCs (29, 98). Differences in inflammatory infiltrates with increase in lymphocytes and macrophages as well as dendritic cells around the ducts were reported in non-alcohol- versus alcohol-related chronic pancreatitis pathologies (20). However the non-alcoholic group comprised a heterogeneous group of patients (including 4/12 patients with associated non-pancreatic immune-disease manifestations), although no major histologic differences were noted between the patients. Thus whether functional and immune response differences exist between alcohol and non-alcohol mediated chronic pancreatitis remains to be defined in studies with inclusion of a large number of patients. In addition, immune cell infiltration and responses are likely dynamic processes that vary with stage and disease progression. In agreement, early disease is associated with moderate inflammatory cell collections or dispersed in the fibrous tissue as compared to later disease stages, where scant lymphocyte infiltration is observed around the ducts and neurons (51). Recent experimental data show that macrophages can influence fibrosis and chronic pancreatitis progression (98). More importantly, immune mediated pathways targeting the IL-4Rα signaling could alter established chronic pancreatitis associated fibrosis and slow disease progression. Thus immune targets might offer novel future therapies in chronic pancreatitis, although the dilemma of diagnosing early clinical chronic pancreatitis remains.

5. Conclusion

To date, experimental therapies used for AP treatment have demonstrated some success, but have given more challenges and unanswered questions from the clinical side. Numerous pre-clinical studies often gave diverse, and in some cases, the opposite results. Unfortunately, inconsistency in conclusions after drug testing does not give us an open window for translation of these drugs into clinical trials. Turning our direction, and changing our approach from classic, standard AP drug treatments, towards targeting immune response might bring more promising results and significantly better disease outcome. Also, it might turn out to be very rewarding to try combinational therapies targeting immune cells together with classic approaches. These questions and assumptions stay to be answered in a near future. Chronic pancreatitis adds another challenge where immune responses are quite different when compared to acute pancreatitis responses. In addition, translation of experimental findings will require better diagnostic criteria that can identify patients with early and late disease stages.

6. References


