Immunology of pancreatitis and environmental factors

Bomi Lee, Qinglan Zhao*, and Aida Habtezion*

Purpose of review
This report reviews recent aspects of pancreatitis immunology and environmental factors that link to development and progression of disease.

Recent findings
Limited human and animal model studies have recently attempted to understand immune mechanisms that lead to the pathogenesis of acute and chronic pancreatitis. Based on these studies innate immune responses emerge as critical elements in disease pathogenesis and severity of inflammation. The immune basis for environmental factors such as smoking, which are highly associated with disease progression highlight novel cross talk mechanisms between immune and nonimmune pancreatic cells such as the pancreatic stellate cells.

Summary
Better understanding of immune responses and signaling pathways are emerging as important contributors in pancreatitis development and progression. Such mechanisms are likely to offer future targetable therapies that can either halt or reverse disease progression.

Keywords
acute pancreatitis, chronic pancreatitis, fibrosis, inflammation, pancreatic stellate cells

INTRODUCTION

Acute pancreatitis is characterized by pancreatic acinar cell injury and abnormal pancreatic enzyme activation leading to cell death and inflammation [1]. Severe acute pancreatitis has a mortality rate as high as 10–30%, leading to rapid systemic organ failure [2]. Acute pancreatitis can be caused by alcohol, gallstone, and other factors. Access to clinical samples and development of therapeutics for acute pancreatitis have been challenging. However, animal models developed to study acute pancreatitis have increased our understanding of the mechanisms of disease. As with all animal models, there remains the challenge of extrapolating to human disease.

Recurrent acute pancreatitis can lead to chronic pancreatitis, a progressive and fibroinflammatory disease characterized by acinar cell death, pancreatic duct dilation, inflammation, and fibrosis leading to both endocrine and exocrine dysfunction [3]. Although it is a less common problem for acute pancreatitis, the field has been hampered by the lack of ways to diagnose early chronic pancreatitis. Similar to acute pancreatitis, several chronic pancreatitis animal models have been developed to study mechanisms and test therapeutics but aside from supportive management active FDA-approved treatments are currently not available. In this report, we discuss recent findings pertaining to the immunology of pancreatitis and immune-environmental factor interactions that promote progression of disease.

IMMUNOLOGY OF ACUTE PANCREATITIS

Initially acute pancreatitis is associated with sterile inflammation. Intracellular components released from injured acinar cells into the extracellular space stimulate damage associated molecular patterns (DAMPs). Several DAMPs are found to mediate sterile inflammation including high-mobility group box protein 1 (HMGB1) and heat shock protein 70

Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, California, USA

Correspondence to Aida Habtezion, 300 Pasteur Drive, Always Bldg. M211, Stanford, CA 94305, USA. Tel: +1 650 725 6511; fax: +1 650 723 5388; e-mail: aidah@stanford.edu

*Bomi Lee and Qinglan Zhao contributed equally to the writing of this article.

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KEY POINTS

- Recent studies highlight immune mechanism for pancreatitis development and progression.
- Innate immune responses involving macrophages and dendritic cells play critical roles in both acute and chronic pancreatitis.
- Recent studies demonstrate a cross talk between immune and nonimmune cells such as pancreatic stellate cells to drive environmental factors (such as smoking) mediated disease progression.

(HSP 70). Both extracellular HMGB1 and HSP70 increase further pancreatic tissue injury through toll-like receptor 4 (TLR4) in rodent models of acute pancreatitis by increasing pro-inflammatory cytokine release [4,5]. In contrast, intracellular HMGB1 was found to inhibit acute pancreatitis in mice by reducing nuclear catastrophe and inflammatory nucleosome release [6]. Similarly, HSP70 protects against acute pancreatitis by reducing the levels of inflammatory cytokines such as, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and trypsinogen activation peptide (TAP) [7]. Previous reports have shown that TLR4 has a pro-inflammatory role and is involved in the pathogenesis of severe acute pancreatitis [8,9]. Consistent with this finding, inhibition of macrophage TLR4 complex expression through carbon monoxide-releasing molecule-2 (CORM-2) treatment ameliorates experimental acute pancreatitis and leads to a reduction in macrophage TNF-α secretion [10]. Another toll-like receptor implicated in acute pancreatitis is TLR9, where deletion of TLR9 and pretreatment with TLR9 antagonist decreased pancreatic edema and inflammation in cerulein-induced and taurolithocholic acid 3-sulfate-induced acute pancreatitis [11].

Following a TLR priming event, the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome, also has a role in acute pancreatitis. Mice deficient in inflammasome components such as apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), NLRP3, and caspase-1 have reduced severity of cerulein-induced acute pancreatitis [11]. In addition to these components, a recent report indicates a pivotal role for other types of inflammasomes such as nucleosome-induced absent in melanoma 2 (AIM2) in acute pancreatitis. The receptor for advanced glycation end products (RAGE) was shown to activate AIM2 inflammasome and pro-inflammatory signaling in macrophages of L-arginine-induced and cerulein-induced acute pancreatitis suggesting nucleosome-RAGE-AIM2 signaling as a potential signaling therapeutic target in AP [12▪]. Furthermore, innate immune components such as caspase recruitment domain (CARD) proteins are also implicated in the pathogenesis of acute pancreatitis. For example, Card9, a member of the CARD protein family, is highly expressed in circulating mononuclear cells of patients with severe acute pancreatitis and proposed to augment production of inflammatory cytokines such as IL-6, IL-1b, IL-17A, and TNF-α [13]. Using the sodium taurocholate-induced severe acute pancreatitis rat model, it was shown that the regulation of pro-inflammatory cytokine levels by Card9 were mediated through an NF-κB and P38MAPKs pathway [14].

In the later phase of acute pancreatitis, translocation of gut bacteria and bacterial products plays an important role in disease severity and progression [15]. Experimental models of acute pancreatitis have been used to show how gut microflora activate innate immune components such as the nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and lead to downstream transcriptional responses (via NF-κB and STAT3) culminating in upregulation of inflammatory chemokines that recruit immune cells [16].

In addition to innate immunity, adaptive immune responses are also involved in acute pancreatitis. Clinical studies suggest immunosuppression with reduced T cells and serum immunoglobulins in severe acute pancreatitis, which points to functional impairment of lymphocytes [17]. Moreover, several cytokines have been implicated in acute pancreatitis. A clinical study reported higher levels of IL-17, IL-6, endotoxin, and bacterial load in serum of patients with severe acute pancreatitis with organ dysfunction. This study proposed that earlier and elevated IL-17 level can be used as early prognostic biomarker for severe acute pancreatitis in predicting length of hospitalization, organ failure, and death [18]. In experimental models of acute pancreatitis, pancreatic IL-22 levels are depleted, and treatment with IL-22 confers protection. IL-22 originates from leukocytes and activates IL-22 receptor on pancreatic epithelial cells to signal through STAT3 and promote regeneration; thus, the IL-22 pathway offers an important cross talk between pancreatic acinar cells and immune cells [19].

IMMUNOLOGY OF CHRONIC PANCREATITIS

Both innate and adaptive immunity play important roles in chronic pancreatitis. Among innate immune cells, dendritic cells are known to bridge between innate and adaptive immunity by affecting T-cell differentiation and activity. Innate response

molecules such as TLR4 or the myeloid differentiation primary response gene 88 (MyD88)-independent TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway play an important role in pancreatic fibrosis and inflammation. Interestingly, blockade of MyD88 accelerates pancreatic stromal inflammation through augmenting dendritic cells activation of T (Th2) cells [20]. Macrophages make up a key innate immune population in chronic pancreatitis. Macrophage infiltration is found in the pancreas of experimental and human chronic pancreatitis [21,22,23▪]. Unlike in acute pancreatitis, alternatively activated macrophages (AAMs, M2) are dominant in chronic pancreatitis. Mice lacking myeloid-specific IL-4Rα, and those treated with IL-4/IL-13 (cytokines that induce M2 polarization) inhibitor are less susceptible to chronic pancreatitis development and progression [23▪]. Although other cells likely contribute, pancreatic stellate cells were shown as a key source for IL-4/IL-13 and have the ability to induce M2 or AAMs [23▪]. Although adaptive immune responses are less well studied, T cells are found in abundance [24,25] and likely to play an important role in chronic pancreatitis. The proportion of T-helper (Th)1, Th2, and Th17 cells in the peripheral blood of patients with chronic pancreatitis was significantly increased as compared to controls [26]. Patients with chronic pancreatitis with diabetes have higher circulating Th1 cells and intra-islet colocalization of Th1 and Th17 cells as compared to control and patients with chronic pancreatitis without diabetes, which suggests that T cells mediate islet inflammation and β-cell dysfunction in chronic pancreatitis [27].

IMPACT OF ENVIRONMENTAL FACTORS IN PANCREATITIS

Although many diseases are closely connected with genetic mutations, environmental factors play an important role in disease onset and progression. Therefore, the impact of environmental factors on pancreatitis have been emphasized and extensively appreciated in recent years. Environmental factors can modulate epigenetic modifications leading to changes in gene function and disease progression [28]. Here we highlight the impact of environmental factors on development and progression of acute pancreatitis and chronic pancreatitis.

ALCOHOL AND PANCREATITIS

Alcohol abuse is a well known risk factor and common cause of acute pancreatitis and chronic pancreatitis in different regions of the world [29,30]. In acute pancreatitis, alcohol is the second most frequent etiologic factor next to gallstones, and also closely associated with recurrent acute pancreatitis and progression to chronic pancreatitis [31]. Alcohol increases the risk for organ failure and pancreatic necrosis in patients with acute pancreatitis by promoting severe acute pancreatitis associated with higher mortality [32]. Susceptibility genes for pancreatitis have been identified [33,34]. For example the expression and function of cystic fibrosis transmembrane conductance (CFTR) are disrupted by ethanol, fatty acids or fatty acid ethyl esters and contribute to the development of pancreatitis in human, mouse, and guinea pig models [35▪]. The PRSS1–PRSS2 locus polymorphism in patients with acute pancreatitis is highly associated with alcohol consumption and cigarette smoking [36]. Furthermore, Ren *et al.* [37▪] recently reported that ethanol induces pancreas edema, acinar atrophy, macrophage-mediated inflammation, endoplasmic reticulum stress, and oxidative stress with a mouse model of binge ethanol exposure.

Interestingly, a recent study showed a protective effect of moderate alcohol consumption on all types of pancreatitis in women and on recurrent acute pancreatitis and chronic pancreatitis in men [38], consistent with the fact that only a small minority of heavy alcohol drinkers develop pancreatic diseases and that alcohol may require additional risk factors such as genetic and other environmental factors to induce pancreatitis. Mechanistic studies suggest how alcohol can both protect and also damage the pancreas through the unfolded protein response (UTR) of the endoplasmic reticulum [39,40]; alcohol-induced mild endoplasmic reticulum stress activates spliced X-box binding protein 1 (XBP1) that mediates adaptive responses to maintain cellular homeostasis by generating endoplasmic reticulum chaperones, foldases and antioxidant factors. However, in the presence of heavy alcohol consumption and additional risk factors severe endoplasmic reticulum stress can lead to impaired adaptive responses and pancreatitis.

Although enhanced inflammatory responses in acinar cells have been attributed to ethanol metabolism [41], the effect of alcohol and alcohol metabolites including fatty acid ethyl esters and acetaldehyde on immune cells in pancreatitis remain to be elucidated.

SMOKING AND PANCREATITIS

An association between cigarette smoke and pancreatitis has been described in many reports over the last three decades [42,43]. Although cigarette smoking and alcohol consumption coexist in many patients with chronic pancreatitis [44], a number

of papers suggest that smoking is an independent risk factor in the development of chronic pancreatitis and has emerged as a critical factor for acute pancreatitis and for recurrent acute pancreatitis [45]. It has been reported that smoking confers a two-fold to three-fold increased risk for pancreatitis.

Acute pancreatitis remains a major cause for hospital admissions among gastrointestinal diseases across the world [46,47]. Therefore, identifying and removing major risk factors for acute pancreatitis is important. Recent studies including meta-analysis provide supportive evidences that smoking may enhance the risk of acute pancreatitis [48–51]. Smoking is reported as one of the predictors for respiratory failure and mortality in the early phase of acute pancreatitis [52]. It is also recognized that heavy smoking is not only an independent risk factor for acute pancreatitis, but also augments the effect of alcohol and lowers age of recurrent acute pancreatitis onset and subsequent chronic pancreatitis development [53–56]. Recently, Lee *et al*. [57] reported that continued smoking accelerates pancreatic calcification in chronic pancreatitis based on the evaluation of computed tomography imaging studies in patients with chronic pancreatitis.

Nicotine is one of the major compounds studied in cigarette smoke. At a cellular level, pancreatic stellate cells are activated by cigarette smoke components, alone and in combination with ethanol through nicotinic acetylcholine receptors (nAChRs), suggesting the combined effects of alcohol and smoking on pancreatic fibrosis to facilitate chronic pancreatitis [58▪]. However, nicotine, a selective cholinergic agonist, is reported to have an anti-inflammatory effect by inhibiting HMGB1 release [59]. Activation of a 'nicotinic anti-inflammatory pathway'' mediates the α7 subunit of the acetylcholine receptor (α7nAChR) and reduces tissue injury and inflammatory response in an acute pancreatitis mouse model [60]. A more recent study reported nicotine-mediated immunosuppression in severe acute pancreatitis to be mediated through $CD4^+$ $CD25^+$ regulatory T cells [61]. These findings are controversial in view of the negative effects of smoking in epidemiologic studies, which means nicotine may not be a major negative effector and/or nicotine in combination with the other compounds in cigarette smoke has different effect relative to when present or is tested alone. Moreover, nicotine effect might vary depending on the stage or severity of acute pancreatitis. Thus, studies assessing the impact of smoking in tissue inflammation and injury have to be performed with consideration of the feature of smoke with multiple effects from many cigarette compounds. In addition, other host factors are likely to account for the heterogeneity and susceptibility of individuals to smoking related effects.

Cigarette smoke consists more than 5000 different compounds including almost 100 hazardous chemical compounds such as benzopyrene and dioxin. Some clinical and experimental studies have attempted to elucidate the mechanism underlying the effect of smoking on pancreatitis by searching for direct causative molecules and signaling pathways.

Endothelin-1 (ET-1) is secreted in excessive amounts by damaged endothelial cells and is elevated in plasma and the pancreas of smoking patients with chronic pancreatitis implying that ET-1 mediates an effect of smoking on the endothelium in chronic pancreatitis [62]. Our group recently reported the effect of smoking as a basis for immune-mediated dysfunction leading to progression of chronic pancreatitis in experimental models of chronic pancreatitis [63▪]. In this study, aryl hydrocarbon receptor (AhR) ligands in cigarette smoke promoted pancreatic fibrosis, a major characteristic of chronic pancreatitis, via induction of IL-22 and enhancing pro-fibrogenic effects of pancreatic stellate cells [63▪]. Notably, circulating levels of IL-22 were elevated in patients with chronic pancreatitis. Although the study needs to be replicated in a larger cohort of patients, those who continued to smoke had higher circulating IL-22 and more severe endocrine insufficiency suggesting that smoking-mediated AhR activation might cause both exocrine and endocrine dysfunction in chronic pancreatitis. These results offer potential immune mechanisms for the effects of smoking on chronic pancreatitis and further emphasize the importance of smoking cessation in the treatment of chronic pancreatitis. This notion is also supported by the fact that smokers with chronic pancreatitis experience a worse outcome and quality of life [64,65].

DIETARY FACTORS AND PANCREATITIS

There is a strong correlation between fat intake and obesity, which is a rising risk factor for pancreatic diseases [66,67]. Increased serum triglyceride levels in patients with acute pancreatitis correlate with persistent organ failure [68], and acute pancreatitis is associated with multisystem organ failure in obese humans potentially as a result of fatty acid-induced lipotoxicity [69]. Other mechanisms proposed include inflammatory cytokines, such as IL-18 and IL-12, which are elevated in diet-induced obesity and associated with disease severity and duration [70,71].

Vegetable and fish consumption may have a beneficial effect in the prevention of nongallstone-related acute pancreatitis [72,73]. Recent reports suggested a protective effect of coffee consumption in

acute pancreatitis [74]. Huang *et al.* [75▪] reported that caffeine reduces pathological calcium signaling in pancreatic acinar cells via the inhibition of inositol 1,4,5-triphosphate receptor-mediated calcium release and suggested caffeine and its metabolites as potential therapeutics for acute pancreatitis. Because oxidative stress plays a critical role in the pathogenesis of chronic pancreatitis, the effect of antioxidants such as, vitamin A, C, and E have been investigated and reported in some studies to have a beneficial influence on chronic pancreatitis by reducing pain and response to supportive treatments [76–78]. However, the effect of antioxidants on chronic pancreatitis and pain reduction needs further exploration and clear evidence since other studies failed to show beneficial effects [79,80].

CONCLUSION

Understanding mechanisms involved in exocrine pancreatitis and its progression is critical for developing targeted therapies. Recent studies reveal immune cells as significant contributors for the pathogenesis of acute and chronic pancreatitis. Innate immune responses can have dual function, where protection and exacerbation of disease can be context dependent as demonstrated with HMGB1 and HSP70 [4–7]. Furthermore, innate immune components such inflammasome and CARD protein activation further elucidate mechanism for pancreatitis development and their downstream products as potential biomarkers [13,14]. In addition to endogenous molecules, epidemiologic studies and more recent experimental findings identify environmental factors such as alcohol and smoking as critical contributors for the development of pancreatitis. Cigarette smoke components promote fibrosis in chronic pancreatitis via AhR activation-mediated IL-22 generation, and IL-22 induces pancreatic stellate cells to upregulate extracellular matrix gene expression. Such mechanism highlights a novel role for immune-pancreatic stellate cell cross talk in the progression of chronic pancreatitis [63▪]. Future studies are needed to identify the complex cellular interactions that contribute to pancreatitis pathogenesis and progression.

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Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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