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Role of Immune Cells and Immune-Based Therapies in Pancreatitis and Pancreatic Ductal Adenocarcinoma

Lei Zheng*, Jing Xue, Elizabeth M. Jaffee, and Aida Habtezion*

Stanford University School of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, Stanford, California and The Sidney Kimmel Cancer Center at Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Abstract

Immune cells are important in pathogenesis of acute pancreatitis (AP) and determine disease severity. Results from cytokine-based clinical trials for AP have been disappointing, so strategies that target and alter the behavior of infiltrating immune cells require consideration. Recurrent AP can progress to chronic pancreatitis (CP). CP is a well-described risk factor for pancreatic ductal adenocarcinoma (PDA). However, most patients with CP do not develop PDA, and most patients with PDA do not have history of pancreatitis. Interestingly, CP and PDA tissues have similarities in their desmoplasia and inflammatory infiltrates, indicating overlapping inflammatory responses. Further studies are needed to determine the differences and similarities of these responses, improve our understanding of PDA pathogenesis, and develop specific immune-based therapies. Immune cells in PDA produce immunosuppressive signals that allow tumors to evade the immune response. Unlike single therapeutic agent studies that block immunosuppressive mechanisms, studies of combination therapies that include therapeutic vaccines have provided promising results.

Keywords

acute pancreatitis; chronic pancreatitis; pancreatic ductal adenocarcinoma

Introduction

Premature activation of digestive enzymes in pancreatic acinar cells initiates autodigestion of the pancreas¹. However, acute pancreatitis (AP) is also an inflammatory disorder; a complex cascade of immunologic events affects disease pathogenesis and progression. Although intra-acinar or interstitial activation of trypsinogen is an early event in the development of AP², in recent years, many researchers have focused on the roles of leukocytes³. Macrophages, granulocytes, and T cells have important roles in the pathogenesis of AP. We review the contribution of the different leukocyte populations to pathogenesis and their role in therapy.

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*Correspondence to: (Dr Aida Habtezion and Dr Lei Zheng), Dr Aida Habtezion, Stanford University School of Medicine, Department of Medicine, Division of Gastroenterology & Hepatology, 300 Pasteur Drive, Stanford, CA 94305, USA; aidah@stanford.edu. Dr Lei Zheng, Department of Oncology, Johns Hopkins University School of Medicine, 1650 Orleans Street, CRB1 Room 488, Baltimore, MD 21287, USA; Lzheng6@jhmi.edu.

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Alcohol and gallstones are the most common etiologic factors in AP. Recurrent AP can lead to chronic pancreatitis (CP), a progressive inflammatory and fibrotic disease (Figure 1). CP eventually leads to irreversible damage and in late stages, pancreatic exocrine and endocrine deficiencies⁴. Histologic features of CP include pancreatic fibrosis, acinar cell atrophy, chronic inflammation, and distorted and blocked ducts⁵. Population-based studies reported that 20%–45% patients have a recurrence of AP, with the highest rates been among those with alcohol-related AP^{6–9}. Progression to CP following recurring AP has been reported in 4%–24% of patients—again more commonly among those with alcoholic recurrent AP^{7–9}. In a recent US population-based study, Yadav et al. found that readmission for a primary diagnosis of AP was 22%, whereas subsequent admission for CP was only 6%¹⁰. Consistent with previous studies, the risk for recurrent AP and progression to CP were greatest among users of alcohol and tobacco. Interestingly, in a long-term prospective study (1976–1992) of patients who had recurring AP and continued to consume alcohol, disease progressed to CP in 78%¹¹. A 30-year Danish follow-up study found that AP (alcohol-related and idiopathic) progressed to CP with a mean interval of 3.5 years¹².

Chronic inflammatory conditions of the pancreas are risk factors for the development of pancreatic ductal adenocarcinoma (PDA)^{13–17}. Patients with CP have a higher incidence of PDA¹⁸, and individuals with hereditary pancreatitis have a 40% cumulative risk of developing PDA in their lifetime¹⁹. The earliest genetic changes within chronically inflamed pancreatic tissue lead to production of cytokines that initiate a chain of events that results in tumor formation, growth, metastasis, and suppression of anti-tumor immune responses^{13–17}. Studies have shown that immune responses change as PDAs progress from low-grade pancreatic intraepithelial neoplasias (PanINs) to high-grade PanINs and invasive adenocarcinomas²⁰ (Figure 2). However, most patients with PDA have no history of AP or CP. Since most patients who present with AP have mild and self-limited disease, it is not clear whether patients that develop PDA have experienced bouts of subclinical or asymptomatic pancreatitis. Alternatively, sporadic PanIN lesions could induce a fibro-inflammatory response, similar to that found in patients with CP, that promotes development of PDA. Herein, we review the roles of adaptive and innate immune cells in pancreatitis and pathogenesis of PDA, and their potential as therapeutic targets.

Adaptive and Innate Immune Cells and Their Roles in AP

In AP, the initial inflammatory process leads to migration of monocytes and neutrophils into the pancreas, mediated by a multi-step process that involves adhesion molecules²¹. Hyperstimulation with caerulein leads to upregulation of intracellular adhesion molecule 1 (ICAM-1) in the pancreas, which mediates adhesion of neutrophils to the site of inflammation²². Neutrophils have been proposed to have important roles in the early phase of the disease development, contributing to activation of trypsinogen and progression to severe AP^{23, 24}. In support of this, ICAM-1 deficiency and systemic depletion of neutrophils were each shown to reduce the severity of AP and lung injury²⁵.

Monocytes and macrophages are key inflammatory cells involved in the pathogenesis of AP; the degree of macrophage activation might be an important determinant of the severity of AP. These infiltrating cells produce cytokines and inflammatory mediators such as tumor necrosis factor (TNF) α , interleukin (IL)1 β , IL6, monocyte chemotactic protein (MCP)1, and platelet activating factor (PAF)²⁶. Some of these mediators are initially released by pancreatic acinar cells, leading to the recruitment of neutrophils and monocytes (Figure 1). These leukocytes, in turn, produce cytokines and chemokines that recruit more leukocytes and increase the inflammatory response in the pancreas, followed by involvement of distant organs such as the lung²⁷. CC chemokines such as MCP-1, macrophage inflammatory protein (MIP)-1 α , and RANTES (CCL5) are believed to activate primarily monocytes.

Bhatia et al. showed that administration of bindarit, a blocker of MCP-1 synthesis, significantly reduced the severity of AP, indicating that MCP-1 is an inflammatory mediator during early stages of AP²⁸. Depletion of macrophages via injection of clodronate liposomes, protected against caerulein mediated pancreatitis in mice²⁹. Monocyte and macrophage populations, however, are heterogeneous, with great phenotypic and functional plasticity. Recently, CD11b^{hi}Gr-1^{low}CD11c⁻ monocytes (also Ly-6C^{hi}CCR2⁺), which originate in the bone marrow, were shown to be involved in progression of AP in mice²⁹.

Although macrophages are a major leukocyte population found in the inflamed pancreas, T cells are also present, in smaller numbers, at sites of inflammation. Based on studies of T-cell depletion and lymphopenic animals, activated T cells appear to be necessary for progression of AP³⁰. As AP progresses, there are changes in the number and ratio of CD4⁺ and CD8⁺ T cells. This could be because CD4⁺ T cells contribute to activation of macrophage, via antigen presentation and release of inflammatory cytokines³¹. In contrast to total depletion of CD4⁺ T cells, and consistent with functional heterogeneity of CD4⁺ T cells, recent data indicate that subset of CD4⁺ IL22⁺ T cells likely protect against AP in mice³².

Extra-Pancreatic Immune Responses

Although most episodes of AP are mild, some patients develop severe disease, with local and extra-pancreatic complications³³. The incidence of pulmonary complications is high among patients with severe pancreatitis, ranging from 15% to 55%³⁴. The severity of pulmonary complications ranges from mild hypoxemia, without clinical or radiological abnormalities, to severe acute respiratory distress syndrome. Renal dysfunction is another severe complication; it results from inadequate fluid resuscitation and/or septic complications. In contrast to lung and renal injury, hepatic injury is usually mild, but can still contribute to the systemic inflammatory response³⁵.

Pancreatic necrosis is the most severe local complication, and is associated with high risk of infectious complications. Infection and bacterial colonization of the necrotic pancreas are the most feared complications, and significantly increase mortality. Bacterial colonization and its associated inflammation likely result from failure of intestinal barrier function and translocation of intestinal microflora or their products into the splanchnic vascular bed³⁶. Pathogen-associated molecular patterns (PAMPs) derived from the intestinal microflora activate the host innate immune system via pattern recognition receptors, such as Toll-like receptors (TLRs) and nucleotide-binding domain and leucine-rich repeat containing molecules (NLRs)^{37, 38}. Activation of TLRs and NLRs likely mediates the mechanism by which bacterial translocation leads to severe AP. Consistent with this, mice that lack TLR4 develop less severe forms of AP³⁶, and polymorphisms in *TLR* genes have been associated with susceptibility to AP^{39, 40}.

Li et al.⁴¹ associated upregulation of TLR4 with increased expression of TNF α in peripheral blood mononuclear cells during early stages of AP, supporting an important role for TLR-4 in the pathogenesis of AP. In mice with caerulein-induced AP, pancreatic inflammation required the presence of intestinal flora, which acted via intracellular PAMP nucleotide-oligomerization domain 1 (NOD1)⁴². Furthermore, they found that NOD1 facilitated migration of CCR2⁺ monocytes to the pancreas in response to MCP-1. Therefore, NOD1 signaling promotes pathogenesis of caerulein-induced AP via its ability to respond to the intestinal commensal bacteria.

Interestingly, although blocking TLR4 through an MyD88-independent pathway protects against AP, MyD88 inhibition exacerbates pancreatic inflammation and neoplastic transformation, by increasing dendritic cell ability to promote development of T-helper

(Th)2 cells⁴³. Dendritic cells could therefore have dual roles, via TLR4 signaling checkpoints, that protect against inflammation but also produce inflammatory responses that lead to neoplastic progression. Thus, dendritic cells might be important therapeutic targets.

Immune Cell-Based Therapy for AP

Despite the important roles of cytokines in development of AP, and the abilities of IL-10, PAF antagonists, and anti-TNF α agents to reduce pancreatic inflammation in experimental models^{44, 45}, there have been discouraging and/or few results on the effects of cytokine manipulation from clinical studies. There have been conflicting results from randomized double-blind studies of the ability of IL10 to prevent endoscopic retrograde cholangiopancreatography-induced AP. One study found no difference between IL10 and control, and the study population of another, which reported effects of IL10, had an unusually high incidence of AP^{46, 47}. Similarly, the PAF receptor antagonist (lexipafant) did not reduce local complications, multi-organ failure, or mortality in a trial of patients with severe AP⁴⁸.

The high risk for infectious complications has precluded clinical studies of anti-TNF agents in patients with severe AP, although in a case report, infliximab reduced symptoms of AP in a patient with inflammatory bowel disease⁴⁹. Cytokine-based therapies might be difficult to develop for patients with AP, because cytokine levels vary among patients and the disease has a wide spectrum of presentation. However, strategies to target and alter the activities of immune cells that contribute to AP could have long-term therapeutic effects for a disease that has a broad range of presentation.

The roles of macrophages in the pathogenesis and progression of experimental AP make these cells interesting therapeutic targets. The heterogeneity and plasticity of macrophages, which sequentially exhibit pro- and anti-inflammatory properties, indicate that they not only mediate inflammatory injury but might also be induced to modify the sequence of events that occur during development of AP. Initial experimental strategies focused on inhibiting or depleting macrophages. Macrophage inhibitors (compounds such as gadolinium chloride, liposome-encapsulated dichloromethylene diphosphonate, and PAF antagonists) were shown to modulate the systemic inflammatory response⁵⁰. However, most studies administered the inhibitors before AP was induced, which is clinically less relevant, because most patients present following pancreatic injury.

Other approaches to modify the activities of macrophages have been tested. IL4 and IL13 have been tested for their abilities to convert pancreatitis-activated M1 peritoneal macrophages into reparative M2 macrophages. However, despite their ability to change M1 pro-inflammatory macrophages into M2 reparative cells in vitro, these cytokines were degraded and their activities were lost in vivo, before effects on the macrophages could be observed on experimental AP⁵¹.

Transfer of hemin-activated macrophages protects mice from AP injury,⁵² and administration of hemin protects against early and late stages of experimental AP⁵³. Heme-activated macrophages express high level of hemoxygenase-1, which promotes production of anti-inflammatory agents such as biliverdin and carbon monoxide, which induce IL10, IL22, and p38 MAPK^{32, 54}. Strategies to change macrophages, either in vivo or ex vivo, into cells with anti-inflammatory properties are therefore viable approaches to therapy. In a study involving a xenogenic system, human bone marrow-derived clonal mesenchymal stem cells (hcMSCs) were administered to rats with mild or severe AP⁵⁵. The hcMSCs induced Foxp3⁺ T regulatory (Treg) cells and suppressed pancreatic infiltration by T cells. Although more studies are needed in this area of research, stem cell-based immunosuppressive strategies could be developed as allogenic therapies for AP.

Roles for Adaptive and Innate Immune Cells in CP

Our understanding of fibrogenesis in the pancreas of patients with CP improved with the finding that pancreatic stellate cells (PSCs) regulate synthesis and degradation of the extracellular matrix proteins that comprise fibrous tissue⁵⁶. Under homeostatic conditions, PSCs are quiescent. However, PSCs are activated by toxic factors such as ethanol and its metabolites or by inflammatory cytokines and chemokines (Figure 1), which are up-regulated in pancreatic tissues of patients with CP. Such factors induce PSCs to proliferate and transform into myofibroblast-like cells⁵⁷. Moreover, activated macrophages were shown to stimulate collagen and fibronectin synthesis by cultured PSCs⁵⁸. The significance of infiltrating myeloid cells, and particularly macrophages, was further demonstrated by the requirement of myeloid (rather than acinar cell) nuclear factor (NF)- κ B p65 subunit to promote fibrosis in experimental CP⁵⁹.

Human pancreas samples were found to have significant increases in CD4⁺ and CD8⁺ T-cell infiltrates and *perforin* mRNA-expressing cells in CP lesions, compared with healthy pancreatic tissue, indicating the involvement of cell-mediated cytotoxicity⁶⁰. Although there were no differences in total leukocyte or T-cell populations, samples from patients with CP had increased numbers of CD4⁺ and CD8⁺ central memory T-cell subsets (CCR7⁺), compared to controls⁶¹. This increase in circulating memory T cells was apparent even in samples from patients with CP who had undergone pancreatic resection 0.5 to 3 years earlier, indicating ongoing, abnormal T-cell responses in patients with CP.

A more recent study investigated pancreas-specific T cell responses to antigens from lysates of human CP lesions, obtained during surgical resection⁶². Although T cells from healthy individuals, patients with pancreatic cancer, and patients with CP responded similarly to tetanus toxoid, T cells from CP patients had higher levels of IL10-based (mediated via CD4⁺CD25^{high}FoxP3⁺CD127⁻) responses to pancreatitis-associated antigens. In contrast, T cells from patients with pancreatic cancer responded to pancreatic carcinoma-associated, but not pancreatitis-associated, antigens and produced high levels of interferon- γ . Unlike patients with CP or pancreatic cancer, T cells from healthy individuals did not respond to either antigen, supporting the association between CP and changes in tissue- and disease-specific memory and regulatory T-cell responses. However, there are still no immune-based therapies for CP, probably because the disease is generally considered to be fibrotic and irreversible. This idea is likely to change based on above findings and the roles of PSCs and their interactions with immune and other pancreatic cells.

Acute and Chronic Pancreatitis Animal Models

Several animal models of AP have been created, using methods that range from simple and noninvasive to relatively complex and more invasive⁶³. Although these do not necessarily recapitulate all aspects of human AP, they have increased our understanding of the pathophysiologic mechanisms that contribute to inflammation, leukocyte recruitment, pancreatic acinar cell injury, and necrosis. The relative difficulty of accessing and analyzing human pancreatic tissue makes animal models necessary. Due to variations in responses among the different species and models, use of more than one model should be considered, especially for testing potential therapeutic agents. Experimental approaches that reduce established disease or prevent progression of disease as compared to those that test disease prevention are more likely to lead to effective therapies.

Similar to AP, several animal models of CP have been described and are based on repeat or prolonged pancreatic injury (eg, caerulein injections and duct ligation)⁶⁴. Genetic models have also been generated to allow for studies of specific gene products in pathogenesis, such as PRSS1, PRSS2, SPINK1, and CFTR. Transgenic mice that express Elastase-IL-1 β at an

early age develop CP that resembles the human disease⁶⁵. Interestingly, as these mice age, they develop acinar–ductal metaplasia, supporting the role of immune signaling in the development of CP and progression to metaplasia.

Innate and Adaptive Immune Cells in PDA Development

Genetically engineered mouse models of pancreatic cancer have facilitated evaluation of inflammatory factors within the developing tumor microenvironment⁶⁶. This is a relatively new area of study, but a number of different immune cell types, innate and the adaptive, have been found to contribute to the progressive inflammatory changes that lead to PDA (Table 1). The activities of these cells, and their roles in immune regulation during tumor development, have not been completely elucidated. However, tumor cells appear to alter the activities of immune cells to promote tumor growth and progression; the presence of these immune cells in PDAs has been associated with poor prognosis. Interestingly, a few of the best-characterized cell types promote tumor development, however these cells have the ability to alter their polarization and inhibit tumor development.

Tumor-Associated Macrophages

PanINs and PDAs have significant increases in macrophage infiltration, compared to normal pancreatic tissue. Tumor-associated macrophages develop from peripheral blood monocytes in response to tumor-induced cytokines such as macrophage colony-stimulating factor (m-CSF), chemokines, VEGF, etc^{67–69}. These macrophages are functionally divided into M1 and M2 subtypes⁶⁹. The M1 subtype is characterized by production of high levels of IL12 and IL23, toxic intermediates, and inflammatory cytokines such as TNF α . The M2 subtype is characterized by production of transforming growth factor (TGF) β and IL10 and promotes adaptive Th2-mediated immunity⁶⁹. The M2 subtype has immunosuppressive activities and has been associated with a poor prognosis⁷⁰. Although the M1 subtype is inflammatory, production of TNF α by these tumor-associated macrophages promotes tumor growth and epithelial to mesenchymal transition⁷¹.

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a group of myeloid-derived cells that lack markers of mature myeloid cells^{72, 73}. They have immunosuppressive activities and can inhibit T-cell functions. Increased numbers of MDSCs with suppressive activities have been found in PanINs and PDAs, compared with normal pancreatic tissue⁷⁴. Tumor-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) was recently shown to promote development of MDSCs and their recruitment to the tumor microenvironment of PDAs^{75, 76}.

Neutrophils

Neutrophils are often the first effector cells recruited to sites of acute inflammation. Although neutrophil infiltrates are rarely found in PDAs, they have been associated with undifferentiated types of PDAs and poor prognosis⁷⁷. Neutrophils have been functionally characterized into N1 (anti-cancer) and N2 (oncogenic) subtypes⁷⁸. The N2 subtype might be associated with early stages of PanIN progression (Keenan and Jaffee, unpublished data).

Mast cells

Mast cells are another type of cell in the innate immune response; they respond to tumor development by producing inflammatory cytokines⁷⁹. However, these cytokines appear to promote PDA growth and development^{80–82}.

Th and Treg Cells

Numbers of CD4⁺CD25^{high}Foxp3⁺ Treg cells are significantly increased in mice with late-stage PanINs and PDA⁷⁴. Their presence is associated with reduced numbers of CD4⁺ and CD8⁺ T cells⁷⁴. Treg cells have been identified in human PDA tissues, in association with tumor stroma, in significantly higher quantities than in non-neoplastic, inflamed pancreatic stroma. Higher levels of Treg cells have been associated with more poorly differentiated tumors, with high-grade PanINs, and with invasive PDA, compared to low-grade PanINs, and are also associated with poor prognosis^{74, 83}. In some cases, Treg cells are accompanied by effector CD4⁺ T cells with Th2 cell functions. The higher ratio of Th2 to Th1 cells in tumor infiltrating lymphocytes has been associated with shorter times of disease-free and overall survival^{84–86}.

CD8⁺ T Cells and Natural Killer Cells

Immune effector cells such as CD8⁺ cytotoxic T and natural killer cells are usually decreased in PanINs and PDA⁷⁴. In contrast to other inflammatory cells, increasing numbers of tumor antigen-specific CD8⁺ T and natural killer cells are associated with better prognosis^{87, 88}. However, even if immune effector cells are present in PDAs, they are not functional or are poorly cytotoxic.

Stromal Fibroblasts

PDA-associated stromal fibroblasts, which are PSCs, are not considered to be an immune component of developing PanINs and PDA. However, PSCs are activated and desmoplastic stroma forms during development of CP. The role for PSCs in the pathogenesis PDA is supported by evidence showing that PSCs increase proliferation of PDA cells, as well as tumor cell migration and metastasis^{89, 90}. Findings from Kraman et al. indicated that stromal cells support tumor growth through an immune mechanism. These investigators created transgenic mice with fibroblast activation protein (FAP) α , a protein expressed by human stromal fibroblasts, ablation to study the role of stromal cells in promoting tumor growth⁹¹. Depletion of FAP-expressing stromal cells from transplanted lung tumors and subcutaneous, Kras-induced pancreatic tumors in mice caused immune-mediated, hypoxia-induced necrosis of tumor and stroma cells. It is therefore possible that the desmoplastic stroma formed during development of CP could help create an immunosuppressive microenvironment that causes immune failure and tumor evasion.

The Immunopathogenesis of PDA

Many of the immune components of the PDA microenvironment are inflammatory cells or factors. However, these do not support anti-tumor immunity. Instead, these inflammatory components (which include macrophages, neutrophils, and mast cells) promote tumor growth and invasion. These cell types have been observed in low-grade premalignant lesions, and their numbers increase when the low-grade premalignant lesions progress into high-grade lesions and invasive PDAs. Many of these cell types are also involved in the establishment of CP^{13–17}. It is common to see evidence for CP in resected PDA specimens. PDAs can develop in patients who have had CP for long periods of time, although pancreatic carcinoma is more frequently accompanied by subclinical, pathology findings of CP. One hypothesis for the development of a carcinogenic inflammatory response in patients with CP is that immune cells are initially recruited and activated in response to an acute stimulus, such as during AP, and are followed by a different group of immune cells, which attempt to clean up damage from the acute insult (Figure 1). In this model, the acute stimulus could be delivered by AP, but is on most occasions subclinical, because AP rarely precedes the development of PDAs. As the acute insult becomes chronic, genetic changes occur in the pancreatic tissue. Alternatively, the tumor initiation process (occurring via

genetic alterations in early-stage PanINs and/or phenotypic changes in pancreatic ductal, acinar, and/or progenitor cells) induces an inflammatory response that mimics CP. Initially the inflammatory changes attempt to eliminate these early-stage, genetically altered pancreatic cells. However, with time, the anti-cancer response is insufficient to eliminate the genetically altered cells and alter their function to produce immunosuppressive signals. The inflammatory cytokines or toxic intermediates that are produced during this transition to a carcinogenic state can promote tumor growth. Once the tumor is established, the tumor microenvironment has a highly immunosuppressive composition (increased tumor-associated macrophages with an M2 phenotype, increased neutrophils with an N2 phenotype, Th2 cells, and Treg cells) that contributes further to immune evasion (Figure 2). There is evidence that the accumulation of genetic alterations during progression of CP to invasive PDA affects the inflammatory response, by providing additional factors such as IL-8 (via mutated Kras) and TGF β (via SMAD4) that promote recruitment of carcinogenic inflammatory cells^{13–17}.

Mouse Models to Study Inflammatory Responses and PDAs

Mouse models of PDA have accelerated our understanding of the inflammatory responses associated with their development and progression. The genetically engineered mouse model that most closely resembles the development of human PDAs was established via knock-in of a conditional Kras^{G12D} allele under the control of the Pdx1 or Ptf1a/p48 promoters⁹². These mice spontaneously develop PanINs that progress to invasive PDAs, much like the human disease. Human PanINs associated with activating mutations in Kras are thought to progress to PDA following a series of alterations in the p16/CDKN2A and TP53, and in the TGF β signaling pathway associated with the DPC4/SMAD4, TGF β RI, and TGF β RII. Transgenic expression of one or more of these proteins with Kras^{G12D} speeds the development of PDA, and tumors form with distinct pathologic features⁶⁶. These mouse models have been used to study the changes in the immune response during progression from PanINs to PDA⁷⁴.

Mice that express Kras^{G12V} under control of the elastase promoter in acinar cells are useful for studying the role of pancreatitis in PDA pathogenesis. The ductal-like cells observed in the PanINs that form in these mice do not arise through transformation of normal ductal cells^{93, 94}. Instead, PanINs originate by abnormal differentiation of elastase-expressing acinar cells or their progenitor cells. These mice develop PanINs and PDAs with high penetrance following induction of AP or CP with caerulein. Studies with this mouse model have indicated that pancreatitis might promote initiation of PanIN lesions and PDAs by reprogramming adult acinar cells. The tissue damage that results from pancreatitis might induce a tumorigenic inflammatory response mediated by macrophages and T cells. This mouse model has therefore become the leading model for the study of the relationship between pancreatitis and PDA.

Immune-Based Therapeutic Strategies

For many years, researchers believed that human PDAs were poorly immunogenic. However, our current understanding of the role of inflammation in development of PanINs and progression to PDA contradicts this concept. PDAs evade the immune response by sending immunosuppressive signals to the microenvironment, and inflammation promotes formation of early, premalignant lesions and their progression. The inflammatory response in PanINs resembles that observed in patients with CP (Table 2). Nonetheless, they are not the same—they likely involve different pathways, and many patients with pancreatitis do not develop PDAs. Based on the gross similarities between cells that infiltrate CP and PDA tissues⁹⁵, regardless of the differences in etiologic or pathogenic mechanisms involved, the

inflammatory outcomes (fibrosis and immune cell recruitment) could be similar. Studying the similarities and differences between CP and PDA, particularly using immune cell sub-type and functional analyses, could lead to immune-based anti-tumor therapies.

Inflammation can also induce immunosuppressive signals that allow tumors to evade the immune response. So the good news is that the immune system does gain access to the pancreas during transformation and stages of pre-malignancy. However, in order for immunotherapies to have effects on tumors, their immunosuppressive mechanisms must be defined and antagonized. In addition to inhibiting the carcinogenic inflammatory signals, it is important to induce a strong anti-tumor inflammatory response for an optimal therapeutic effect. Otherwise, a weak inflammatory response, like that which occurs during the early stages of PanIN development, might not be sufficient to eradicate the tumor cells. Therefore, an ideal immunotherapeutic strategy would be to combine agents that induce strong anti-tumor immunity with those that inhibit immunosuppression.

Vaccine-based immunotherapy can induce tumor antigen-specific immune responses. Several vaccine-based approaches have been developed, including an allogeneic, GM-CSF-secreting tumor cell vaccine (GVAX)^{96, 97} and a Muc1-pulsed dendritic cell vaccine⁹⁸. Each vaccine has been shown in phase I and II studies to prolong survival of patients with resected PDA compared with patients who did not receive the vaccines⁹⁶⁻⁹⁸. However, therapeutic agents that block immunosuppressive mechanisms were found to be ineffective as single agents in patients with metastatic pancreatic cancer^{99, 100}. Nonetheless, when ipilimumab, an antibody that inhibits the immune checkpoint CTLA-4, was given in combination with GVAX to patients with metastatic PDA, clinical and immune responses were observed that were associated with increased overall survival, compared to patients treated with only ipilimumab (Le et al. 2012 GI ASCO abstract - J Clin Oncol 30, 2012 (suppl 4; abstr 211)). These findings support the development of combination therapies, comprising vaccines and immune checkpoint inhibitors, for patients with PDA.

We are now realizing the complexity of the inflammatory changes associated with the development and progression of PDA. This complexity, however, provides numerous avenues for exploring targets for immunotherapy. All components of the inflammatory response are present in PDAs. However, there is an imbalance between these components and their respective functions, resulting in an immunosuppressive tumor microenvironment. The same inflammatory components that promote tumorigenesis might therefore be targeted to reestablish an anti-tumor microenvironment. This concept is supported by a recent study showing that an antibody agonist of CD40, given to patients with metastatic PDA in combination with gemcitabine, activated infiltrating macrophages to kill tumor cells and also increased the cytotoxic effects of gemcitabine chemotherapy, possibly by depleting tumor stroma¹⁰¹.

Tumor-derived GM-CSF promotes development of PDAs by recruiting MDSCs,^{75, 76} whereas secretion of a high intra-dermal concentration of GM-CSF, secreted by the tumor cell vaccine, facilitates anti-PDA immune response by activating dendritic cells^{96, 97}. Therefore, targeting macrophages, neutrophils, and mast cells is a promising strategy along with traditional immunotherapy that activates T cells. Furthermore, the stromal fibroblasts provide a protective environment that not only supports and promotes PDA tumor growth and progression, but also likely suppresses the development and/or access of anti-tumor immune responses. Thus, strategies to deplete the desmoplastic stroma before immune therapy is instituted could promote robust response against tumor cells.

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Figure 1. Immune cell involvement in pancreatic ductal adenocarcinoma, acute and chronic pancreatitis

Recurrent and continued pancreatic injury can lead to chronic pancreatitis (CP). CP can progress to pancreatic ductal adenocarcinoma (PDA), although most PDA likely arises independent of pancreatitis. Cytotoxic T lymphocyte (CTL), polysaccharide (LPS), myeloid derived suppressor cells (MDSC), natural killer cells (NK), pancreatic stellate cells (PSCs), platelet derived growth factor (PDGF), reactive oxygen species (ROS), regulatory T cells (Tregs), T helper cells (Th).

Figure 2. Immune cell infiltrates during pancreatic tumor progression

Infiltration by immune cells with immunosuppressive activities allows for an environment that fosters tumor growth and progression. Sequential acquisition of mutations in proto-oncogene *K-RAS* and the tumor suppressors *INK4A*, *TP53*, *DPC4*, *BRCA2*, and over-expression of cyclin D1 and mesothelin lead to pancreatic tumor progression. Effector T cells (Teff), myeloid derived suppressor cells (MDSC), natural killer cells (NK), pancreatic ductal adenocarcinoma (PDA), pancreatic intraepithelial neoplasia (PanIN), regulatory T cells (Tregs), T helper cells (Th).

Table 1
Summary of immune cells in the tumor microenvironment of pancreatic adenocarcinoma

Immune cell types	Infiltration and distribution			Associated cytokines	Functions	PDA-associated prognostic factors
	Pancreatitis	PanINs	PDA			
Neutrophil	high	present	rare	IL-8, C5a, MMP	Proinflammatory, Procancer	Undifferentiated types of PDA
Mast Cell	evenly distributed	evenly distributed	present at infiltrating edges of tumor	GM-CSF, INF γ , TNF α , IL-4, IL-13	Proinflammatory, Procancer	Poor survival
TAM	M1	high	present	INF γ , IL-12, IL-23, TNF α	Proinflammatory, Procancer	
	M2	low	high	IL-4, IL-10, IL-13, TGF β	Immunosuppression	Poor survival
MDSC	present	low	high	GM-CSF, VEGF, TGF β	Immunosuppression, Decreased CD8 $^{+}$ T cell infiltration	Poor survival
T cell	Treg	present	low	TGF β , IL-6	Immunosuppression, Decreased CD8 $^{+}$ T cell infiltration	Poor survival
	T helper	present	low	IL-2, INF γ	Immune activation	Good survival
		present	high	IL-4, IL-5, IL-6 IL-13	Immunosuppression	Poor survival
	Teff	present	low	INF γ , TNF α , IL-2	Anti-tumor immunity	Good survival

PanINs, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma ; TAM, Tumor associated macrophages; Treg, regulatory T cells.

Table 2

Comparison of pathological features between chronic pancreatitis and PanINs

Chronic pancreatitis	PanINs
Loss of acini	Cytological atypia and hyperchromatic nuclei with loss of polarity
Distorted and blocked ducts, terminal ductular proliferation	Adjacent parenchymal atrophy
Pancreatic fibrosis	Fibrotic foci
Chronic inflammation with mononuclear cell infiltrate	Inflammatory cell infiltration (mononuclear)