TOPIC HIGHLIGHT

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Complex role for the immune system in initiation and progression of pancreatic cancer

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Abstract

The immune system plays a complex role in the development and progression of pancreatic cancer. Inflammation can promote the formation of premalignant lesions and accelerate pancreatic cancer development. Conversely, pancreatic cancer is characterized by an immunosuppressive environment, which is thought to promote tumor progression and invasion. Here we review the current literature describing the role of the immune response in the progressive development of pancreatic cancer, with a focus on the mechanisms that drive recruitment and activation of immune cells at the tumor site, and our current understanding of the function of the immune cell types at the tumor. Recent clinical and preclinical data are reviewed, detailing the involvement of the immune response in pancreatitis and pancreatic cancer, including the role of specific cytokines and implications for disease outcome. Acute pancreatitis is characterized by a predominantly innate immune response, while chronic pancreatitis elicits an immune response that involves both innate and adaptive immune cells, and often results in profound systemic immune-suppression. Pancreatic adenocarcinoma is characterized by marked immune dysfunction driven by immunosuppressive cell types, tumor-promoting immune cells, and defective or absent inflammatory cells. Recent studies reveal that immune cells interact with cancer stem cells and tumor stromal cells, and these interactions have an impact on development and progression of pancreatic ductal adenocarcinoma (PDAC). Finally, current PDAC therapies are reviewed and the potential for harnessing the actions of the immune response to assist in targeting pancreatic cancer using immunotherapy is discussed.

Key words: Immune system; Pancreatitis; Pancreatic ductal adenocarcinoma; Immunosuppression; Immunotherapy; Inflammation

Core tip: The development and progression of pancreatic cancer is heavily influenced by the immune response. Inflammation of the pancreas (pancreatitis) is a significant risk factor for pancreatic cancer. Immune cells recruited to the inflamed pancreas release additional cytokines and potentiate damage to the tissue. Pancreatic cancer is characterized by profound immune suppression thought to be caused by signals originating from the tumor cells. Additionally, a subset of immune cells has been shown to support the growth of pancreatic cancer cells. Novel therapies for pancreatic cancer aim to utilize this unique immune environment to target this deadly disease.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, and with a 5-year survival rate of 6%, it is one of the deadliest cancers worldwide[1]. The development and progression of PDAC is strongly influenced by the presence of inflammation[2]. Inflammation of the pancreas (pancreatitis) is a strong risk factor for PDAC development[3,4], and has been described as a critical factor in the initiation[5] and maintenance[6,7] of pancreatic disease. Additionally, PDAC is characterized by marked immunosuppression[6], which is thought to promote tumor progression and invasion.

This review highlights the role(s) of the immune response in the development of PDAC, focusing primarily on inflammation. Inflammatory conditions of the pancreas that can lead to increased risk for PDAC (pancreatitis), as well as the role of the immune response in the progressive stages of pancreas disease development, are discussed. Data from clinical studies and rodent models are integrated to present an up-to-date consensus of the role of inflammation in the initiation and progression of pancreatic cancer.

IMMUNE SYSTEM: A BRIEF OVERVIEW

The immune system is characteristically activated in response to infection by a foreign pathogen. In the case of cancer, tumor-specific antigens are recognized by the immune system, turning the cancer cell, in essence, into a foreign pathogen[8]. This allows the immune system to act as an extrinsic tumor-suppressor system. However, over time the chronic immune response to the tumor drives immunoselection of tumor cells that are able to thrive in an immunocompetent environment[9], much like the resistant bacterial strains that emerge as a result of exposure to antibiotics.

The mechanism by which the immune system can initially protect a host from tumor growth, but in some cases subsequently promotes cancer progression, is termed cancer immunoediting[10]. Cancer immunoediting is a dynamic process consisting of three phases, (1) elimination, when the immune system overcomes and eliminates the tumor before it can progress to a clinically relevant disease; (2) equilibrium, when the immune system does not eliminate the tumor, but controls tumor growth; and (3) escape, which occurs when the tumor has overcome the immune system and progresses to a clinically apparent disease. This third stage is generally seen as a failure in the adaptive immune system to provide long-term protection from tumor development due to selection of less immunogenic tumor cell variants during the equilibrium stage. Additionally, tumor escape can be facilitated by active immunosuppression induced by the tumor itself or some form of immune compromise or immune deficiency[11]. It is through this process that the cells of the immune system can act as both friend and foe to the body in the face of cancer.

Innate immunity

The innate immune system is composed of those immune cells that are already present in the body and can be immediately recruited to a site of infection during the process of "inflammation". Innate immune cells include granulocytes, macrophages, mast cells, natural killer cells (NKCs) and dendritic cells (DCs). Table 1 reviews the mechanism(s) of action attributed to the innate immune cells in both the normal immune response and during the process of cancer development. Neutrophils are by far the most abundant granulocytes in the body and typically one of the first cell types to respond; they secrete cytokines and chemokines, modulating other cells in the immune response[12]. Macrophages, another major cell type of the innate immune response, remove dead or dying cells and associated debris *via* phagocytosis, as well as play a role in the adaptive immune response. Macrophage maturation in response to various signals produces one of two cell types, M1-polarized cells, which initiate the inflammatory response, and M2-polarized cells, which restrain the inflammatory response[13]. M2-polarized macrophages are immunosuppressive and can limit adaptive immunity by inhibiting T-lymphocyte proliferation, thus impeding the T-lymphocyte response[14].

In the context of cancer, two types of macrophages emerge, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSC). Both cell types are similar to M2 macrophages and are recruited by tumor cells to thwart the anti-tumor immune activity. TAMs inhibit T-lymphocyte responses[15] and secrete cytokines that promote the tumor phenotype and metastasis[16,17]. In addition to their ability to directly induce T-lymphocyte apoptosis[18], TAMs produce arginase-1[19], a metalloenzyme that metabolizes and depletes the environment of arginine, an essential compound for T-lymphocyte proliferation[20,21].

The second type of immunosuppressive macrophage often found in the tumor microenvironment is the MDSC. Similar to TAMs in function, they differ mainly by their cell surface markers. MDSCs have been shown to exhibit powerful immunosuppressive properties, in part through production of reactive oxygen species and peroxynitrite[22,23], expression of arginase-1[24,25], induction of regulatory T-lymphocytes (Tregs)[26], and depletion of available cysteine, another amino acid required by T-lymphocytes[27].

NKCs are circulating immune cells that are able to kill target cells *via* induction of programmed cell death. NKCs have been shown to eliminate tumor cells, and treated cancer patients with high circulating NKCs have significantly longer metastasis-free survival[28]. The gap between the innate and adaptive immune systems is bridged by DCs[29]. Activated, antigen-presenting-DCs travel to the lymphoid organs where they interact with, and activate, B- and T-lymphocytes. In this way, activation of DCs by foreign pathogens can lead to the activation of both the innate and adaptive immune responses, allowing the body to fully respond to the perceived threat[30].

Table 1 Major functions of the innate immune cells in both inflammation and cancer

Inflammatory cell	Immune function	Role in cancer
Neutrophil	Secretion of cytokines/chemokines to modulate other cells in the immune response	Maintenance of pro-angiogenic phenotype[242]
	Release of cytotoxic granules	Suppression of anti-tumor immunity[243]
	Phagocytosis	Promotion of metastasis[244]
Mast cell	Release of cytotoxic granules	Suppression of anti-tumor immunity[245]
	Enhancement of immune cell recruitment	Stimulation of angiogenesis[152]
	Permeabilization of blood vessels	Direct stimulation of cancer cell growth[246]
		Secretion of mitogenic factors[157]
Macrophage	Phagocytosis	
	Promotion of T-lymphocyte activation	
M1	Initiation of immune cell response	
	Antigen presentation	
M2	Wound repair	
	Immunosuppression	
	Tissue remodeling	
	Resolution of immune response	
TAM		Secretion of Arginase-1[19]
		Support of Treg activation[247,248]
		Promotion of angiogenesis[16]
		Enhancement of tumor metastasis[17]
MDSC	Suppression of NKC and T-lymphocyte activation	Production of ROS[22]
		Secretion of peroxynitrite[23]
		Secretion of Arginase-1[24,25]
		Induction of Treg[26]
		Depletion of cysteine[27]
NKC	Release of cytotoxic granules	Tumor cytotoxicity[28]
DC	Antigen presentation	

TAM: Tumor-associated macrophage; MDSC: Myeloid-derived suppressor cell; NKC: Natural killer cell; DC: Dendritic cells.

Adaptive immunity

Innate immunity is a powerful first defense against invading pathogens; however, it is most effective against cells bearing antigens that are common to many pathogens, and can be subverted by quickly evolving cell types, as in the case with cancer cells. The adaptive arm of the immune system is responsible for controlling those pathogens that have overcome the innate response and can provide long-lasting immunity against specific infectious agents.

T- and B-lymphocytes comprise the adaptive arm of the immune response. T-lymphocytes are grouped into classes based on their cell-surface proteins which mediate distinct effector functions. Cytotoxic T-lymphocytes express CD8 and are responsible for killing cells expressing foreign antigen by activating the target cell's apoptosis program, leading to subsequent cell death[30]. Helper T-lymphocytes express CD4 on their surface and assist in the activation of CD8+ T-lymphocytes, B-lymphocytes and macrophages *via* secretion of specific cytokines, thus extending their function across both the innate and adaptive immune responses[30]. A subset of CD4+ lymphocytes, Tregs, protects the body from autoimmune responses. Tregs suppress T-lymphocyte activation in a cytokine independent, cell-contact-dependent manner[31].

B-lymphocytes are one of the main cell types responsible for the body's ability to mount a long-term pathogen-specific response. Each B-lymphocyte produces a single species of antibody, and once activated, proliferates into an antibody-secreting effector cell. It is largely through the action of B-lymphocytes that the body is able to maintain an immunological memory and initiate and immediate response to foreign pathogens it has already encountered[32].

Although inflammation serves to protect the body from harm, it also plays a major role the development of disease, including cancer[33]. Pancreatic cancer, in particular, is heavily influenced by the inflammatory response associated with pancreatic injury and disease. Pancreatitis, or inflammation of the pancreas, is a relatively common condition that often leads to irreversible pancreatic damage and leaves the pancreas vulnerable to the development of neoplastic disease.

PANCREATITIS

The pancreas is comprised of both exocrine (acinar and ductal cells) and endocrine (islets of Langerhans) cells. The exocrine pancreas functions to produce and secrete digestive enzymes into the small intestine whereas the endocrine pancreas is primarily responsible for producing hormones crucial for glucose homeostasis. Dysfunction of the pancreas due to disease or injury can lead to impaired digestion, hypoglycemia and diabetes[34]. Acute pancreatitis (AP) is one of the most commonly diagnosed gastrointestinal diseases, with over 200000 patients admitted to the hospital each year[35]. Patients typically present with acute abdominal pain which may be accompanied by nausea and vomiting, and display increased serum concentrations of amylase and lipase[36]. Pathologically, AP

presents as acinar degranulation, increased occurrences of autophagosomes, formation of dilated acinar lumina and autodigestive fat necrosis[37]. Risk factors for AP include alcohol consumption, gallstones, and smoking, however many cases are idiopathic[38-41]. Although the clinical symptoms of AP often resolve completely, more severe cases can lead to serious complications and even death in a small percentage of patients[42]. Complications associated with AP include pancreatic necrosis[43], infection leading to sepsis[44,45], and systemic inflammatory response syndrome (SIRS) leading to distant organ damage and failure (multiple organ dysfunction syndrome, MODS)[46]. These complications significantly increase the risk of mortality from AP. Additionally, recurrent bouts of AP lead to fibrosis/damage and chronic pancreatitis (CP), a risk factor for the development of pancreatic cancer[4,47].

CP is characterized by acinar loss, extensive fibrosis and immune cell infiltrate, and is a strong risk factor for pancreatic cancer[47-49], which may develop 10-20 years following CP diagnosis[3]. Although CP shares many of the same risk factors, causes, and symptoms as AP, the clinical presentation differs dramatically. Serum levels of pancreatic enzymes amylase and lipase are elevated in AP due to the acute damage caused to the pancreas. In contrast, these enzymes are either normal or only mildly elevated in CP[50]. The chronic inflammation that is the hallmark of CP leads to permanent damage and loss of pancreatic function, leading to diabetes and pancreatic insufficiency[51]. Comprehensive reviews of the diagnosis and etiology of these diseases, as well as factors that distinguish CP from AP, are available[52-54].

Studies from both clinical cases and rodent models of pancreatitis have contributed to the understanding of the inflammatory response to AP and associated syndromes. AP is thought to originate with uncontrolled activation of pancreatic acinar cells and release of digestive enzyme stores leading to autodigestion of the pancreatic cells. This autodigestion releases cellular contents, triggering the recruitment of inflammatory cells. Those inflammatory cells release cytokines and other modulating factors that can amplify the inflammatory response, causing systemic inflammation that can progress to SIRS and MODS. The events responsible for initiating the premature activation of digestive enzymes are not fully understood, but include trypsin auto-digestion[55], generation of reactive oxygen species, disturbances in microcirculation[56], calcium overload, and leukocyte overstimulation[57], and are reviewed in detail elsewhere[58-61].

There is a close relationship between the inflammatory response to AP and clinical severity of the disease[62]. Indeed, overstimulation of leukocytes, specifically neutrophils, results in systemic activation and is thought to be a major cause for severe AP-associated death[57,63]. Many animal models for AP exist in which pancreatitis is induced by pancreatic injury or surgical blockage. While insights into the possible mechanisms of the immune response in the pancreatic disease process can be gained from these models, they do not exactly replicate the clinical disease. Additionally, it should be noted that whereas rodents are the model of choice to study the immune system and its interaction with organ systems, significant differences exist between the human and rodent immune systems in the balance of leukocyte subsets, and in the expression of inflammatory mediators, cytokines and cytokine receptors, as well as the significantly different immunological environments occupied by either species[64]. For these reasons, animal models of pancreatitis will be addressed separately in this section of the review.

Inflammatory response to AP: Clinical findings

AP is characterized by early recruitment and activation of polymorphonuclear cells, the majority of which are neutrophils[57]. Activation of neutrophils can be identified by rising levels of serum neutrophil elastase in the early course of AP, typically within the first two days of diagnosis[62,65,66]. This is accompanied by the detection of metabolically hyperactive granulocytes in the pancreatic tissue of AP patients within 48 h of admission to the hospital, suggesting that granulocyte activation is an early AP event[67]. Neutrophil recruitment is followed by recruitment of the macrophage-monocyte system in the subsequent 2-3 d, as determined by rising levels of C-reactive protein (CRP) in the serum[62,68]. As elastase and CRP are released by neutrophils and macrophages, respectively, the presence of these proteins in the blood of pancreatitis patients is used as an indirect indicator of the recruitment and activation of these inflammatory cells in the pancreas.

Complications associated with AP can be grouped into two phases: immune overactivation and immune suppression. In the first phase, control is lost over the local inflammatory response, leading to excessive and uncontrolled systemic activation of inflammatory cells and mediators[69]. This often leads to the development of SIRS and MODS, and is associated with death within one week of disease[70]. In a subset of patients, the body responds to systemic inflammation with compensatory anti-inflammatory response syndrome (CARS)[71]. CARS initiates the second phase of complications associated with AP and can lead to immune deficiency or suppression, rendering the body susceptible to infection. These patients go on to develop AP-associated infections[44] that are associated with excessive CARS.

Systemically, a decrease in circulating lymphocytes, including B- and T-lymphocytes (both CD4^{+} and CD8^{+}), as well as NKCs, is often seen in AP[67,72,73]. A decrease in circulating lymphocytes is associated with more severe disease and is often predictive of AP-associated systemic infection[74-76]. Kylänpää-Bäck *et al*[77] demonstrated a significant decrease in monocyte surface expression of human leukocyte antigen-DR (HLA-DR), a hallmark for systemic immunosuppression, in the first 24-48 h following AP diagnosis. Many independent studies have confirmed that a decrease in serum lymphocytes, as well as monocyte HLA-DR, correlates with more severe disease and increased mortality[73-75,77,78].

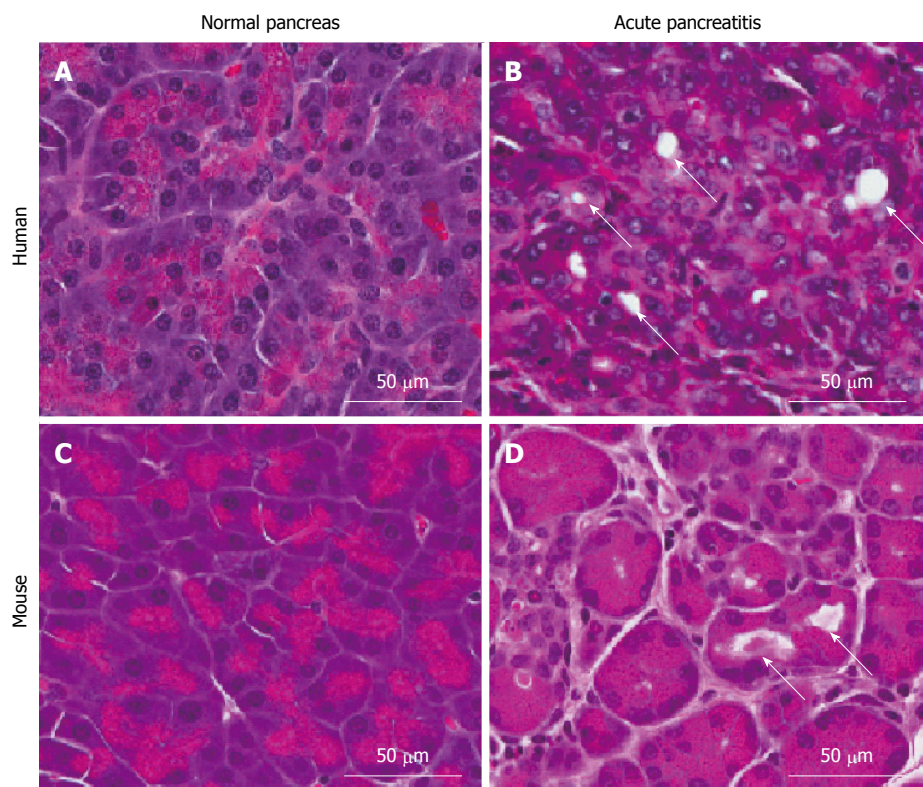It is important to understand that the clinical timeline of inflammatory response to AP is a relative concept, as

Figure 1 Comparison of histology of human and mouse pancreatic tissue. A: Normal human pancreas; B: Human acute pancreatitis (AP); C: Normal mouse pancreas; D: Mouse AP. Arrows indicate acini with dilated lumina, as commonly seen in AP.

data is dependent on the timing of the patient's admission to the hospital. Additionally, as biopsies are not routinely performed on AP patients, a detailed histological analysis of the pancreatic tissue is often not available. It is these limitations of clinical analysis that led to the use of rodent models in an attempt to more fully understand the mechanism of disease initiation and progression.

Animal models of AP

Several animal models have been developed to study the complex interactions that occur between the pancreatic epithelium and the many inflammatory cell types recruited to the pancreas. These models can be used to more precisely map the time course of the inflammatory response in AP, as well as to determine the mechanism(s) of damage and recovery in order to identify potential therapeutic targets. Although many species have been used to evaluate AP, this review will focus on data generated using rodents. Rodent models are used most often because of their cost-effectiveness and ease of characterization and genetic manipulation; however, no one model completely recapitulates all components of human disease.

The most common method of modeling pancreatitis in rodents is secretagogue hyperstimulation leading to premature intrapancreatic activation of digestive proteases. In this model, administration of high concentrations of the intestinal hormone cholecystokinin, or its molecular ortholog, caerulein, leads to autodigestion of the pancreas[79] and pancreatitis-like pathology including vacuolization, edema, acinar degranulation, dilated acinar lumina, necrosis, lung injury, and cytoplasmic destruction of pancreatic acini[80,81]. Figure 1 shows the histological similarities between human AP and caerulein-induced rodent AP. Another model for AP is surgical ligation of the pancreatic duct. Although this method was designed to mimic clinical gallstone-induced pancreatitis, it often produces a milder form of the disease and is more technically demanding and invasive[82]. Other models of AP include administration of high concentration of L-arginine leading to acinar necrosis[83], feeding of a choline-deficient diet, leading to severe necrotizing pancreatitis[84,85], and overstimulation of the immune system using bacteria or toxins[86].

Inflammatory response to AP: Animal models

Animal models of AP allow histological analysis of all stages of the disease, providing much information regarding the pathogenesis of AP. Although induced AP can present differently depending on animal model utilized, nearly all models result in a recruitment of neutrophils within hours of treatment (Figure 2), thus confirming neutrophils as one of the first responders to pancreatic damage[81,87-91]. Neutrophils have been shown to mediate systemic remote organ injury and death in a murine model of hemorrhagic pancreatitis[92]. Significant macrophage infiltration to the pancreas is observed shortly after caerulein-induced AP (Figure 2), and macrophage-derived macrophage-inflammatory protein-2 (MIP-2) is known to play a role in progression of AP through attraction of leukocytes, promoting tissue injury[93]. Therefore, results
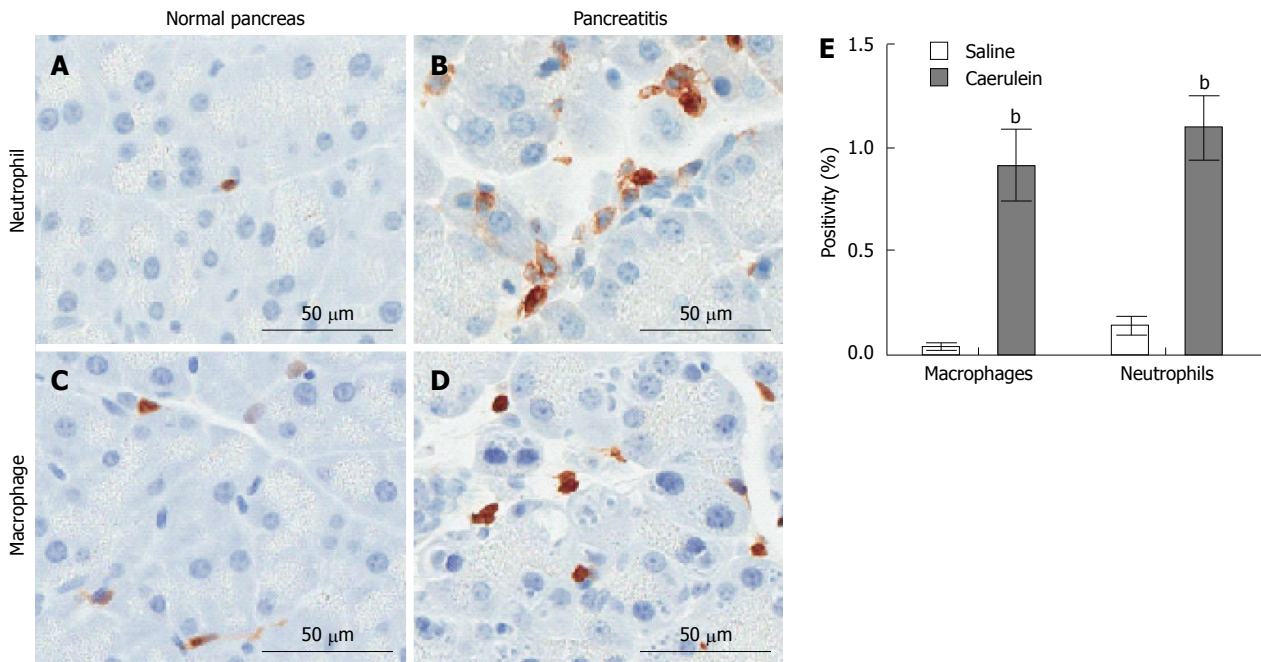
Figure 2 Induction of innate immune response in a mouse model of acute pancreatitis. Mice were injected intraperitoneally with 50 μg/kg caerulein or saline hourly for 7 h (Mayo Clinic IACUC protocol A48510). The pancreas was isolated one hour following the last injection and analyzed immunohistochemically for the presence of neutrophils (Ly6B.2: AbSerotec) or macrophages (Mac-2; Cedarlane Diagnostics). A: Neutrophil infiltration in saline-treated mice; B: Neutrophil infiltration in caerulein-treated mice; C: Macrophage infiltration in saline-treated mice; D: Macrophage infiltration in caerulein-treated mice; E: Stained slides were imaged using ScanScope XT (Aperio, Vista, California) and immunohistochemical staining was quantified using the Aperio ImageScope reader. Mean ± SD is plotted for each group of *n* ≥ 4 mice, [b]*P* < 0.01 *vs* saline group.

from caerulein-treated mice are consistent with clinical data that macrophages also contribute to the early inflammatory response to AP[93].

Mast cells are present in the normal pancreas and undergo degranulation upon induction of AP[87,94]. Inhibition of mast cell degranulation decreases pancreatic inflammation without affecting pancreatic damage[94], suggesting that mast cells primarily play a role in releasing or activating additional inflammatory mediators. DCs are rare in the normal pancreas, but pancreata of caerulein-treated mice exhibit a significant increase in mature DCs. These DCs are crucial for pancreatic viability during injury, as their depletion during caerulein- and L-arginine-induced AP leads to massive pancreatic cell death[95].

A distinct decrease in B- and T-lymphocytes is seen in serum from patients with AP, suggesting a systemic inhibition of the immune system. In support of impaired cell-mediated immunity, interleukin (IL)-2, a product of T-lymphocytes, is decreased in mononuclear splenic cells in a murine model of AP[96]. However, it has been theorized that decreases in circulating lymphocytes are not due to immune suppression but instead to a redistribution of lymphocytes from the blood pool to the pancreas[67]. This concept is difficult to test clinically, as most AP patients will not undergo pancreatic biopsy. In support of this theory, Demols *et al*[97] demonstrated that T-lymphocytes, specifically CD4^{+} cells, are increased in the murine pancreas following induced AP. This study showed that T-lymphocytes have a role in mediating tissue injury, as depletion of CD4^{+} T-lymphocytes, or elimination of T-lymphocytes using genetic models, reduced the severity of AP, and this injury was reversible by a T-lymphocyte transfer[97].

Role of cytokines in AP

AP is characterized by excessive recruitment and activation of leukocytes within the pancreas, and in many cases, other organs. Recently, a theory has emerged that the damaged pancreatic epithelium itself is responsible for expression of the first wave of inflammatory mediators, effectively launching the inflammatory cascade that leads to recruitment and activation of immune cells. This is supported by the fact that acinar cells are capable of producing a number of inflammatory mediators in response to damage or noxious stimuli[98-100]. When activated or stressed, isolated acinar cells have been shown to express cytokines[101-104] and chemokines[105] (Table 2 and Figure 3). Detailed reviews regarding the major roles of these mediators in AP and associated conditions are available[106-108]. In addition, activated immune cells secrete numerous chemotactic factors which are capable of perpetuating the immune cell activation cascade.

IL-1β and tumor necrosis factor (TNF) α production by pancreatic cells is thought to be a relatively early event in the activation of the cytokine cascade in AP, with IL-6 secretion occurring later in the disease process[109]. Serum IL-6 correlates with disease severity in humans[110,111] and is useful as an early diagnostic marker of AP[112,113]. In a rat model of pancreatitis, TNFα levels were shown to rise over time following induction of pancreatitis[114], and neutralization of TNFα *via* antibody improved all aspects of pancreatitis (elevated serum amylase, hematocrit, ascites) supporting an important role for TNFα in the
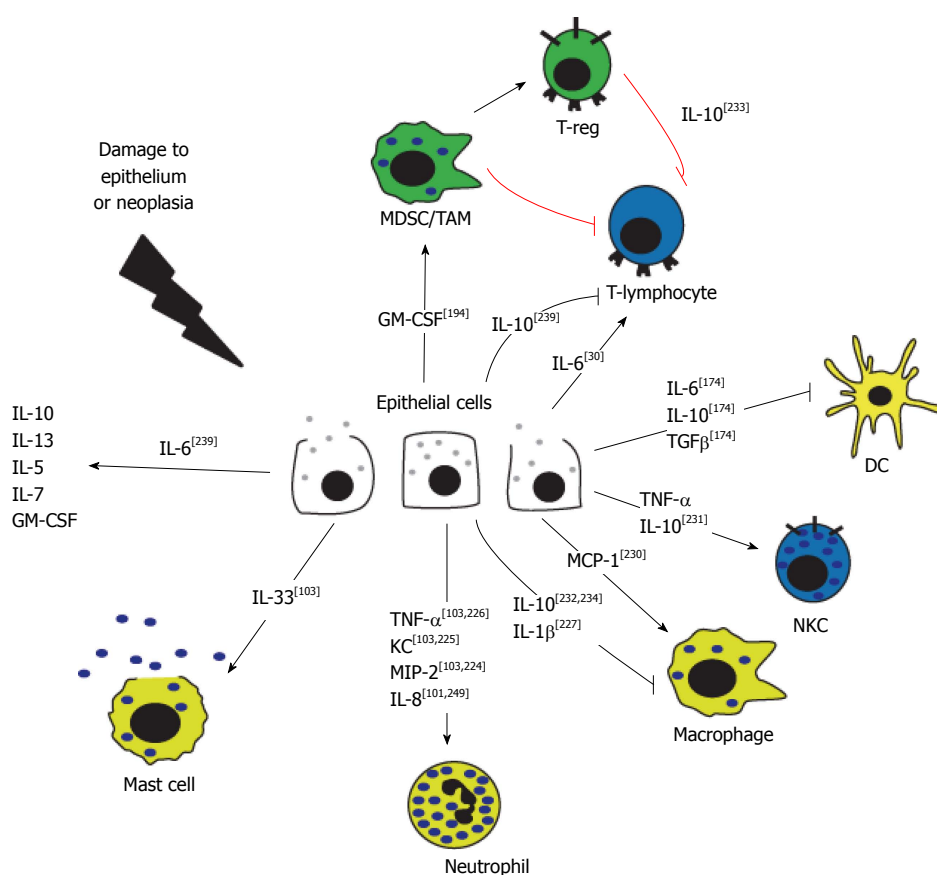
Figure 3 Cytokine signaling in the pancreatic epithelium. In response to damage or disease, the pancreatic epithelial cells release cytokines and chemokines. Activated immune cells secrete numerous chemotactic factors in response to, and in addition to, those secreted by the epithelial cells. TNF: Tumor necrosis factor; IL: Interleukin; MDSC: Myeloid-derived suppressor cells; TAM: Tumor-associated macrophage.

Table 2 Cytokines released during pancreatic injury

Cytokine	Expressed by	Acts on
GM-CSF	Acini[194]	Neutrophils[231], MDSCs[194]
IL-1β	Acini[104,238], activated macrophages[237], neutrophils[236]	Macrohpage (inhibition)[227]
IL-6	Acini[239], monocytes[30], macrophages[30], endothelial cells[30] fibroblasts[30], smooth muscle cells[30], IL-1b[30], TNFα[30]	CRP[228,229], T-lymphocyte[30], DCs (inhibition)[174]
IL-8	Acini[105], IL-1[241], TNFα[241], macrophages[241], neutrophils[235]	Neutrophils[223]
IL-10	Acini[240], Treg[233]	T-lymphocytes (inhibition)[233], DCs (inhibition)[174], macrophages (inhibition)[232,234]
IL-12	Activated macrophages[231], dendritic cells[30]	NKCs[231]
IL-33	Acini[105]	Mast cells[103]
KC	Acini[105], monocytes and neutrophils[250]	Neutrophils[225]
MCP-1	Acini[105]	Macrophages[230]
MIP-2	Acini[105], activated macrophages and neutrophils[93]	Neutrophils[224]
TNFα	Acini[105], activated macrophages[99], neutrophils[236], mast cells[226]	Neutrophils[226], NKC[231]

IL: Interleukin; MCP-1: Monocyte chemotactic protein-1; MIP-2: Macrophage inflammatory protein 2; TNFα: Tumor necrosis factor-α; MDSC: Myeloid-derived suppressor cell; NKC: Natural killer cell; DC: Dendritic cells.

pathogenesis of AP[114]. Blockade of IL-1 signaling using IL-1 receptor antagonist (IL-1ra) attenuates the rise in both IL-6 and TNFα, as well as lessens pancreatic damage in the context of AP, supporting early expression of IL-1 and confirming its role as an important mediator for subsequent cytokines[99].

IL-8 is another cytokine implicated in the early stages of the disease process. IL-8 is an established secretory product of activated macrophages[115], but has also been detected in the pancreatic epithelial cells of clinical and pre-clinical pancreatitis tissue[101,102], suggesting a damaged pancreatic epithelium as a possible source of IL-8. Gross *et al*[116] determined that IL-8 in the serum of pancreatitis patients correlates with disease severity, and showed a significant positive correlation between serum IL-8 and neutrophil elastase, a marker of neutrophils found upregulated in the early stages of AP. As IL-8 is a potent neutrophil activator[117], infiltration of neutrophils may be initiated by
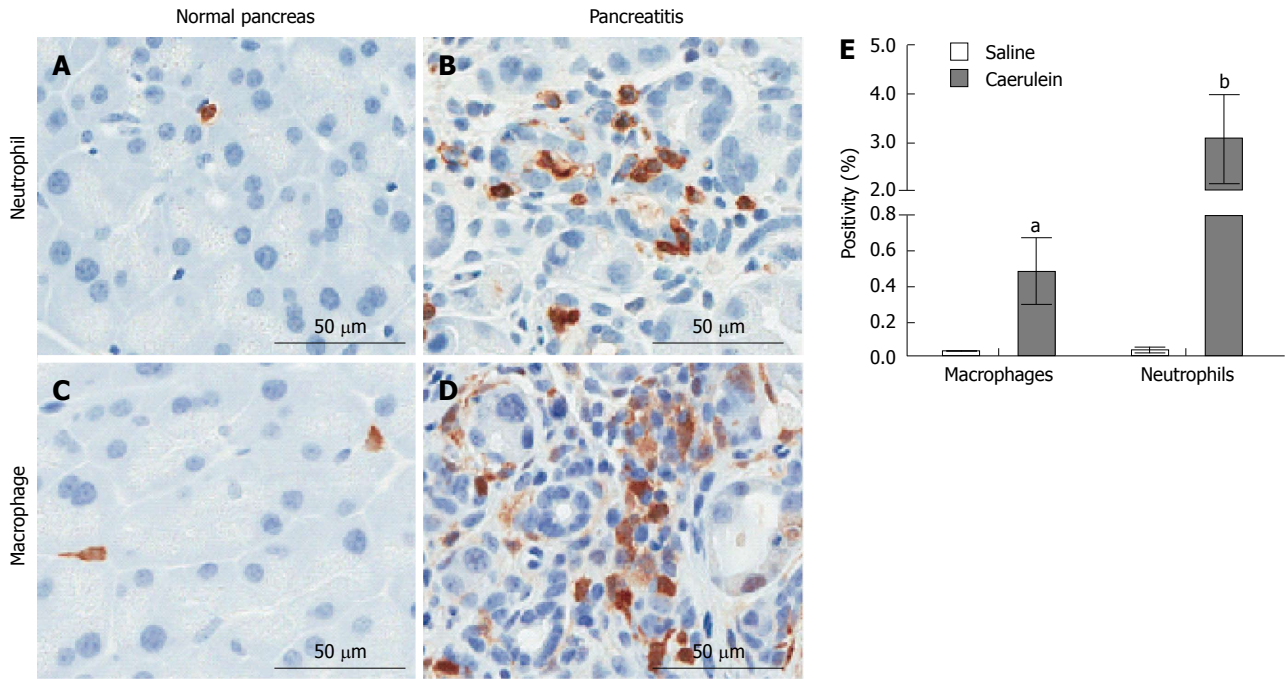
Figure 4 Induction of innate immune response in a mouse model of chronic pancreatitis. Mice were injected intraperatoneally with 250 μg/kg caerulein or saline twice daily, six days per week for two weeks (Mayo Clinic IACUC protocol A48510). The pancreas was isolated 24 h following the last injection and analyzed immunohistochemically for the presence of neutrophils (Ly6B.2: AbSerotec) or macrophages (Mac-2; Cedarlane Diagnostics). A: Neutrophil infiltration in saline-treated mice; B: Neutrophil infiltration in caerulein-treated mice; C: Macrophage infiltration in saline-treated mice; D: Macrophage infiltration in cearulein-treated mice; E: Stained slides were imaged using ScanScope XT (Aperio, Vista, California) and staining was quantified using the Aperio ImageScope reader. [a]$P < 0.05$, [b]$P < 0.01$ *vs* corresponding saline treated control. Mean ± SD is plotted for each group of $n \geqslant 5$ mice.

secretion of IL-8 from the pancreatic parenchyma. Resident and infiltrating macrophages can also secrete IL-8, recruiting more neutrophils and effectively reinforcing the cycle of inflammation.

Based on clinical studies and analyses of murine models of AP, our knowledge of the inflammatory events in AP can be summarized as: Damage to the pancreas, either caused by, and/or resulting in pancreatic autodigestion, leads to the release of inflammatory mediators from acinar cells. Many of these mediators are potent neutrophil attractants and are likely responsible for neutrophil recruitment to the pancreas as the first wave of inflammatory response. Once in the pancreas, activated neutrophils secrete additional cytokines, thus amplifying the inflammatory response by recruiting additional neutrophils as well as macrophages and other cells of the innate immune response. These cell types are often able to resolve the damage to the pancreas, with limited activation of the adaptive immune response. However, recurrent bouts of AP can lead to a much more serious condition, CP, which presents as a significant risk factor for the development of pancreatic cancer.

Whereas AP is often a self-limiting condition, CP is defined as longstanding inflammation of the pancreas that leads to progressive and irreversible changes. Clinically, CP is characterized by macrophage and T- and B-lymphocyte infiltration into the pancreas[118-122], although peripheral T-lymphocytes appear to decrease[123,124]. Infiltrating mast cells have also been described in the pancreas of CP patients[120,125,126], positively associating with pancreatic fibrosis[126] and pain[120,125]. Finally, immunosuppressive Tregs have been identified in the bone marrow, blood and lesions of CP patients[127].

In general, animal models of CP are generated by repeated induction of AP[128]. In a rat model of CP, both macrophages and CD8^{+} T-lymphocytes were prevalent in the connective tissue and parenchyma, and CD8^{+} T cells infiltrated the pancreatic lobules[118]. In a mouse model of CP, a significant increase in both macrophages and neutrophils were observed in response to caerulein (Figure 4), and depletion of these inflammatory cell types significantly reduced pancreatic injury as determined by serum amylase release, and pancreatic lesion formation and fibrosis[129,130]. Similar to clinical findings, mast cells were found to play a significant role in the pathogenesis of pain in a mouse model of CP[125]. Although a serious disease on its own, CP is also a significant risk factor for PDAC and may represent a condition that promotes PDAC development[131].

PANCREATIC CANCER

PDAC is the most common form of pancreatic cancer. PDAC is thought to develop by progression through a distinct series of pre-cancerous stages, pancreatic intraepithelial neoplasias (PanINs), before advancing to adenocarcinoma[132]. Greater than 90% of all pancreatic adenocarcinomas contain an activating mutation in codon 12 of the Kirsten rat sarcoma viral oncogene homolog (Kras)[133]. This mutation is thought to occur early in
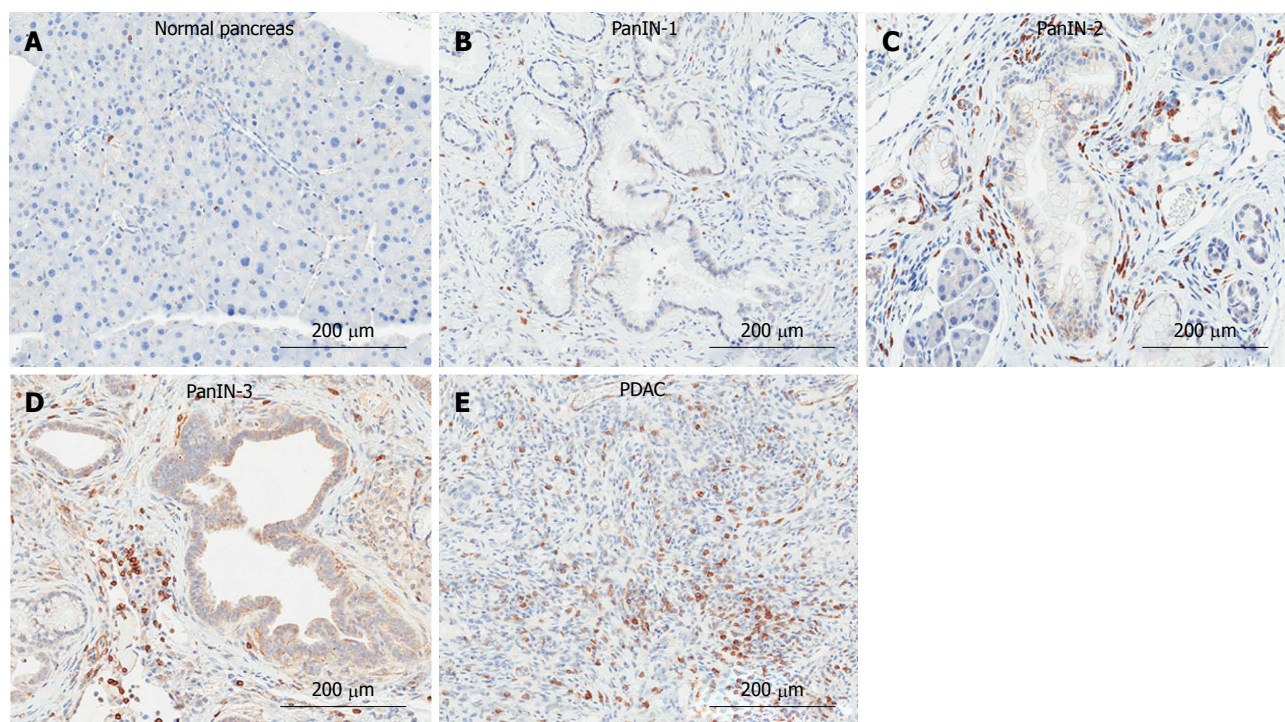
Figure 5 Immunohistochemical detection of T-lymphocytes in the Kras^{G12D}-induced mouse model of pancreatic ductal adenocarcinoma. A: Normal pancreas; B: Pancreatic intraepithelial neoplasia (PanIN)-1; C: PanIN-2; D: PanIN-3; E: Pancreatic ductal adenocarcinoma (PDAC) tissue was analyzed by immunohistochemistry for the presence of T-lymphocytes (CD3: abcam).

the disease process and drive initiation and progression of PDAC. Indeed, Kras mutations are prevalent even in early stage PanINs and their presence correlates with disease progression[134]. In a classic PDAC mouse model, tissue specific expression of oncogenic Kras^{G12D} from the endogenous Kras promoter (Lox-Stop-Lox- Kras^{G12D}; Pdx-1-cre, KC mouse model) in the mouse pancreatic epithelium recapitulates the full spectrum of human PDAC, including development of PanINs, with progression to adenocarcinoma at approximately 1-2 years of age[135]. For this reason, Kras^{G12D}-expressing mouse models are commonly used to study the initiation and development of PDAC.

It is well established that inflammation plays a major role in the development and progression of pancreatic cancer. Chronic inflammation of the pancreas (pancreatitis) is a significant risk factor for development of PDAC, and PDAC itself is characterized by marked leukocyte infiltration[4,47,48]. Notably, multiple immunosuppressive cell types are observed in pancreatic cancer tissue, suggesting a dysfunction of the immune response, likely mediated by the cancer itself (as described below). Immune dysfunction in PDAC is typified by (1) the recruitment and activation of immunosuppressive cell types; (2) the presence of tumor-supportive immune cells; and (3) a lack of immunity due to defective or absent immune cells. Clinical data and experimental rodent models contribute to the current understanding of the inflammatory response to PDAC as well as the impairment of that response that is a common evasion tool of most cancers.

Immunosuppression in PDAC

Clinical and murine evidence support the existence of profound immunosuppression in PDAC tissues. Clark *et al*[6] characterized the immune cell influx in various stages of PDAC ranging from normal pancreas to PanINs to invasive carcinoma using the KC mouse model of pancreatic cancer. The study describes an early immunosuppressive phenotype in pancreatic cancer, challenging the classic immunoediting "elimination" phase and suggesting that tumor cells "escape" from immune control almost immediately. At the early PanIN stages, Tregs and MDSCs dominate the immune infiltrate. As the disease progresses to PDAC, CD4$^+$ and CD8$^+$ cells are inconsistently found associated with the tumor, and those CD8$^+$ cells associated with the tumor lack evidence of activation, suggesting a suppressed immune environment[6]. In all stages of disease, there is a strong inverse correlation between MDSCs and CD8$^+$ T-lymphocytes, suggesting that MDSCs are a mediator of tumor immunosuppression[6].

Clinically, lymphocytes are prevalent in pancreatic cancer. CD8$^+$ cells are elevated in the circulation of PDAC patients[136], and leukocytes, the majority of which are T-lymphocytes, surround the pancreatic lesion[137]. T-lymphocytes are found more frequently in the fibrotic interstitial tissue than in the intraepithelial area of the pancreatic cancer[138], and distribute heterogeneously within the tissue, presenting as both scattered cells and focal areas of high accumulation[139]. An example of T-lymphocyte accumulation in progressive stages of the KC model of PDAC is shown in Figure 5.

The majority of the T-lymphocytes in PDAC are

CD4^{+} Tregs, supporting an immunosuppressive phenotype. Tregs are significantly increased in the blood of PDAC patients as well as in the pancreatic tissue[140]. They are found typically in the stromal areas of the tumor, and only occasionally in association with tumor epithelial cells[141]. Hiraoka *et al*[141] examined clinical samples of pre-malignant lesions and found that Treg accumulation correlates with the progression of both of the major preneoplastic lesions in pancreatic cancer, PanINs and intraductal papillary mucinous neoplasms (IPMN). The association of Tregs with IPMN progression has been independently confirmed[142]. Additionally, Tregs correlate with metastasis[143] and tumor grade, and negatively correlate with patient survival[141].

Treg infiltration in the development of pancreatic cancer is confirmed in murine models of PDAC. A significant accumulation of Tregs is found in the KC model of PDAC[6]. In another murine tumor model, subcutaneous injection of mouse pancreatic tumor cells into syngeneic mice results in a significant increase in Tregs in the spleen and tumor-draining lymph nodes of these mice[144]. Tan *et al*[145] have shown in both human PDAC and a murine model of pancreatic cancer that tumor cells produce elevated levels of ligands for the CCR5 receptor, a receptor preferentially expressed by Tregs. Interruption of this receptor-ligand signaling reduces tumor size, as well as Treg recruitment to that tumor, suggesting Tregs are likely recruited in response to direct signaling from the tumor cells.

MDSCs are another immunosuppressive cell type prevalent in PDAC that contributes to immune dysfunction. Whereas MDSCs are absent in the healthy human pancreas, they are readily detected in the stroma of PDAC, comprising approximately 67% of the infiltrating leukocytes[146]. MDSCs are also found in the blood and bone marrow of PDAC patients, and are significantly higher in cases of metastatic disease compared to patients with local tumor[146-148], supporting a previous report that MDSC count correlates with disease stage[149]. Additionally, circulating MDSC numbers are found to be an independent prognostic factor for survival[147].

The functional role of MDSCs in tumor promotion has been characterized in murine models of PDAC[146,147,150]. Following subcutaneous injection of the non-metastatic pancreatic cancer cell line, Pan02, MDSC infiltration is detected in bone marrow, spleen, and tumor[146]. This increase is associated with decreased levels of CD4 and CD8 and increased levels of Tregs in circulation. These MDSCs are able to suppress CD8^{+} T-lymphocytes *in vitro* and promote initial tumor growth when co-injected with Pan02[146]. Similarly, in a spontaneous murine model of tumor formation [driven by pancreas-specific overexpression of transforming growth factor (TGF) α and loss of Trp53], MDSC numbers increase in the lymph nodes, blood and pancreas as early as the pre-malignant lesion stage, and increase further upon tumor development. *In vitro*, these tumor-associated MDSCs were shown to possess arginase activity and suppress T-lymphocyte

responses[151]. In the KPC model of metastatic pancreatic cancer (driven by pancreas-specific expression of oncogenic Kras[G12D] and mutant Trp53[R172H]), MDSCs accumulate in tumor and spleen and comprise 20%-30% of all leukocytes. MDSCs are closely associated with tumor cells and metastases, suppress proliferation of T-lymphocytes, and express high levels of arginase and nitrite upon stimulation[150]. Collectively, these animal models strongly support a role for MDSCs in tumor promotion through T-lymphocyte inhibition and resulting immunosuppression.

To summarize, both clinical and animal models provide strong evidence for accumulation of immunosuppressive cells types, and subsequent inhibition of immune function in PDAC. The consequence of a suppressed immune system is not direct tumor promotion, but rather alleviation of an important barrier to tumor growth. As described by the cancer immunoediting model, a functional immune system can act as an extrinsic tumor suppressor, identifying and eliminating tumor cells[10]. Alterations in tumor immunogenicity or suppression of the immune response are crucial mechanisms by which this barrier is surmounted, allowing the cancer to progress.

Tumor-supportive immune cells

In addition to the immunosuppressive cell types that inhibit effector T-lymphocyte immunity, other immune cells adopt a tumor-supportive role in the context of PDAC. Once a tumor is established, factors secreted by effector immune cells can often promote, rather than prevent, tumor progression. Mast cells accumulate in PDAC tissue and, along with macrophages, express tumor-promoting factors including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)[152]. VEGF and FGF have been shown to stimulate the growth of pancreatic tumors and maintain blood vessels[153,154]. Mast cell accumulation correlates with higher tumor grade, worse survival[155], lymphatic and microvascular invasion[156], and lymph node metastasis[152,156]. In support of the idea that mast cells can support tumor growth, *in vitro* analyses demonstrate that mast cell-derived factors can promote PDAC cell migration, proliferation, and invasion[155,157]. Interestingly, the importance of mast cells in PDAC appears to be zone specific, with the presence of mast cells in the intratumoral border zone, and not in the intratumoral center or peritumoral zones, correlating with microvessel proliferation, lymph node metastases, and lymphatic and microvascular invasion[156]. Additionally, mast cell accumulation within the intratumoral border is an independent prognostic factor for survival[156]. The significance of mast cell localization likely stems from the ability to establish a pro-tumor microenvironment by degrading tissue surrounding the tumor to promote invasion and by remodeling blood vessels[156]. Taken together, preclinical PDAC models support a role for mast cells in tumor growth and for mast cell accumulation and function at the tumor border[158].

Inflammation can drive the early stages of pancreatic

lesion formation in mouse models[159]. A recent study demonstrated that two macrophage secreted inflammatory cytokines could mediate the effects of infiltrating macrophage on acinar cell metaplasia[5]. Matrix metalloproteinase (MMP)-9 and RANTES (regulated on activation, normal T cell expressed and secreted), secreted by pancreas-infiltrating macrophages, are implicated in driving the very first stages of pancreatic lesion formation in a mouse model of cearulein-induced pancreatitis[5].

Immune factors that are responsible for pathogen killing can also promote tumor cell migration and metastasis[160]. Additionally, tumor-promoting immune factors can promote tumor cells to adopt a more fibroblast-like morphology[155,157,160-162] and remodel the tumor environment[156,163] to facilitate migration. In these ways, neutrophil- and B-lymphocyte-derived proteins are implicated in PDAC invasion[160-163]. Neutrophil-derived elastase has been shown to mediate epithelial-mesenchymal transition (EMT) of PDAC cells *in vitro*[161,162]. Neutrophil-derived MMP-9 promotes PDAC tumor growth and angiogenesis[163], and MMP-9-producing neutrophils have been identified at the leading edge of PDAC in a murine model of pancreatic cancer as well at the invasive front of PDAC metastases[164]. Neutrophils, typically one of the body's first lines of defense, are not prevalent in PDAC epithelia[165]. However, they are associated with the predominant stromal component of PDAC, and several reports describe a significant increase in neutrophils when measuring levels in both PDAC tissue and stroma together[162,166]. In PDAC, neutrophils are associated with micropapillary carcinoma of the pancreas and correlate with poor prognosis[165,167]. Although density of neutrophil infiltrate does not correlate with tumor clinical stage[162], a patient's neutrophil: lymphocyte ratio is an independent prognostic indicator of survival following resection of PDAC tumor[168,169].

B-lymphocytes are also able to promote the tumor phenotype by secretion of B-lymphocyte activating factor (BAFF). Koizumi *et al*[160] described *in vitro* data demonstrating that BAFF mediates EMT, increases invasion and promotes motility of PDAC cells, supporting a role for B-lymphocyte-secreted BAFF tumor progression and metastasis. Additionally, BAFF-expressing B-lymphocytes surround and infiltrate tumor cells in clinical samples of PDAC, correlating with increased serum levels of BAFF[160].

Defective immune cells in PDAC

DCs are the link between innate and adaptive immunity, recognizing the presence of foreign pathogens and alerting the adaptive immune cells *via* antigen presentation. However, in a tumor environment, DCs often display an immature phenotype and are defective in their antigen-presenting abilities[170-173]. Tumors express various molecules which are thought to repress DC maturation[174] including IL-10[175,176], IL-4[177], VEGF[178], TGF-β[179], IL-6[180,181], and macrophage-colony stimulating factor[181]. Muc-1, a protein highly expressed on PanIN cells pro-

foundly affects the maturation of DCs. Monti *et al*[182] demonstrated that DCs exposed to tumor mucins do not fully mature and are characterized by a tolerogenic/regulatory cytokine profile, expressing high levels of IL-10 and low levels of IL-12. Peripherally, circulating DCs are significantly decreased in patients with PDAC[183-186], and demonstrate an impaired ability to stimulate T-lymphocyte proliferation[184].

Absent immune cells in PDAC

Similar to chronic pancreatitis, few NKCs are found in PDAC tissue[138]. The basal systemic NKC activity is decreased in PDAC patients, as well as the NKC response to interferon-α, a potent enhancer of NKC activity[187,188]. Decreased NKC activity has been linked to poor patient prognosis[188] and may be indicative of a suppressed innate immune response.

A role for epithelial cells in dysfunction of the immune response

Several lines of evidence point to a strong role for the neoplastic pancreatic epithelial cells in establishing a dysfunctional immune environment. As previously mentioned, PDAC cells can recruit Tregs[145], promote mast cell migration and activation[157], and repress DC maturation[182]. *In vitro*, human PDAC cells inhibit T-lymphocyte proliferation and migration, and this is accompanied by an increase in immunosuppressive cytokines including IL-8 and TGFβ[189]. Immunosuppressive compounds TGFβ and IL-10 are upregulated in PDAC and pancreatitis patient sera[190]. Finally, Muc-1, a mucin highly expressed in PanIN-1 early lesions, can suppress T-lymphocyte proliferation[191,192] and it has been suggested that Muc-1 is responsible for tumor escape from recognition and destruction by immune cells[193].

Murine models of PDAC provide additional support for the role of pancreatic epithelial cells in maintaining an immunosuppressive tumor environment. In the KC mouse model, Kras^{G12D}-dependent upregulation of granulocyte macrophage-colony stimulating factor (GM-CSF) is detected in PanINs, and results in recruitment of MDSCs and concomitant inhibition of CD8$^+$ T-lymphocytes[194]. This data is supported by the work of Bayne *et al*[150] who describe a model in which tumor-derived GM-CSF recruits myeloid inflammatory cells resulting in the negative regulation of CD8$^+$ T cells.

Interaction(s) between immune cells and stroma in PDAC

The development of PDAC is marked by increasing desmoplasia, resulting in the development of a vast stroma that often equals or exceeds the epithelial component of the tumor. The stroma is composed of extracellular matrix proteins and contains various non-epithelial cell types including stellate cells, endothelial cells, and immune cells, but the majority of the stromal cells are activated pancreatic stellate cells (PSCs) and fibroblasts[195]. Activated PSCs promote cancer cell growth and immune cell dysfunction

in PDAC[196,197]. PSCs have been implicated in promoting transformed growth, cellular invasion and EMT of PDAC cells, as well as in promoting PDAC tumor incidence, size, and metastasis in an orthotopic mouse model[198-200].

PSCs can have profound effects on the immune cell milieu of PDAC, and have been shown to express a number of growth factors and cytokines[201]. Inflammatory cells recruited to the tumor site are most often found in the stroma rather than infiltrating the epithelial cells[202]. Indeed, activated PSCs have been shown to attract and adhere to CD8^{+} T-lymphocytes, sequestering them in a juxtatumoral compartment (< 100 μm from tumor) and preventing their access to PDAC tumor cells[197]. One report showed that 94% of tumor-associated T-lymphocytes were either inactivated or did not make it to tumor because they were trapped in the tumor stroma[190]. A potential mechanism by which PSCs may regulate T-lymphocyte trafficking in PDAC is *via* CXCL12 expression, as PDAC T-lymphocytes express elevated levels of the CXCL12 receptor[197]. Additionally, activated PSCs express a number of cytokines and adhesion-mediating molecules[203], and have been shown to produce MDSC-promoting cytokines, including IL-6, M-CSF, and VEGF, and to promote differentiation of MDSCs from peripheral blood mononuclear cells[204]. Finally, PSCs stimulate mast cell activation, and, conversely, mast cell-derived factors can stimulate PSC proliferation[157]. These data suggest that not only can activated stellate cells directly promote the cancer cell phenotype, but they also contribute to the immunosuppressive phenotype that characterizes PDAC and hampers immunotherapy efforts.

Cancer stem cells

In addition to the immune dysfunction that is prevalent in PDAC, new evidence is emerging to support a role for immune-cytokines in promoting cancer metastasis *via* interaction with cancer stem cells (CSCs). CSCs are characterized by their ability to self-renew, capability to develop into multiple lineages, enhanced tumor-initiating ability, and resistance to typical cancer therapies[205]. CSCs are thought to be responsible for cancer progression, resistance to standard therapies and tumor relapse. Specifically, pancreatic CSCs have been shown to play a crucial role in the aggressive nature of pancreatic cancer and the resistance to therapy that is a hallmark of this cancer (reviewed in Dorado *et al*[206]). They share an intimate relationship with the tumor microenvironment, regulating, and being regulated by, cells present therein[207,208]. Specifically, TAMs regulate CSC tumorigenicity and anticancer drug resistance through production of specific growth factors[208]. Additionally, inflammatory cytokines play a role in mediating CSC self-renewal[209].

Recent work suggests that inflammatory cells can promote dissemination of pancreatic CSCs. Rhim *et al*[210] reported that pancreatic cancer cells exhibiting cancer stem cell properties, including enhanced tumor-initiating capacity, survival, and self-renewal, left the pancreas and were detected in the circulation at the immediate early stages of pancreatic cancer development (PanIN formation), prior to overt tumor formation. The presence of CSCs in the circulation was significantly elevated following induction of pancreatitis, and conversely, treatment with an anti-inflammatory drug, dexamethasone, resulted in a significant decrease in the circulating CSCs. These data support a role for inflammation in promoting dissemination of pancreatic CSCs and potentially in PDAC metastasis.

THERAPY FOR PDAC

Immunotherapy, therapeutic modulation of the immune response, has emerged as a promising line of treatment for many cancers including PDAC[211,212]. Types of immunotherapy that are currently being tested in clinical trials for pancreatic cancer include whole cell, peptide/DNA, antigen pulsed DC, and monoclonal antibody vaccines. Whole cell vaccines typically use irradiated pancreatic cancer cells as the immunogen. These cells have the potential to elicit a robust immune response because they express the full complement of tumor-associated antigens. There have been significant survival advantages reported in resected pancreatic cancer patients using whole cell vaccines such as Algenpantucel-L, an irradiated, live combination of two allogenic pancreatic cell lines[213]. Vaccines comprised of peptides or DNA corresponding to tumor antigens are designed to enhance the cytotoxic T-lymphocyte response. Peptides corresponding to oncogenic Ras, telomerase, VEG-F receptor, carcinoembryonic antigen (CEA), survivin, and Muc-1 have all been successful at prolonging life in pancreatic cancer patients in clinical trials[211,213].

Antigen-pulsed DC vaccines take advantage of the antigen-presenting abilities of DCs to elicit a robust adaptive immune response specific to a tumor antigen of choice[214]. DCs pulsed with Muc-1 and CEA antigens have both been used in clinical trials for pancreatic cancer[215]. Finally, monoclonal antibodies against cell surface tumor antigens are used to induce antibody-dependent cell cytotoxicity[211]. Clinical trials have evaluated antibodies against mesothelin, CEA, and epidermal growth factor receptor for pancreatic cancer treatment[211,215]. Of importance, the success of an anti-cancer vaccine relies on its ability to elicit an immune response in the host, and thus may not exhibit uniform effectiveness in all patients depending on the ability of their immune system to generate a response to treatment.

The stromal compartment has drawn recent interest as a target for PDAC therapy. The stroma creates an inflammatory environment and promotes tumor progression; and the extent of activated stroma has been identified as a novel independent prognostic marker in PDAC[216]. Several potential PDAC therapies have targeted the stroma[217-219]. One method in particular aims at overcoming the immunosuppression often found in, and potentially caused by, cells of the stroma. CD40 agonists take advantage of the inflammatory cells found within the stroma[218].
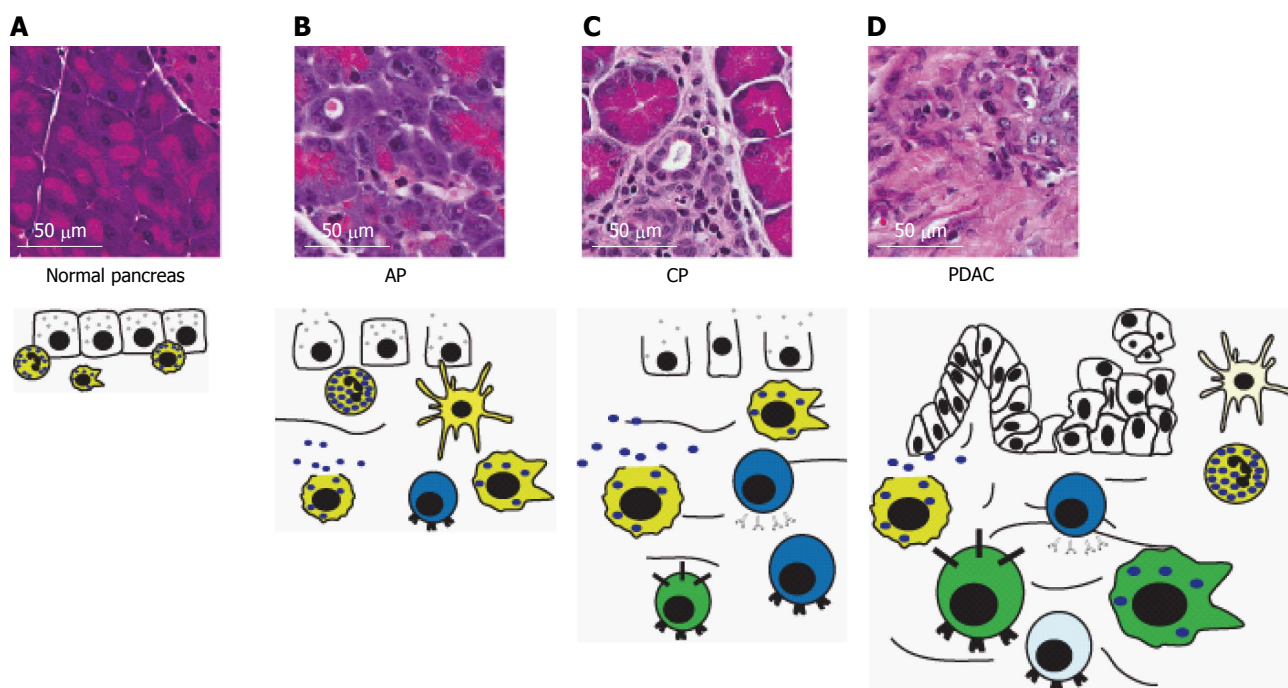
Figure 6 Immune cells in progressive pancreatic disease. A: The normal pancreas contains sparse, mostly innate, inflammatory cells and lacks the dense stroma typically seen in chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC); B: Acute pancreatitis (AP) is characterized by acinar degranulation, edema and recruitment of mostly innate inflammatory cells, but also some T-lymphocytes in response to acinar damage. Pancreatic mast cells begin to degranulate; C: CP is characterized by development of stroma surrounding degranulated acinar cells, acinar-to-ductal metaplasia and edema. Additionally, there is an increased presence of macrophages, and T- and B-lymphocytes, and further degranulation of mast cells; D: Development of pancreatic intraepithelial neoplasias and subsequent PDAC leads to a significant increase in immunosuppressive cell types including tumor-associated macrophages, myeloid-derived suppressor cells, and Tregs. Degranulated mast cells, neutrophils, dendritic cells and B- and T-lymphocytes are also present. However, T-lymphocytes and dendritic cells are typically inhibited and defective, respectively. Size of immune cell represents relative abundance. Cell color denotes immune cell type: yellow, innate immune cells; blue, adaptive immune cells; green, immunosuppressive cells. For description of graphical representation of cell types, see Figure 3 legend. Cells that are inactivated or defective are represented by a lighter color.

CD40 is the co-stimulatory factor needed for activation of the T-lymphocyte-dependent anti-tumor response of the immune system. It is thought that activating stromal T-lymphocytes may overcome the immunosuppressive environment that is a hallmark of PDAC. Co-treatment with a CD40 agonist and gemcitabine showed therapeutic efficacy in patients with metastatic PDA[218].

Whereas cancer immunotherapy has typically involved treatment with cancer antigens to stimulate or boost the anti-cancer immune response, the immunosuppressive environment found in PDAC presents obvious challenges. Vaccination with self-antigens has been associated with induction of immunosuppressive cell types, thus potentiating, rather than inhibiting, tumor growth[220]. Successfully overcoming the immunosuppressive environment that characterizes PDAC will likely require a multifaceted approach due to the multiple mechanisms by which tumor-associated immune dysfunction seems to occur. However, new evidence suggests that immunotherapy can be successful for pancreatic cancer, if stimulation of the immune system is combined with control over the immunosuppressive environment[221,222]. Developing new methods to overcome immunosuppression or exploit the immune response to target PDAC may be utilized in combination with conventional or novel chemotherapy to enhance the survival of this currently deadly disease.

CONCLUSION

The immune system protects the host from invading pathogens and foreign materials. The aberrant expression profile and uncontrollable proliferation that characterizes tumor cells should allow for recognition as non-self by the immune system. However, we now know that from a very early stage in PDAC development, the ability of the immune system to identify and eliminate neoplastic cells is compromised. This suggests that an immunosuppressive environment is established early in tumor development to effectively thwart the immune response to neoplastic cells at the onset of tumor development. Figure 6 depicts the progressive changes in immune cell infiltrate found during various stages of pancreatic disease.

Based on data reviewed here, neoplastic cells produce compounds (such as GM-CSF) at a very early stage of pancreatic cancer development, that recruit immunosuppressive immune cells, potentially facilitating progression to later PanIN stages and PDAC[190-192,194]. Once PDAC cells are present, they actively prevent the maturation of dendritic cells, inhibiting their antigen presenting activity and effectively cutting off a major communication between the innate and adaptive immune response[182]. In addition, immunosuppressive Tregs and MDSCs accumulate in the blood, stroma and PDAC tissue and inhibit T-lym-

phocyte proliferation[6,140,141,144-146]. In this setting, even immune cell types considered pro-inflammatory add to the tumor-supportive environment: neutrophils accumulate in the stroma and secrete proteases that aid in EMT, tumor motility and invasion[161,163] and mast cells accumulate in the tumor tissue and express factors used by the tumor to sustain its growth[152]. It is clear that a complex relationship exists between the immune system and the developing pancreatic cancer, and that these interactions have important implications for disease prevention and control. Immunotherapy can potentially be a powerful component of PDAC treatment. Further study of the mechanisms by which immunosuppression is initiated in PDAC, and ways to overcome it, will facilitate the development of this treatment option[22,104,223-249].

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