Pathophysiology of Acute Pancreatitis: Potential Application from Experimental Models and Human Medicine to Dogs

Caroline Mansfield

The cellular events leading to pancreatitis have been studied extensively in experimental models. Understanding the cellular events and inciting causes of the multisystem inflammatory cascades that are activated with this disease is of vital importance to advance diagnosis and treatment of this condition. Unfortunately, the pathophysiology of pancreatitis in dogs is not well understood, and extrapolation from experimental and human medicine is necessary. The interplay of the inflammatory cascades (kinin, complement, cytokine) is extremely complex in both initiating leukocyte migration and perpetuating disease. Recently, nitric oxide (NO) and altered microcirculation of the pancreas have been proposed as major initiators of inflammation. In addition, the role of the gut is becoming increasingly explored as a cause of oxidative stress and potentiation of systemic inflammation in pancreatitis.

Key words: Cytokine storm; ROS; Pancreatic necrosis.

A considerable amount of work has elucidated the cellular events that initiate pancreatitis and the resultant inflammatory cascades over the past 3 decades. Most of the investigations have been conducted on experimental rodent models, with a resultant extrapolation to naturally occurring disease that may not always be appropriate.1

Pancreatitis develops when there is excessive activation of trypsin and other pancreatic proteases within the pancreas that overwhelms both the local safeguards within the acinar cell and antiproteases in the circulation.2 This initial activation may be caused by oxidative stress or hypotension, and it is worsened by low acinar pH and high intracytosolic calcium concentrations.3–7

After initial production of active pancreatic enzymes, local inflammation ensues. Neutrophil migration then occurs directly attributable to activation of trypsin and chymotrypsin, and subsequent production of reactive oxygen species (ROS) and NO contributes to ongoing inflammation.8 There is a complex and closely interrelated set of pathways between cytokine production and this inflammation. Interleukin-8 (IL-8) initiates neutrophil migration early in the course of the disease, and upregulates intercellular adhesion molecule-1 (ICAM-1) to promote adhesion of neutrophils to the endothelial wall.9,10 Major factors that then play a role in progression of disease include altered pancreatic microcirculation, ischemia-reperfusion injury, a shift from acinar cell apoptosis to necrosis, and complex interaction and stimulation of a variety of inflammatory pathways (complement, kallikrein, renin angiotensin system [RAS]).

Unfortunately, despite increased understanding of the pathophysiology of pancreatitis, a 5–15% mortality rate is still reported in human medicine.11 This may reflect the fact that laboratory models tend to focus on one particular intervention or disease pathway. Consequently, the clinical manifestations and multiple pathways present in naturally occurring disease may not be taken into account. Despite flaws in translating experimental knowledge to clinical cases, an improved understanding of the pathophysiology of acute pancreatitis at a cellular level likely will serve to improve therapeutic interventions in dogs.

Animal Experimental Models

To fully interpret the advances made in understanding the pathophysiology of pancreatitis, it is important to have an understanding of the experimental models used and how they may relate to naturally occurring pancreatitis in dogs. These methods have been well summarized1 and are reviewed briefly here. The most commonly applied noninvasive method is administration of a cholecystokinin (CCK) analog (cerulein) IV, SC, or intraperitoneally. It has been applied to a number of species, including dogs.12 This results in a mild, self-limiting form of pancreatitis that is useful for

Abbreviations:
PSTI pancreatic secretory trypsin inhibitor
IL Interleukin
TNF tumor necrosis factor
NF nuclear factor
ROS reactive oxygen species
NO nitric oxide
NOS nitric oxide synthase
ICAM intercellular adhesion molecule
RAS renin angiotensin system
NK natural killer
CCK cholecystokinin
PLA-2 phospholipase A_2
PAF platelet activating factor

From the Faculty of Veterinary Science, The University of Melbourne, Werribee, Vic, Australia. All writing was undertaken at the University of Melbourne, and was not supported by a grant or other funding body.

Corresponding author: C. Mansfield, Faculty of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria, Australia 3030; e-mail: cmans@unimelb.edu.au.

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studying pulmonary pathology, but is probably only reflective of a proportion of dogs with pancreatitis seen in veterinary practice. A dietary model based on feeding a choline-deficient diet with ethionine is simple and highly reproducible, and causes a severe necrotizing form of pancreatitis.¹³ This model correlates well with severe acute pancreatitis seen clinically in dogs because it results in hypovolemia, hypoxia, pancreatic necrosis, and acidosis. It is very useful for assessing interventions that might decrease mortality, but is limited by sex (female) and species (mice) specificity.¹⁴ Other noninvasive methods such as ethanol administration, gene knockout, or immune modulation are difficult to reproduce and costly to perform, and seldom are undertaken unless studying a specific genetic abnormality or pharmacologic intervention. A recently developed protocol is administration of high-dose arginine, which results in dose-dependent pancreatic necrosis.¹⁵ Long-term administration results in chronic pancreatitis, which also may be of benefit in canine medicine as it could allow for evaluation of the insulin-acinar axis, and evaluation of the sentinel acute pancreatitis event (SAPE) theory which hypothesizes that chronic pancreatitis results from continued inflammation after an initial acute insult to the pancreas.¹⁶

One invasive method of investigating pancreatitis includes the closed duodenal loop model, which closes off the pancreatic duct and diverts bile to the jejunum.¹⁷ This model induces necrosis rather than inflammation and is associated with a high mortality rate, often precluding assessment of therapeutic interventions. If the loop is temporarily closed, and trypsin along with sodium taurocholate is injected into the duodenum, the pancreatitis is milder and therapeutic interventions are easier to study.¹⁸ It is unknown how much of a role duodenal reflux plays in causing pancreatitis in dogs, and this model may not be valid for comparative studies. Perhaps the most promising methodology for canine disease is combined hypersecretion (injection of CCK) and low-dose infusion of glycodeoxycholic acid into pancreatic ducts.¹⁹ This method causes widespread necrosis and inflammation of the pancreas, but poses some technical challenges. Other invasive methodologies such as antegrade pancreatic duct perfusion, duct ligation, ischemia-perfusion models, or biliopancreatic duct injections of taurocholate all produce severe pancreatitis, but are technically complex.

Pathophysiology of Pancreatitis

**Normal Protective Safeguards Against Pancreatitis**

The pancreas is able to protect itself from autodigestion in a number of ways. First, some digestive enzymes are stored in the acinar cells as inertzymogens, and theoretically are only activated after secretion into the lumen of the duodenum.²⁰ Second, within the acinar cell, the zymogen granules remain physically separate from the lysosomal granules enclosed in membrane-bound organelles.²¹ Lysosomal enzymes are produced on ribosomes attached to rough endoplasmic reticulum in the same manner that zymogens are produced, but additionally are glycosylated and phosphorylated as they pass through the Golgi complex.²²,²³ Studies have shown that these 2 enzyme groups are kept physically apart throughout all stages of their production.²² Third, location of pancreatic secretory trypsin inhibitor (PSTI) within the acinar cells allows for immediate inhibition of trypsin should it be activated within the acinar cells. PSTI is produced and stored in the same cellular location as the digestive enzymes.²⁴,²⁵ Finally, should any activated trypsin be released into the circulation, larger antiproteases in the blood theoretically have the capacity to deactivate some circulating trypsin.

**Initiating Cellular Events**

Electron microscopy and ultrastructural studies have confirmed that the earliest event in pancreatitis is activation of trypsinogen to trypsin within the acinar cell.²⁶,²⁷,²⁸,²⁹ A secretory (or apical) block develops, and zymogen granules are not secreted normally into the intestinal lumen for degradation by enterokinase.³⁰ As a result of this apical block, colocalization of zymogen granules and lysosomal proteases develops, and trypsinogen is activated to trypsin by lysosomal proteases, mainly cathepsin B.³¹,³² This colocalization theory is depicted in Figure 1, and has become widely accepted as the main initiating step in pancreatitis. However, concurrent administration of a cathepsin B inhibitor significantly decreased development of pancreatitis in 1 rodent study but did not completely prevent it.³³ Other mechanisms also may be involved in trypsin activation because cathepsin B deficient mice can develop pancreatitis, albeit of lesser severity than mice without cathepsin B deficiency.³⁴ In addition, hypotension has been shown to cause trypsin activation before appearance of proteases at the apical surface, suggesting there are other cellular mechanisms not yet fully elucidated that lead to premature trypsin activation.³⁵,³⁶ Colocalization may occur because of a dysfunction of the normally coded separation of the 2 components.³⁷

The role of inappropriate trypsin activation in initiating pancreatitis is exemplified by a mutation in the cationic trypsinogen gene linked to recurrent pancreatitis in people.³⁸,³⁹ This mutation may result in failure of the protective mechanism against activation, and so prematurely activated trypsin accumulates in the acinar cell.³⁹ A genetic cause of pancreatitis is present in some canine breeds, such as Miniature Schnauzers, but the exact genetic abnormalities are yet to be established.⁴⁰,⁴¹ Experimental models have shown that the pH of the acinar cell is very important in determining the likelihood of lysosomal and zymogen granules colocalizing.⁴² The most recent of these studies showed that low acinar pH alone did not lead to development of pancreatitis, but it did increase zymogen activation after cerulein stimulation.⁴³ Trypsinogen activation also
appears to require the presence of intracytosolic calcium in some in vitro experiments.\textsuperscript{33,34} Calcium located in the zymogen granules may have a protective function against activation of trypsin, but within the cytosol it acts in conjunction with CCK as an intracellular messenger for the release of lysosomal proteases.\textsuperscript{33} In fact, blockade of the calcium signaling system within cells stops production of trypsinogen activation peptide.\textsuperscript{32,34,35}

Trypsin and other activated pancreatic enzymes are likely to be directly damaging to the acinar cell when production overwhelms local PSTI. In particular, Phospholipase-A\textsubscript{2} (PLA\textsubscript{2}) causes hydrolysis of cell membrane phospholipids, elastase causes degradation of elastin in blood vessel walls, chymotrypsin activates xanthine oxidase (thereby perpetuating oxidative damage), and lipase causes fat hydrolysis.\textsuperscript{36} Early experimental models favor the notion that the spilling over of activated enzymes into the interstitium is the triggering factor for progression from mild to severe pancreatitis.\textsuperscript{27} Premature activation of trypsin within the acinar cells is highly dependent on intracellular pH and calcium, and can be induced by hypotension and exacerbated by genetic abnormalities in local safeguards.

**Recruitment of Neutrophils and Production of Reactive Oxygen Species**

After this direct damage to the pancreas, inflammation ensues. Inflammation involves vasodilatation of local blood vessels, increased permeability of local capillaries, clotting of fluid in the interstitial spaces, migration of granulocytes to affected tissue, and swelling of the tissue cells.\textsuperscript{37} Neutrophils contain oxidizing agents such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl ions (OH$^-$) that are collectively termed ROS. ROS are directly toxic to cells and further increase cellular and vascular permeability, as well as enhancing the expression of inflammatory mediators, such as eicosanoids released by leukocytes.\textsuperscript{37} Severity of pancreatitis was shown to be decreased in mice with neutrophil depletion.\textsuperscript{38} However, other studies have shown that blockade of ROS does not ameliorate pancreatitis, and thus other factors also must contribute to development of severe disease.\textsuperscript{39,40}

Chemokines are a group of small molecular weight cytokines that have chemotactic properties, and therefore play a large role in recruiting leukocytes to areas of injury. In general, they are subdivided into the CXC and CC subfamilies based on their amino acid sequence.\textsuperscript{9} The CXC chemokines that possess the conserved amino acid sequence ELR immediately before the first cysteine residue at their N terminus are potent neutrophil attractors, and the best example of this group is IL-8. Neutrophil migration to the pancreas is thought to be initiated mainly by IL-8 early in the course of inflammation.\textsuperscript{21,41}

The adhesion of leukocytes to the endothelial wall occurs via expression of ICAM-1 and selectins mediated by IL-8 and other chemokines.\textsuperscript{9,42} ICAM-1 is an inducible molecule normally only expressed at low levels on endothelial surfaces, but expression has been shown to be increased in experimental pancreatitis.\textsuperscript{10} ICAM-1 knockout mice are protected to some extent against the development of acute pancreatitis and associated organ damage, demonstrating the role of this molecule in perpetuation of disease.\textsuperscript{10}
One study recently analyzed the role that elastase and trypsin play in neutrophil migration in acute pancreatitis. The authors used an in vitro model to demonstrate that both trypsin and elastase directly upregulate expression of adhesion molecules on white blood cells and endothelial cells. Activated neutrophils then follow a chemokine gradient into pancreatic tissue. Leukocyte accumulation occurs initially in the perivascular areas of the pancreas, and then after edema and the resultant change in permeability they egress to the pancreatic body, perpetuating the inflammatory pathway within the pancreas.

Neutrophils also have been implicated in causing a shift from apoptosis to necrosis in pancreatitis. Necrotic cells release cytosolic contents into the extracellular space and elicit a profound inflammatory response. Apoptotic cells on the other hand are rapidly phagocytized by macrophages and do not elicit an inflammatory response. Milder forms of experimental pancreatitis are characterized by apoptosis than necrosis, and vice versa. Necrosis is intricately linked to pancreatic microcirculation, and is proportional to the degree of oxidative stress. Vasactive mediators also have been implicated in the development of necrosis (eg, endothelin-1, PLA-2). Neutrophil migration into the pancreas is directly stimulated by activated trypsin and chymotrypsin, and indirectly by upregulation of ICAM-1 by inflammatory mediators, especially IL-8. Neutrophils themselves perpetuate an inflammatory state and are involved in progression from apoptosis to necrosis within the pancreas.

**NO and Oxidative Stress**

NO is a small inorganic molecular compound that has been shown to regulate pancreatic exocrine secretion, promote capillary integrity, inhibit leukocyte adhesion, and modulate pancreatic microvascular blood flow. The biological functions and effects of NO are complex, and NO can act as both a pro- and anti-inflammatory agent. NO isoforms are named after their initial site of location in the vascular endothelium (eNOS), neurons (nNOS), or macrophages (iNOS). When NO concentration increases within cells owing to decreased circulation, regional vasodilatation occurs, but if this persists, mitochondrial respiratory function is impaired and NO becomes peroxynitrite. Peroxynitrite is an oxidant that can cause lipid peroxidation, protein nitration, DNA strand damage, and cell necrosis. Furthermore, NO can directly produce ROS, thus perpetuating oxidative stress. In experimental pancreatitis, NO has been shown to be deleterious by increasing oxidative stress or causing hypotension. Conversely, NO has been shown experimentally to have a protective effect in pancreatitis by increasing blood flow through the pancreas.

The differences in studies assessing NO may be explained by the different biological effects of the different NOS isoforms. In the pancreas, eNOS and nNOS are constitutively expressed and are calcium-dependent enzymes that stimulate low levels of NO production. On the other hand, iNOS is calcium insensitive and produces large amounts of NO that circulate throughout the body. Studies have shown that eNOS is downregulated during later stages of severe pancreatitis and upregulated in mild disease, whereas iNOS is increased in proportion to disease severity. Tumor necrosis factor-α (TNF-α) also decreases expression of eNOS in bovine endothelial aortic cells, and both nuclear factor kappa-B (NF-kB) and IL-8 increase expression of iNOS in the lung and pancreas during experimental pancreatitis in rodents. NO derived from nNOS is poorly studied in pancreatitis, but could potentially play a role in amplifying neurogenic inflammation mediated by substance P, as discussed later.

The exact role of NO in naturally occurring human pancreatitis still remains unclear. One recent study showed a significant positive correlation between plasma NO and severity in a group of people with acute pancreatitis. Oxidative stress was greatest in the group that developed systemic complications. Cigarette smoking and alcohol consumption may also increase the incidence or severity of acute pancreatitis by uncoupling eNOS from the NO production, and causing increased iNOS. Despite these assumptions, therapeutic manipulation of the NO system has had no proven clinical benefit to date in human medicine in preventing endoscopic retrograde cholangiopancreatography-induced pancreatitis. Additional studies elucidating the role of NO and the various NOS isoforms are needed in canine pancreatitis. There is experimental evidence that eNOS may be protective in pancreatitis, whereas iNOS is associated with more severe pancreatic inflammation.

**Pancreatic Microcirculation**

The blood flow to, and capillary structure of, the pancreas are complex and allow integration between the endocrine and exocrine functions of the pancreas, which requires a relatively high blood flow to maintain function. In animal models, vasoconstriction within the pancreas appears to be an early event in acute pancreatitis. In addition, administration of phenylephrine (a potent vasoconstrictor) converts mild, edematous pancreatitis to a severe, necrotizing form. In people, early onset spasm of large pancreatic vessels has been shown to correlate with poorly perfused areas of the pancreas, and subsequently with high mortality rates. One experimental study in rats also identified that lower pancreatic perfusion was associated with more severe pancreatitis.

Disturbed pancreatic microcirculation usually is multifactorial in origin and can occur as a result of increased vascular permeability resulting from inflammatory cytokines and microthrombi formation resulting from hypercoagulability. Increased capillary permeability leads to edematous changes in the acinar
cells, and further migration of inflammatory cells (Fig 2). Necrotizing pancreatitis causes a progressive reduction in capillaries after acinar cell injury that cannot be reversed by fluid resuscitation. The pancreas is intrinsically susceptible to ischemia, and subclinical pancreatitis frequently is identified in people with hypovolemia. It remains unknown what degree of hypovolemia is required to disturb pancreatic microcirculation in dogs.

Disturbances of pancreatic microcirculation commonly occur in experimental models of pancreatitis, and contribute to the inflammatory state.

**Kallin-Kallikrein System**

An early and direct action of pancreatic proteases is to activate the kallikrein system. The pancreas has a high tissue concentration of kallikrein, generally stored

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![Diagram](Fig 2). Pancreatic microcirculation in health and during inflammation. PMN = neutrophil; ICAM = intercellular adhesion molecule.
as prekallikrein.\textsuperscript{74} Pancreatic tissue kallikrein releases kallidin, whereas plasma kallikrein releases bradykinin. Kinins precipitate edema by vasodilatation of arteries and increased capillary permeability. They are also potent activators of nociceptive neurons, can attract cytotoxic factors into tissue, and enhance production of prostaglandins.\textsuperscript{74} There are 2 major kinin receptor types, with B\textsubscript{2} constitutively expressed in tissue and becoming the major receptor during acute inflammation. The nociceptive and vascular effects of the kinin system are predominantly mediated by B\textsubscript{2} receptors. Production of kallikreins in acute pancreatitis has been confirmed in experimental models.\textsuperscript{74,75} Kinins have a short biological half-life, and are degraded by kininase II which is structurally identical to angiotensin-converting enzyme.\textsuperscript{76} Along similar lines, B\textsubscript{2} receptor antagonists appear experimentally to have beneficial effects on pancreatic blood flow, necrosis, and survival in necrotizing models even when given after induction of disease.\textsuperscript{77,79} The kinin system may worsen pancreatic edema, and interventions targeting B\textsubscript{2} kinin receptors theoretically may be of some benefit in treatment of disease.

**The Complement System**

The complement system has been shown to be activated early in people with acute pancreatitis, and to a greater extent in those with severe disease.\textsuperscript{80} It remains unknown to what extent or in what fashion this occurs in canine acute pancreatitis. Most likely the alternative complement pathway is initiated in pancreatitis, as there is an absence of antibodies or microbes as the primary trigger.\textsuperscript{36} C5a is one of the most potent mediators of the complement system, and substantially enhances vascular permeability. Surprisingly, in a study using mice that either lacked C5a receptors or did not express C5a, the resultant pancreatitis was more severe.\textsuperscript{81} It remains unclear as to why C5a appeared to have an anti-inflammatory effect in that study, but it may well be that C5a limits the recruitment of proinflammatory mediators.\textsuperscript{9,26} In contrast to this finding, 1 group of researchers found a strong association between expression of C3a and necrotizing versus edematous pancreatitis.\textsuperscript{82} In addition, blockade of complement receptor-1 led to amelioration of local pancreatic inflammatory and downregulation of the leukocyte-endothelial interaction. The conflicting conclusions of these studies may be because of the different experimental models used. Alternatively, and more likely, the complement system may have opposing and synergistic actions within its cascade. The role of the complement system in acute pancreatitis is complex and not fully elucidated in experimental models to date.

**Substance P and the Renin Angiotensin System**

Substance P is a peptide produced by nerve endings that binds to NK-1 receptors. As well as mediating pain, Substance P likely increases vascular permeability, and therefore may play a role in progression of pancreatitis. A study with a mouse model has demonstrated upregulation of NK-1 receptors and the amount of Substance P subsequently expressed in acute pancreatitis.\textsuperscript{64} Expression of Substance P appeared to be particularly related to lung injury, and genetic deletion of NK-1 receptors was protective against the systemic effects of pancreatitis.\textsuperscript{64} NO and Substance P also may interact and synergistically amplify pain and inflammation.\textsuperscript{49} No studies to date have assessed the role of Substance P, or the potential benefit of treatment with NK-1 receptor antagonists, in canine pancreatitis.

One area that has been poorly investigated in both experimental and naturally occurring acute pancreatitis is the role the RAS may play in the initiation and perpetuation of pancreatic inflammation.\textsuperscript{43} Angiotensin II receptors have been identified in the vascular and ductular endothelium of the rodent pancreas.\textsuperscript{83} Expression of genes encoding these receptors and metabolites of the RAS have been shown to be upregulated in acute pancreatitis, and this may result directly from pancreatic hypoxia.\textsuperscript{84} The functional effect of this upregulated RAS has not been established, but it is logical that local vasoconstriction would ensue. Substance P inhibition theoretically could decrease pain and lung-associated injury, and the pancreatic RAS may stimulate vasoconstriction in acute pancreatitis.

**Cytokine “Storm”**

Before, during, and after inflammatory cells have appeared within the pancreas many cytokines and chemokines are activated. This is a highly complex phenomenon, and has been referred to as “cytokine storm”, with many cytokines contributing to the inflammatory state.\textsuperscript{42,85,86} Cytokines are small proteins produced in response to stimuli, and act to up- or downregulate various aspects of the inflammatory process. Many cytokines are produced by T cells to either drive or facilitate a Th1 or Th2 immune response. Th1 responses mediate cellular immunity and activate neutrophils, macrophages, and NK cells. Th2 responses mediate humoral immunity by B cell activation or recruitment of eosinophils. Each cytokine has the capacity to act synergistically with others, and there is a large overlap in their functions (Fig 3).

The initiating step of this process in pancreatitis, as in many other systemic inflammatory diseases, appears to be the activation of NF-κB.\textsuperscript{87,88} NF-κB is a transcription factor that modulates the expression of most cytokines. High NF-κB expression has been identified in peripheral blood mononuclear cells from people with acute pancreatitis, and correlates with development of systemic complications.\textsuperscript{89} NF-κB also stimulates production of adhesion molecules and iNOS.\textsuperscript{62}

In 1 rodent model, cytokine activity within the pancreas was determined by tissue mRNA and RT-PCR, and TNF-α and IL-1β were the first cytokines to appear.\textsuperscript{90} The amplitude of cytokine expression correlated with the severity of pancreatic inflammation.
by TNF-α and IL-1β, and is increased early in the course of experimental pancreatitis. IL-6 is a relatively easy cytokine to measure and has been negatively correlated with prognosis in naturally occurring acute pancreatitis in humans. IL-8 is a proinflammatory chemokine closely involved in neutrophil actions, both as an inciter and product of the Th1 profile. Similar to IL-6, the concentration of IL-8 has been correlated with the severity of naturally occurring pancreatitis in people. Platelet activating factor (PAF) is a cytokine produced predominantly by neutrophils that has been extensively studied in experimental models. It appears to be particularly associated with lung injury, which is one of the major causes of early (<2 weeks) mortality in people with acute pancreatitis. Blockade of PAF has led to reduction in lung-associated complications in people with pancreatitis in some studies, but overall, did not improve mortality or other organ failure.

In experimental studies, IL-10 has been assessed because of its anti-inflammatory effect, which favors a shift to a Th2 cytokine profile and downregulates NF-κB transcription. Some initially promising results demonstrated that IL-10 ameliorated the severity of pancreatitis in experimental models. Pretreatment of rats with IL-10 decreased iNOS concentrations, and increased TGF-β1, which correlated with an increase in the rate of apoptosis as compared with necrosis. In addition, IL-10 knockout mice were shown to develop a more severe form of pancreatitis. These findings, along with demonstration that serum IL-10 was increased in people with severe pancreatitis within the first 2 days of disease, but subsequently decreased, suggested a potential treatment avenue. Unfortunately, IL-10 therapy has had limited success in treatment of acute pancreatitis in humans.

As knowledge in this area continues to increase, many other cytokines (eg, IL-2, -4, -11, and -18) are also being evaluated in experimental pancreatitis. Regulated on activation, normal T cell expressed and secreted (RANTES) is now known as CC chemokine ligand 5 (CCL5), and is produced by circulating T cells under stimulation by TNF-α and IL-1. It is responsible for protection against viruses, and is a potent chemoattractant for recruiting leukocytes. The role of CCL5/RANTES or other potent chemoattractants, such as monocyte chemoattractant proteins (MCP)-9 in canine pancreatitis remains unknown. Whether these inflammatory mediators become clinically important in canine pancreatitis from a therapeutic or etiologic point of view remains to be seen. There are a large number of complex and interdependent cytokines produced in acute pancreatitis. This suggests that intervention targeting a single cytokine is unlikely to be effective in modulating disease.

**Perpetuation of Disease**

With resultant hypovolemia from vomiting, diarrhea, and anorexia, the splanchnic circulation is sacrificed early and may contribute greatly to the perpetuation of

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**Fig 3.** Demonstration of the activation of the cytokine storm in acute pancreatitis. Systemic inflammation results from the cytotoxic effects when the proinflammatory mediators (red) overwhelm the anti-inflammatory mediators (blue). TNF, tumor necrosis factor; IL-, interleukin; ra, receptor antagonist; NO, nitric oxide; ROS, reactive oxygen species or superoxide; AA, arachidonic acid; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell expressed and secreted. Solid lines represent stimulatory pathways, whereas dotted lines represent inhibitory pathways.
from the capillary network to the veins occurs closer exquisitely sensitive to villus hypoxia because drainage unbranched to the tip of the villus. In dogs, a similar vascular anatomy of the villi tips susceptible to tissue hypoxia if vasoconstriction occurs. Regardless of the mechanism, this low PO\textsubscript{2} gradient makes the villus susceptible to tissue hypoxia if vasoconstriction occurs. In dogs, a similar vascular anatomy of the villus is present, with a single arteriole rising unbranched to the tip of the villus. Dogs may be exquisitely sensitive to villus hypoxia because drainage from the capillary network to the veins occurs closer to the tip of the villus than in other species. The intestine is susceptible to ischemia during acute pancreatitis, and upon reperfusion may be the initiator or perpetuator of distant inflammation by a variety of mechanisms.

**Pathophysiology in Experimental Models Related to Potential Etiologies in Dogs**

The oxidative theory of pancreatitis was first proposed in human medicine in the early 1980s. According to this theory, the pancreas is exposed to oxidative stress either through the systemic circulation, or via reflux of bile containing reactive metabolites into the pancreatic duct. In people, the most common oxidative stressors include alcohol and high-fat diets. However, oxidative stress currently is thought to accompany but not directly cause pancreatitis without other factors also being present.

Experimental evidence also suggests that low-protein, high-fat diets induce pancreatitis in dogs, and that high-fat diets in dogs cause more severe pancreatitis than fat-restricted diets. These studies, however, did not assess whether pancreatic necrosis or inflammation was present, rather the volume of pancreatic secretion and the protein concentration of those secretions was assessed. Overweight dogs are considered at greater risk of pancreatitis, and this risk may be associated with abnormal dietary intake or may indicate a general predisposition to inflammation.

A chronic inflammatory state is associated with adipose tissue and adipokines in people, and a similar association likely also occurs in dogs. In 1 retrospective survey, dogs with recent ingestion of unusual food items and garbage ingestion showed increased risk of developing pancreatitis, rather than dogs that appeared to have a higher intake of treats and snacks. This study suggested, but could not prove, that inappropriate food rather than the fat and protein content of food may be most important in the development of pancreatitis.

Concurrent diseases, particularly diabetes mellitus, hypothyroidism, and hyperadrenocorticism, are reported commonly in dogs with pancreatitis. Some possible connections between diabetes mellitus and pancreatitis include subclinical pancreatitis causing progressive islet cell destruction or autoantibodies directed against insulin-secreting cells promoting generalized pancreatic inflammation. Acidosis within the pancreas in diabetic ketoacidosis (DKA) may enhance or initiate trypsin activation, rather than DKA being caused by pancreatitis. Endocrinopathies are associated with changes in serum lipid concentrations, and the association between hyperlipidemia and pancreatitis cannot be discounted. Alterations in bile composition with varying lipid status may directly lead to toxic changes in pancreatic acinar cells by changing cellular metabolism. Acute pancreatitis also may directly result from hypoxia and ductal hypertension, by an effect on the pancreatic microcirculation.
may account for the high incidence of pancreatitis reported after abdominal surgery, especially adrenalectomy.\textsuperscript{132,139} Despite the experimental association between premature trypsin activation and hypercalcemia, common conditions such as lymphoma that cause hypercalcemia are not reported to cause pancreatitis in dogs. The lack of reported association between pancreatitis and hypercalcemia in dogs may be because of the fact that the increase in calcium is more gradual than that seen in people, where hypercalcemia-associated pancreatitis most often is seen with cardiopulmonary bypass.\textsuperscript{138,140} It also is possible that the pancreatitis is subclinical and escapes diagnosis in these dogs.

**Development of Complications**

Acute renal failure may develop secondary to hypovolemia, ischemia, intravascular coagulopathy, and direct inflammation resulting from peritonitis.\textsuperscript{141} NF-κB activation also can cause aggregation of activated neutrophils in the glomeruli.\textsuperscript{142} Endotoxin may promote renal vasoconstriction contributing to renal failure attributable to high affinity binding on the renal artery.\textsuperscript{142} Other systemic complications include disseminated intravascular coagulation and cardiac arrhythmias, all mediated by the many systemic inflammatory cascades initiated by acute pancreatitis.\textsuperscript{143,144}

Acute lung injury is most closely linked to PAF, although PLA-2, TNF-α, and IL-1β may also play a role.\textsuperscript{145} The histologic changes in lungs undergoing acute injury during experimental pancreatitis include neutrophilic infiltration, damage of endothelial cells, interstitial edema, and intra-alveolar hemorrhage.\textsuperscript{146} In addition, there may be changes in pulmonary surfactant or apoptosis of type II pneumocytes.\textsuperscript{146} Acute lung injury is considered one of the major early complications in people, and is associated with high morbidity.\textsuperscript{3} The actual incidence of lung injury in dogs with acute pancreatitis remains unknown, but a study assessing clinical severity did identify alterations in respiratory function as significantly affecting clinical outcome.\textsuperscript{147}

Late onset complications include chronic relapsing pancreatitis and subsequent development of exocrine pancreatic insufficiency, both of which have been described in dogs.\textsuperscript{148,149} Recently in people, subclinical exocrine pancreatic insufficiency has been demonstrated after a bout of severe acute pancreatitis.\textsuperscript{150,151} Subclinical exocrine pancreatic insufficiency is difficult to diagnose in dogs, but is highly probable.\textsuperscript{152}

Acute fluid collections are defined in the human literature as fluid pockets within the pancreatic parenchyma that develop within the first 6 weeks after a bout of acute pancreatitis.\textsuperscript{153} A pseudocyst on the other hand, develops at least 6 weeks after an episode, does not contain an epithelial lining and its contents are composed of amylase-rich pancreatic secretion, generally occurring in milder cases of acute pancreatitis.\textsuperscript{153} Another late onset (3–4 weeks) local complication is a hypovascular area containing necrotic tissue that in people may become a nidus for infection.\textsuperscript{153}

The veterinary literature suggests development of fluid in the canine pancreas is invariably acute in onset and sterile in nature.\textsuperscript{154–160} This is much different from the clinical situation in people, where infected necrosis is one of the major determinants of mortality. These findings suggest that the local complications within the pancreas of the dog instead should more consistently be called acute fluid collections, unless they are confirmed to be infected. The reported mortality rates for dogs with pancreatic abscesses are high (>50%), but this may be reflective of fact that all have been treated surgically with subsequent complications.\textsuperscript{154–160} Current recommendations in human gastroenterology are to not surgically debride sterile fluid collections, and if infection is documented treatment with antimicrobials should be employed for as long as possible before surgical debridement.\textsuperscript{167}

**Conclusion**

Acute pancreatitis has a complex pathogenesis that involves multiple inflammatory pathways. The pancreas itself is exquisitely sensitive to circulatory and ischemic events. Even if these events are not the cause of the pancreatic inflammation, their development during the ensuing clinical dehydration has the potential to worsen disease. In recent times, the roles of the kinin–kallikrein system, substance P, pancreatic microcirculation, and perpetuation of disease by the intestinal tract all have received substantial attention. Intervention in these areas appears to have the most promise in attenuating disease severity as compared with treatments that counteract single cytokines.

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