Inflammasomes in pancreatic physiology and disease

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Hoque R, Mehal WZ. Inflammasomes in pancreatic physiology and disease. Am J Physiol Gastrointest Liver Physiol 308: G643–G651, 2015. First published February 19, 2015; doi:10.1152/ajpgi.00388.2014.—In this review we summarize the role of inflammasomes in pancreatic physiology and disease with a focus on acute pancreatitis where much recent progress has been made. New findings have identified inducers of and cell specificity of inflammasome component expression in the pancreas, the contribution of inflammasome-regulated effectors to pancreatitis, and metabolic regulation of inflammasome activation, which are strong determinants of injury in pancreatitis. New areas of pancreatic biology will be highlighted in the context of our evolving understanding of gut microbiome- and injury-induced inflammasome priming, pyroptosis, and innate immune-mediated regulation of cell metabolism.

G protein-coupled receptor 109a; G protein-coupled receptor 81; mitochondrial oxidation; NACHT, LRR, and PYD domains-containing protein 3

ACUTE AND CHRONIC PANCREATITIS ARE INFLAMMATORY CONDITIONS OF THE PANCREAS TRIGGERED BY ACINAR CELL INJURY WITH INTRAPANCREATIC TRYPsinogen ACTIVATION BEING A HALLMARK FEATURE (16). However, intrapancreatic trypsinogen activation accounts for only 50% of acinar cell death in experimental acute pancreatitis (16), and trypsinogen and the trypsinogen-activating protease cathepsin B are dispensable for substantial pancreatic injury, inflammation, and fibrosis in experimental chronic pancreatitis (78). Necrotic cell death triggers a robust sterile inflammatory response (37), and there is substantial evidence that this process contributes to pancreatic injury (32). Innate immune sensing components Toll-like receptor 4 (TLR4) (85) and Toll-like receptor 9 (TLR9) (33) in immune cells and nucleotide-binding oligomerization domain-containing protein 1 (NOD1) (89) in pancreatic acinar cells sense damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) in the injured pancreas. These pathways induce activation of NF-κB (25) and the expression of NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome components and effectors (24, 33). The NLRP3 inflammasome is a macroscopic cytosolic protein complex consisting of NLRP3, apoptosis-associated speck-like protein (ASC), and procaspase 1. It functions to proteolytically activate pro-IL-1β and pro-IL-18 (47) and induce release of active IL-1β, IL-18, and high-mobility group protein B1 (HMGB1) (51) in response to a wide range of stimuli, including extracellular ATP, NAD (80), and saturated free fatty acids (93).

We discuss regulation of signals that activate the NLRP3 inflammasome, specifically saturated free fatty acids, necrotic acinar cell death pathways, and DAMP release in the context of pancreatitis. Decreasing saturated free fatty acid production with the lipase inhibitor orlistat limits injury in experimental pancreatitis (62). The NLRP3 inflammasome effectors IL-1β, IL-18, and HMGB1 are in turn all major determinants of pancreatic inflammation, parenchymal cell injury, and disease resolution in acute or chronic pancreatitis (66, 73, 76, 79). Recently, cell surface receptors for metabolites induced by TLR stimulation have been found to suppress NLRP3 inflammasome expression, inflammasome activation, and pancreatic injury in experimental pancreatitis. This provides insights into the metabolic regulation of the inflammasome (19, 31). Finally, cell signaling components that regulate inflammasome signaling have been investigated in pancreatic acinar cells in the setting of pancreatic injury. These recent findings contextualize inflammasome biology in acute pancreatitis to guide further investigation, and each will be discussed in further detail.

Cell-Specific Expression of Inflammasome Components

Acinar cells, ductal cells, and endothelial cells in the normal healthy pancreas of mice, rats, and humans do not express caspase 1, IL-1β, or IL-18. Expression of the inflammasome components NLRP3 and ASC in the resting pancreas has not been reported. Alcoholic pancreatitis accounts for up to 45% of pancreatitis (91), and Gu et al. investigated whether chronic ethanol administration or endotoxemia, which occurs with alcohol ingestion (70), could induce expression of inflammasome components in the rat pancreas (24). A single lipopolysaccharide (LPS) injection or ethanol feeding for 14 wk induced expression of caspase 1 and IL-18 in pancreatic acinar cells. In isolated rat pancreatic acinar cell, LPS treatment induced expression of caspase 1 and IL-18, confirming direct regulation of expression in the acinar cell compartment. Confirming a clinical relevance of these findings, IL-1β, caspase 1, and IL-18 were expressed primarily in pancreatic acinar cells in pancreatic specimens from patients with acute or recurrent acute pancreatitis vs. normal pancreas (24). The authors con-
firm expression of TLR4 in rat and human pancreatic acinar cells and hypothesize that alcohol-medicated sensitization to injury in pancreatitis through endotoxemia induced TLR4-mediated stimulation of caspase 1, IL-18, and IL-1β in acinar cells. High-fat diets have also been shown to promote low-level endotoxemia in mice (7) and in humans (71). Whether high-fat diet-derived endotoxemia induces expression of inflammasome components in pancreatic acinar cells is currently unreported. Acute exposure to ethanol with ethanol gavage induces expression of nlrp3, asc, and caspase1 predominantly in liver mononuclear cells as opposed to parenchymal liver cells such as hepatocytes (72). This result is quite different from the immunonochemical studies in pancreatic specimens of ethanol-fed mice in which parenchymal cells, specifically the acinar cells, are found to express nlrp3, asc, and caspase1. These differences may reflect differences from acute vs. chronic ethanol toxicity. Steatohepatitis induced by methionine and choline deficiency promotes endotoxemia in mice and expression of nlrp3, asc, pro-IL-1β, and caspase1 in hepatocytes (15). Such findings provide initial insights into potential TLR4-mediated induction of NLRP3 inflammasome components in the pancreatic acinar cell compartment (Fig. 1). Bacterial peptidoglycans from normal gut flora are present in the intestinal circulation and are required to prime innate immune responses in neutrophils through NOD1 signaling, demonstrating for the first time a beneficial effect of normal gut flora microbial components on systemic immune responses (12). It is unknown if the leaky gut induced by high-fat diet or ethanol feeding results in increased sensing of bacterial peptidoglycans through NOD1, NOD2, and/or NLRP1. Many parenchymal cell populations, including pancreatic acinar cells, express functional NOD1 (89). It is an intriguing possibility that NOD1 signaling in acute pancreatitis may induce inflammasome components and effectors in acinar cells (Fig. 1). Similarly, TLR3 is also expressed in acinar cells (44), and TLR3 activation plays an important role in high-fat diet-induced dyslipidemia and inflammation in mice fed a high-fat diet (95). The source of TLR3 ligands, either bacterial products or DAMP release from injured host cells (8), remains to be elucidated. TLR3 ligands administered in vivo also strongly induce expression of the inflammatory caspase 11 (45), discussed in detail below. A role for TLR3 priming of pancreatic acinar inflammatory responses remains unexplored.

**Inflammasome Activators**

**Acinar cell necrosis.** The NLRP3 inflammasome is a major sensor of necrotic cell death and required for the sterile inflammatory response to necrosis (37). In many disease states necrosis occurs through a coordinated program termed necroptosis mediated by signaling through receptors harboring a common death domain, suppression of caspase 8 signaling, and signal transduction through the effectors receptor interacting serine/threonine protein kinase 1 and 3 (RIPK1 and RIPK3) (28, 30). The contribution of necroptosis pathways through death domain-containing receptor pathways, including tumor necrosis factor (TNF)-α, Fas, and TNF-related apoptosis-inducing ligand (TRAIL), has been investigated in acute pancreatitis. TNF-α induces necrosis in primary acinar cells (82), and TNF-α deletion or in vivo blockade mitigates pancreatic inflammation and necrosis in experimental acute pancreatitis (17, 55, 65). RIPK3 is required to induce acinar cell necrosis in experimental acute pancreatitis and limits pancreatic inflammation and injury (28). Therefore, acinar cell necrosis in acute pancreatitis occurs predominantly through specific pathways. TRAIL family receptors are expressed de novo in acinar cells in human chronic pancreatitis (27) although the role of TRAIL blockade is unexplored in experimental pancreatitis. Fas is required to limit pancreatic necrosis in experimental acute pancreatitis (38). Fas promotes apoptosis, a noninflammatory form of cell death, in many cell types (1). However, since apoptosis was not identified to be a significant contributor to acinar cell death in murine experimental pancreatitis, the mechanism of Fas-mediated protection in acute pancreatitis remains to be determined (39). CD11c-positive dendritic cells serve a nonredundant function in the phagocytic clearance of injured pancreatic cells in acute pancreatitis and are required to limit pancreatic injury in experimental acute pancreatitis (4). Persistence of necrotic acinar cells therefore promotes an injurious robust sterile inflammatory response in acute pancreatitis. Release of ATP, NAD, HMGB1, and nucleic acids from necrotic cells activates or induces the NLRP3 inflammasome, and these interactions are further discussed below.

**P2X7 and CD39.** ATP and NAD released from necrotic cells promotes NLRP3 inflammasome activation in immune cells by stimulating the cell surface receptor P2X7 (80). Genetic dele-
ation of P2X7, enhanced metabolic clearance of extracellular ATP through apyrase injection, or competitive inhibition of extracellular NAD for P2X7 activation through injection of etheno-NAD all suppress pancreatic inflammation, pancreatic caspase 1 activity, and pancreatic injury in experimental acute pancreatitis (33). Pancreatic stellate cells are the major drivers of pancreatic fibrosis. At low concentrations, extracellular ATP promotes cell proliferation in primary pancreatic stellate cells in culture in a P2X7-dependent manner (26); at high concentrations, extracellular ATP induces stellate cell death with P2X7 dependence. Mice deficient in CD39, the major extracellular space ectonucleotidase that hydrolyzes extracellular ATP to ADP and AMP, are predicted to have higher extracellular concentrations of ATP at sites of injury or inflammation and greater P2X7-mediated pancreatic stellate cell death. CD39-deficient mice have decreased pancreatic atrophy and decreased fibrogenesis in experimental pancreatic fibrosis (49), consistent with decreases in fibrogenic pancreatic stellate cell survival. Of note, CD39 and P2X7 interplay is a strong determinant of cell death in other immune cell compartments, including mast cells (48). Finally, the closely related hepatic stellate cell expresses NLRP3 inflammasome and develops a fibrogenic phenotype in response to the NLRP3 activator monosodium urate crystals (92).

Free fatty acids. Free fatty acids have been implicated as endogenous ligands for TLR4 through binding of the acute phase protein fetuin A (69) and are also direct activators of the NLRP3 inflammasome (93). The unsaturated free fatty acids oleate and linoleate are cytotoxic to isolated pancreatic acinar cells (62). To date, it is unreported if there is enhancement of free fatty acid cytotoxicity to acinar cells in the presence of fetuin A to promote TLR4 activation or in the presence of prior LPS treatment to induce caspase 1 in acinar cells and potentially transduce fatty acid-mediated NLRP3 inflammasome activation. Local free fatty acid production is thought to be mediated by pathological intrapancreatic lipase activity in the inflamed pancreas. Navina et al. identify that regions of pancreatic necrosis colocalize to regions of nonencapsulated adipose tissue in pancreata in human clinical samples in acute pancreatitis, suggesting a significant contribution of lipotoxicity in the human clinical condition. They further demonstrate that in vivo administration of the lipase inhibitor orlistat mitigates experimental pancreatic inflammation and injury (62). Free fatty acids contribute to cytotoxicity in alcoholic pancreatitis through nonoxidative metabolism of ethanol to fatty acid acyl esters (FAAEs), which accumulate in human pancreatitis specimens (99) and are directly cytotoxic to acinar cells (14). FAAEs accumulated in acinar cells with ethanol ingestion are thought to be cleaved to free fatty acids in acinar cells, providing a mechanism for cytosolic free fatty acid accumulation (Fig. 1). The potential for this mechanism to enhance fatty acid-mediated activation of the NLRP3 inflammasome is unexplored. FAAE formation is required to induce injury in alcoholic pancreatitis in vivo in rodent models as evidenced in vivo through use of small molecule inhibitors of FAAE synthase in experimental alcoholic pancreatitis (35). Complementing these findings, genetic deletion or chemical inhibition of alcohol dehydrogenase shifts alcohol metabolism towards fatty acid ethyl ester formation in rodents and human cells, increases pancreatic FAAE production, and induces more severe pancreatic injury in experimental alcoholic pancreatitis (5, 42). A role for TLR4 and NLRP3 activation in experimental alcoholic pancreatitis is currently unreported.

Inflammasome Components NLRP3 and ASC

NLRP3, ASC, and caspase 1 constitute the NLRP3 inflammasome in the setting of NLRP3-activating ligands. Genetic deficiency of NLRP3 or ASC significantly decreases pancreatic inflammation and injury (33). The role of the NLRP3-activating ligands in acute pancreatitis, including extracellular ATP and NAD as well as saturated free fatty acids, are discussed above. Mitochondrial-derived reactive oxygen species are required for NLRP3 inflammasome signaling (29). Recently, hydrogen-infused saline, a scavenger of reactive oxygen species, has been shown to suppress reactive oxygen species, NLRP3 inflammasome activation, inflammation, and cell death in the pancreas when administered postinsult in the caerulein model of experimental pancreatitis in mice (77). A potential role of other NLR family members in caspase 1 activation in pancreatic inflammation has not been reported. The ligands for other well-studied inflammasomes such as NLRP1, NLRC4, and absent in melanoma 2 are of microbial origin, with no clear endogenous ligands detected to date (51). The NLRP6 inflammasome was recently identified as a critical regulator of host defense through production of antimicrobial IL-18 in intestinal epithelial cells (18). Increased gut permeability is an early feature of clinical acute pancreatitis without systemic bacterial translocation (2). NOD1 in acinar cells contributes to inflammation and injury in experimental pancreatitis through recognition of microbial peptidoglycans. Compromise of intestinal epithelial NLRP6 inflammasome-mediated IL-18 release as a mechanism of increased intestinal permeability in acute pancreatitis and increased acinar cell inflammatory responses through NOD1 and TLR4 remain to be investigated (Fig. 1).

Genetic dysregulation of the NLRP3 inflammasome. Recently Itk kinase α (IKKα) was identified as a negative regulator of inflammasome activation independent of RelA and NF-κB pathway activation. IKKα directly interacts with ASC in resting cells quiescenting it away from other inflammasome components. NLRP3-activating signals suppress IKKα kinase activity, dissociate ASC from IKKα, and result in NLRP3-ASC interaction, with NLRP3 inflammasome formation. Moreover, genetic deletion of IKKα results in NLRP3 inflammasome hyperactivation in immune cells, implicating IKKα as an endogenous negative regulator of the NLRP3 inflammasome (57). Specific deletion of IKKα in acinar cell results in spontaneous acinar cell death, pancreatic atrophy, inflammation, and fibrosis in mice (53). A role of inflammasome hyperactivation in acinar cells in this phenotype remains to be explored. Knock-in mice harboring a missense mutation in NLRP3 that induces spontaneous inflammasome activation develop spontaneous neutrophil infiltration in multiple organs, although not in the pancreas (6). The potentially increased susceptibility of such mice to pancreatic injury in experimental pancreatitis models or from ethanol feeding is unexplored.

Caspase 1 and caspase 11. Caspase 1 activation is required not only for IL-1β, IL-18, and HMGB1 processing and release in response to NLRP3 activation but also for inflammatory cell death. Caspase 1 activity is required for significant injury and inflammation in many experimental models of acute pancreatitis. Genetic deficiency of caspase 1 or injection of small
molecule inhibitors of caspase 1 protease activity suppress pancreatic injury and inflammation in experimental acute pancreatitis in mice and rats (33, 76). Independent of NLRP3 inflammasome formation, caspase 1 can mediate an inflammatory form of cell death termed pyroptosis, which requires ASC oligomerization and recruitment of activated caspase 1 into a cytosolic complex independent of NLRP3 termed the pyroptosome. Caspase 11 can form a pyroptosome independent of ASC and NLRP3 with as yet undefined elements (45). Pyroptosis is an inflammatory form of cell death that involves pyroptosome formation, caspase 1 or caspase 11 activation, rapid plasma membrane rupture, and release of proinflammatory cellular contents, including IL-1α and HMGB1 (50). A potential role for caspase 11 in the inflammatory activation in acute pancreatitis derives from experiments in the initially reported caspase 1 null mice. These mice were in fact null for caspase 11 and caspase 1. Recent generation of a caspase 11 null mice has shed light on the important contribution of caspase 11 in innate immunity (45). Curiously, the caspase 1 caspase 11 double knockout mouse had much greater protection from acute pancreatitis than the NLRP3 or ASC mice (33), suggesting either that caspase 11 may have a significant role in acute pancreatitis or that caspase 1 may have NLRP3- and ASC-independent effects, perhaps being directly activated by cathepsin B released from necrotic acinar cells. A direct role of caspase 11 in pancreatic inflammation remains to be explored. TLR3 is a strong inducer of caspase 11 (45), and chronic treatment with TLR3 ligands results in chronic pancreatitis with macrophage and lymphohcytic infiltrate in MLR/Mp autoimmune prone mice (74). TLR3 expression has been found in secretory acinar cells (44) and can induce sterile inflammation through recognition of endogenous ligands (8). Whether or not necrotic acinar cells induce TLR3 stimulation and caspase 11 expression in pancreatic acinar cells with functional significance in sterile inflammation is unreported (Fig. 1). Finally, extracellular HMGB1 was identified as a strong inducer of pyroptosis in one report through receptor for advanced glycation end products internalization, cathepsin B activation, early cathepsin B-mediated and NRLP3-independent caspase 1 activation, and complexation with ASC to form a pyroptosome, with resultant cell death from membrane rupture (96). The role of HMGB1 as an inflammatory effector in acute pancreatitis is more fully discussed below.

Inflammasome Effectors

HMGB1. HMGB1 is described as a ligand for TLR4. TLR4 expression in bone marrow-derived cells is required for full inflammation and injury in experimental acute pancreatitis induced by caerulein hyperstimulation (97). Endotoxin is reported to be undetectable in the pancreas in the caerulein and l-arginine experimental models of acute pancreatitis, and as such TLR4-mediated signaling in immune cells in the inflamed pancreas is thought to occur through recognition of endogenous ligands (85). HMGB1 is strongly implicated as the major endogenous TLR4 ligand in acute pancreatitis. HMGB1 released from immune cells is regulated by caspase 1 activation and is responsible for profound proinflammatory responses in vivo, including late mortality in inflammatory shock models (51). HMGB1 is also passively released from necrotic cells. Serum HMGB1 levels are elevated in clinical pancreatitis and correspond to disease severity (99), consistent with immune cell activation and acinar cell death. The importance of HMGB1 as an effector of pancreatic injury was demonstrated in vivo as HMGB1 masking antibodies mitigate pancreatic inflammation and injury with TLR4 dependence in experimental acute pancreatitis (79). LPS or LPS and chronic ethanol administration promote extracellular release of HMGB1 from pancreatic acinar cells harboring caspase 1 and result in increased serum levels of HMGB1. In this report, acinar cell HMGB1 release occurred in the absence of acinar cell necrosis, suggesting that HMGB1 may be an early effector of pancreatic injury (24). Use of HMGB1 knockout mice to further clarify the proinflammatory functions of this molecule are confounded by its additional functions. Intracellular HMGB1 is required to promote nuclear integrity and to prevent extracellular release of nuclear proteins, including nucleosomes, which are themselves DAMPs released from injured cells. Acinar cell-specific knockout of HMGB1 resulted in more severe experimental pancreatitis consistent with this anti-inflammatory functionality within the cell (41). Extracellular HMGB1 on the other hand complexes with nucleic acid DAMPs released from necrotic cells and promotes TLR9 recognition contributing to innate immune-mediated inflammatory responses (98). TLR9 is identified as a contributor to sterile inflammation in experimental acute pancreatitis (33). Extracellular HMGB1 signaling induces and enhances sterile inflammatory responses through TLR4 and nucleic acid TLRs, such as TLR9, respectively.

IL-1B. IL-1B is recognized to be a major determinant of sterile inflammation and injury responses in acute pancreatitis. Genetic deficiency of IL-1R or injection of IL-1R antagonist mitigates injury responses in experimental acute pancreatitis (63, 64). Constitutive expression of an IL-1β transgene in acinar cells results in severe chronic pancreatitis with pancreatic atrophy and fibrosis, recapitulating the histopathological changes of human chronic pancreatitis and demonstrating that pancreatic IL-1β expression is sufficient to induce pancreatic damage (56). As noted already, IL-1β is expressed in acinar cells in acute and acute recurrent pancreatitis (24). IL-1β is known to sensitize hepatocytes to TNF-α-mediated cell death in hepatocytes (72). If this effect extends to sensitizing pancreatic acinar cells to TNF-α-mediated cell death is unreported. The role of IL-1β in pancreatic stellate cell-mediated fibrosis remains to be investigated. IL-1β is known to promote fibrogenic responses in the closely related primary hepatic stellate cell, and IL-1R is required for fibrosis in murine experimental nonalcoholic steatohepatitis (60). IL-1β induces hypoxia-inducible factor-1α (HIF-1α) stabilization under normoxia and aerobic glycolysis, both of which are required for stellate cell activation, providing additional potential mechanisms for IL-1β-mediated fibrogenic effects (11).

IL-18. The role of IL-18 in acute pancreatitis appears more complex. IL-18 is required for gut homeostasis, specifically intestinal epithelial host defense as it is directly bacteriocidal (18). Increased gut permeability is an early feature of clinical pancreatitis (2), and a leaky gut may prime for enhanced systemic innate immune responsiveness (101) and possibly worse sterile inflammatory-mediated injury in remote organs such as the pancreas in acute injury. Genetic deficiency of IL-18 results in greater injury in clinical mild experimental pancreatitis induced by caerulein hyperstimulation, and pre-treatment with recombinant IL-18 is dose dependently protec-
In this model (90). In the interstitial compartment, IL-18 expression promotes proinflammatory responses in the context of other proinflammatory cytokines such as IL-12. Consistent with this model, coadministration of IL-18 with IL-12 induces acute pancreatitis in genetically obese mice (83) or mice fed a high-fat diet (73). Administration of IL-18 alone did not promote pancreatitis in these models. Elevations in serum IL-18 correlate with disease severity in the human clinical condition. IL-18 is expressed in acinar cells and in immune cells in conjunction with other proinflammatory cytokines such as IL-18 and TNF-α in acute and chronic pancreatitis (24, 81).

IL-33. IL-33 is an IL-1 family member cytokine that is constitutively expressed in endothelial cells and in pancreatic acinar cells but not found in hematopoietic cell lineages (68). Similar to IL-1α, it is passively released from necrotic cells in a full-length biologically active form. IL-33 cleavage and inactivation by caspase 1 has been reported in the literature (9). However, this finding was not reproduced in another extensive investigation that found IL-33 to be a substrate for apoptotic caspases 3 and 7 but not inflammatory caspases 1, 4, or 5 in vitro; caspase 3 seemed to be the major processor of IL-33 in cells in this study (54). Additionally, IL-33 is also a substrate for neutrophil elastase and cathepsin G, resulting in production of hyperactive forms with greater IL-33 receptor activation (52). The IL-33 receptor suppression of tumorigenicity 2 (ST2) and coreceptor IL-1 receptor accessory protein are expressed on mast cells and NK cells, which are implicated in regulation of sterile inflammation. In mast cells, ST2 transduces TH2 proinflammatory cytokine responses with production of IL-5 and IL-13 and promotes mast cell degranulation. ST2 is also expressed on human pancreatic myofibroblasts; IL-33 treatment of these cells induces proliferation and migration and enhances inflammatory cytokine production in response to IL-4 and interferon-γ. Genetic deletion of ST2 increases the severity of pancreatic injury and increases mast cell degranulation in experimental pancreatitis in mice induced by a choline methionine-deficient diet (68). However, exogenous IL-33 administration also increased pancreatic edema and inflammation and mast cell degranulation in experimental pancreatitis induced by bile duct ligation (46). Most recently, ST2 and exogenous IL-33 were found to limit pancreatic injury in coxsackievirus B-induced chronic pancreatitis through an IL-4-dependent induction of M2 macrophages (84). The role of IL-33 in acute pancreatitis is likely to be complex and has only recently been investigated. However, a role for direct caspase 1 functionality in IL-33 processing and release seems increasingly unsupported in the literature.

**Inflammasome Regulation of Metabolism**

**Metabolic pathway regulation.** Caspase 1 activation and NF-kB signaling pathways promote normoxic HIF-1α stabilization, increased expression of glycolytic enzymes, and decreased expression of tricarboxylic acid pathway enzymes with resultant increases in aerobic glycolysis and decreases in oxidative metabolism (Fig. 2). This has been excellently reviewed by O’Neill et al. (67). AMP-activated protein kinase (AMPK) is a ubiquitously expressed cytosolic sensor of energy homeostasis in cells and induces catabolism by promoting glycolysis and β-oxidation as well as by suppressing protein synthesis. It has recently been identified as a key negative regulator of metabolic pathway regulation. Caspase 1, NOD1, TLR4

**Mitochondrial oxidation**

- mtDNA mutations, NRTIs, Valproic acid
- Caspase 1, NOD1, TLR4
- M2 phenotype

**Fig. 2.** Regulation of mitochondrial oxidation by determinants of pancreatitis. Pancreatitis can be induced by mitochondrial DNA mutations, nucleoside reverse transcriptase inhibitors (NRTIs), and valproic acid, all of which suppress mitochondrial oxidation. Major effectors of pancreatitis severity, caspase 1, NOD1, and TLR4, suppress mitochondrial oxidation. Mitochondrial oxidation promotes an anti-inflammatory macrophage phenotype and suppression of caspase 1 and TLR4 proinflammatory macrophage phenotype signals. mtDNA, mitochondrial DNA.

NLRP3 inflammasome activation (93). LPS and free fatty acids suppress AMPK activation in macrophages consistent with AMPK functioning as a negative regulator of TLR and NLRP3 inflammasome signaling (59, 93). Shugrue et al. have recently identified that AMPK suppresses zymogen activation in acinar cells through use of chemical activator and inhibitors (86). A direct role for AMPK in suppression of sterile inflammation in the pancreas is intriguing but remains to be established. The NAD deacetylase sirtuin 1 (SIRT1) is a ubiquitously expressed cytosolic protein that also senses cellular energy homeostasis and similarly induces oxidative metabolism. SIRT1 antagonizes NF-kB signaling pathways (43) and is cleaved and inactivated in adipose tissue by caspase 1 in mice fed a high-fat diet (10). The role of suppressed SIRT1 signaling in disease severity in acute pancreatitis remains to be explored.

**Macrophage polarization.** Changes in the metabolic phenotype of cells are strong determinants of macrophage polarization (22). Briefly, proinflammatory macrophage polarization requires aerobic glycolysis and decreased oxidative metabolism. This immune phenotype is strongly associated with the acute sterile inflammatory responses in line with TLR4 (22), NOD1 (3), and caspase 1 activation (100). Caspase 1 in particular functions to promote mitochondrial breakdown and suppresses mitophagy, contributing to sustained suppression of oxidative metabolism in cells (100) (Fig. 2). Conversely, anti-inflammatory activated macrophage polarization requires fatty acid oxidation (34), and this immune phenotype is associated with resolution of acute inflammation. Evidence of macrophage polarization effecting pancreatic injury in acute pancreatitis rests largely on investigations of hemin-induced anti-inflammatory macrophage polarization in acute pancreatitis. In these studies, hemin-induced anti-inflammatory macrophages decreased sterile inflammation and tissue injury in experimental acute pancreatitis (61), suggesting that anti-inflammatory macrophage polarization may be protective in pancreatic injury. Metabolic perturbations induced by TLR4, NOD1, or caspase 1 activation in pancreatic acinar cells have not been investigated and may potentially contribute to a metabolic environment conducive to macrophage polarization in the pancreas (Fig. 2).

**Evidence of clinical significance.** Drug-induced pancreatitis provides insights into the potential importance of metabolism in acute pancreatitis. Many agents known to induce pancreatitis have mitochondrial toxicity and/or compromise oxidative metabolism. Nucleoside and nucleotide reverse transcriptase inhibitors.
hibitors are well-known causes of drug-induced acute pancreatitis. Nucleoside and nucleotide reverse transcriptase inhibitors induce mitochondrial toxicities in vivo as most commonly documented by type B lactic acidosis and confirmation of pathological ultrastructural changes in mitochondria in human cells induced by such agents in vitro and in clinical specimens (40). Valproic acid is a rare but potentially underreported cause of drug-induced pancreatitis (23). Valproic acid also appears to inhibit fatty acid oxidation at therapeutic doses with carnitine deficiency being the most studied clinical marker of this effect (87). Valproic acid at therapeutic doses decreases the biosynthesis and serum levels of carnitine, a rate-limiting substrate in fatty acid oxidation. Carnitine supplementation is protective from valproic acid-induced hepatotoxicity, suggesting that restoration of β-oxidative function can rescue this phenotype in the clinical setting (20). The most compelling evidence for mitochondrial dysfunction as a key pathological insult precipitating pancreatic disease comes from studies of inherited mitochondrial disorders. Pearson marrow pancreas syndrome is caused by inherited deletions in mitochondrial DNA and exocrine pancreatic insufficiency with pancreatic atrophy, and scarring is the most common manifestation of this syndrome (36). Other inherited mitochondrial disorders have also presented with chronic pancreatitis, including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndromes, for which many mutations in mitochondrial NADH dehydrogenase have been described. Additionally, a patient with inherited deficiency in the β-oxidative pathway component carnitine palmitoyltransferase presented with recurrent pancreatitis (88). In aggregate, compromised mitochondrial oxidative metabolism appears to be a common finding in drug-induced and genetic pancreatitis and suggests that drug-induced or genetic-mediated metabolic perturbation may contribute to pancreatitis (Fig. 2). An increased susceptibility to NLRP3 inflammasome activation in these conditions of perturbed oxidative metabolism remains to be demonstrated. Potential mechanisms include alteration of macrophage phenotype favoring the proinflammatory phenotype as discussed above. Alternatively, alterations in metabolic intermediate composition may effect metabolite receptor signaling and regulation of NLRP3 inflammasome activity as discussed in the next section.

Metabolite receptors. Recent investigation has established that metabolic intermediates such as succinate can regulate NLRP3 inflammasome induction, NF-κB signaling, and inflammatory signaling in immune cells (58). Recently, we identified that TLR-mediated aerobic glycolytic production of lactate negatively regulates TLR4- and TLR9-mediated NF-κB activation, induction of NLRP3 inflammasome components, and NLRP3 inflammasome activation by ATP in LPS-primed macrophages. We further identified that these effects could be reproduced in vivo by lactate injection, which protects from sterile inflammation and pancreatic injury in experimental severe acute pancreatitis. The immune dampening effects of parenteral lactate dosing required the presence of the cell surface lactate receptor G protein-coupled receptor (GPR) 81 in vivo. We further identified that GPR81 is an endogenous limiter of sterile inflammation in experimental acute pancreatitis. Finally, we showed that a small-molecule GPR81 agonist could reproduce these effects in isolated macrophages (31). Additionally, extracellular lactate is required for pancreatic tumor-associated macrophage polarization though HIF-1α-mediated induction of anti-inflammatory macrophage phenotype genes, including arginase 1 in tumor microenvironments; lactate also induced anti-inflammatory macrophage phenotype genes in macrophages in vitro (13). The mechanism of this lactate-mediated effect remains to be more fully characterized. These studies provide a mechanistic basis for the therapeutic benefit of lactated Ringer vs. normal saline infusion in limiting inflammatory organ injury in a randomized clinical trial in acute pancreatitis (94) (Fig. 3). Serum levels of many amino acids increase in acute inflammation (21), and we have recently identified that aspartate levels increase in macrophages in response to TLR4 stimulation in vitro, suggesting that TLR signaling alters aspartate metabolism (19). We further identify that macrophages signaling through the N-methyl-d-aspartic acid (NMDA) receptor component NMDA receptor subunit 2A (NR2A) suppress induction of NLRP3 inflammasome components mediated by TLRs in cell culture and that parenteral aspartate therapy limits pancreatic NLRP3 inflammasome expression, tissue injury, and inflammation in acute pancreatitis. We confirmed that NR2A was required for the therapeutic benefit of aspartate supplementation and had endogenous function in limiting sterile inflammation in a liver injury model. These findings provided one potential mechanism for the well-known immunosuppressive effects of parenteral nutrition (Fig. 3). Whether parenteral amino acid infusion as therapy may be beneficial to limit sterile inflammation early in the course of pancreatitis remains to be investigated.

Finally, we and others have identified that β-hydroxybutyrate, a key intermediate in β-oxidation of fatty acids, suppresses NF-κB signaling in macrophages (75). Recently submitted work from our laboratory identifies the β-hydroxybutyrate production is increased in TLR4 or TLR9 primed primary macrophages and that exogenous β-hydroxybutyrate suppresses NF-κB induction, NLRP3 inflammasome expression, and inflammasome-mediated IL-1β release. Additionally, we show that these effects are mediated by the cell surface β-hydroxybutyrate receptor GPR109a. We further show that β-hydroxybutyrate supplementation can suppress NF-κB activation in tissue macrophages in vivo and protect from experimental acute pancreatitis and drug-induced hepatitis with GPR109a dependence. Moreover GPR109a has endogenous function in limiting sterile inflammation and tissue injury in experimental pancreatitis. In aggregate, these findings suggest that suppression of β-oxidation may promote pancreatic injury.
in susceptible individuals by limiting production of β-hydroxybutyrate and thereby insufficiently activating a negative regulatory pathway of sterile inflammatory responses (Fig. 3).

Future Directions

The NLRP3 inflammasome has now been recognized for a number of years to be an important determinant of sterile inflammation in many diseases, including pancreatitis. Recent attention has turned to the exact role of individual inflammasome components, and their cellular requirement. Identification of the regulators of the inflammasome machinery has also been a very active area and has revealed multiple connections between inflammasome activation, hypoxia-induced transcription factors, and metabolite receptors. The metabolite receptors are particularly promising therapeutic targets in limiting innate immune-mediated tissue injury in pancreatitis (Fig. 3). The role for inflammasome components and effectors in fibrogenic cell types and in immune cell populations in chronic pancreatitis and in malignant cells and tumor-associated cell populations in pancreatic cancer remain to be extensively interrogated.

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DISCLOSURES

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Author contributions: R.H. drafted manuscript; R.H. and W.Z.M. edited and revised manuscript; R.H. and W.Z.M. approved final version of manuscript.

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