Are genetically modified mice useful for the understanding of acute pancreatitis?

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Abstract

Treatment of patients with acute pancreatitis has greatly improved due to a better understanding of the pathophysiology of the disease. This pathophysiology includes the activation and release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas, and a multiple organ dysfunction after their release into the systemic circulation. Moreover, significant evidence exists that synthesis and release of proinflammatory cytokines and chemokines are also responsible for the local injury and systemic dispersion of the inflammation. The use of knockout mice devoid of active pro- or anti-inflammatory mediators allows examination of the effects of a specific cytokine without any drawbacks induced by pharmacological manipulations. The results obtained from these genetically modified mice show that numerous mediators have a major role in the pathophysiology of acute pancreatitis. They also clearly demonstrate that a single genetic deletion cannot completely prevent the occurrence of pancreatic or distant organ injury. However, the fact that the immune system is characterized by redundancies of ligands and [...]
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ABSTRACT

Treatment of patients with acute pancreatitis has greatly improved due to a better understanding of the pathophysiology of the disease. This pathophysiology includes activation and release of pancreatic enzymes in the interstitium, autodigestion of the pancreas, and a multiple organ dysfunction after their release into the systemic circulation. Moreover, significant evidence exists that synthesis and release of proinflammatory cytokines and chemokines are also responsible for the local injury and systemic dispersion of the inflammation. The use of knockout mice devoid of active pro- or anti-inflammatory mediators allows examination of the effects of a specific cytokine without any drawbacks induced by pharmacological manipulations. The results obtained from these genetically modified mice show that numerous mediators have a major role in the pathophysiology of acute pancreatitis. They also clearly demonstrate that a single genetic deletion cannot completely prevent the occurrence of pancreatic or distant organ injury. However, the fact that the immune system is characterized by redundancies of ligands and receptors complicates the full understanding of each report. The utility of such experimental models might have limitations, and a full extrapolation of experimental data from genetically modified mice to humans must be done with caution.—


Key Words: • IL-1 • metallothionein • tumor necrosis factor • NK1R • acute pancreatitis • knockout mice

Acute pancreatitis is an inflammatory process that occurs in a normal organ and is diagnosed mainly by acute abdominal pain associated with a concomitant rise of serum amylase and lipase concentrations. Gallstone migration into the common bile duct and alcohol abuse account for most of the etiologies of the disease. Usually the injury is mild, but 20% of the patients have a severe injury; among those, 15 to 25% will die.

Treatment of these patients has greatly improved owing to a better understanding of the pathophysiology of the disease. This pathophysiology includes activation and release of pancreatic enzymes in the interstitium, autodigestion of the pancreas, and a multiple organ dysfunction after their release into the systemic circulation. In 1988, Rinderknecht (1) first hypothesized that cytokines may play an important role and suggested that inappropriate activation of the immune system might increase the severity of the local disease and the systemic complications. Over the past few years, significant evidence has accumulated that synthesis and release of proinflammatory cytokines and chemokines are responsible for the local injury and the systemic dispersion of the inflammation. Thus, inflammatory mediators produced within the gland increase the pancreatic injury, and spread out in distant organs, transforming a local inflammation into a severe systemic disease. The effects of these mediators and/or their receptors in the pancreas and remote organs have been evaluated using genetically modified mice. Although these studies are important to consider for increasing our knowledge, several drawbacks of these experimental models have to be pointed out. In this study, we will review the results of recent research dealing with genetically modified animals and see how much they have changed our understanding of the disease (Fig. 1).

INTERLEUKIN 1 (IL-1) AND TUMOR NECROSIS FACTOR α (TNF-α)

During pancreatitis, IL-1 and TNF-α are produced in large quantities. Evidence for their role in acute pancreatitis has been shown by studies using pharmacological antagonism. IL-1 is a proinflammatory cytokine whose deleterious effects might be attenuated by a specific IL-1 receptor antagonist (2). The effects of IL-1 are also suppressed by using IL-1 receptor-deficient mice (3, 4). Although the genetic deletion of IL-1 receptor decreases the severity of acute pancreatitis, IL-1 mRNA increases in organs known to produce the cytokines (4). Moreover, in IL-1 and TNF-α receptor knockout mice, the i.v. administration of sterile, cytokine-free ascitic fluid collected from rats with pancreatitis fails to induce lung injury as observed in normal animals, suggesting that acute lung injury during acute pancreatitis might be attributed to the pulmonary activity of IL-1 and TNF-α (5). When strains of knockout mice for IL-1 receptor or TNF-α receptor are used,
the severity and the mortality of acute pancreatitis are significantly attenuated in mice with a single genetic deletion. Identical benefits are observed with the double knockout mice (6). Animals devoid of receptors for both cytokines fail to show further decrease in parameters of severity and have only a modest decrease in the mortality rate. These observations strongly demonstrate the numerous redundancies that characterize the cytokine system.

Similarly, mice devoid of IL-1-converting enzyme, which is responsible for the cellular export of mature IL-1, cannot secrete IL-1 and fail to develop the acute pancreatic syndrome; pulmonary injury, pancreatic necrosis, and mortality are decreased (7).

Interleukin-6 (IL-6)

Genes encoding for proinflammatory cytokines are regulated during cerulein-induced pancreatitis via activation of the nuclear factor κB (NF-κB) (8). As a result, the expression of proteins such as IL-1, IL-6, and TNF-α is enhanced. IL-6 is a multipotent cytokine that influences numerous cells and several steps of the host defense response, including hepatic acute-phase proteins. IL-6 has a pivotal role in propagating the systemic inflammatory response and subsequent multiple-organ dysfunction in patients with severe acute pancreatitis. In experimental pancreatitis induced by cerulein, Suzuki et al. (9) demonstrate that pancreatic interstitial edema is higher in IL-6 transgenic than in wild-type mice, while the administration of anti-IL-6 receptor antibodies significantly decreased the edema in normal mice. Thus, modulation of IL-6 expression in the pancreas might be an interesting target.

Interleukin-10 (IL-10)

The mechanisms by which pancreatic inflammation acts on distant organ function begins to be understood. Evidence exists that the production of proinflammatory mediators (TNF-α, IL-1, IL-6, and IL-8) balances that of anti-inflammatory mediators (IL-10, IL-11, and IL-1 receptor antagonist). IL-10 is an anti-inflammatory cytokine that inhibits the release of proinflammatory cytokines by macrophages from all tissues. In the model of acute pancreatitis induced by a choline-deficient ethionine-supplemented diet, the severity of lung injury is greater in the IL-10 knockout than in the wild-type mice, whereas the severity of pancreatitis is similar in both groups (10). The transfection of a human IL-10 gene in normal mice results in an effectively transcribed DNA into intact mRNA (11). The transfer of this gene decreases the severity of pancreatitis, demonstrating the benefit of gene therapy during this acute inflammatory process (11). Endogenous IL-10 is important in reducing the extent of inflammation in the pancreas as well as in distant organs.
**CheMoaTtractant CytoKine Receptor-1 (CCr-1)**

The activation and trafficking of inflammatory cells involve a multigene family of cheMoaTtractant cytokines known as chemokines. These chemokines are characterized by numerous redundancies of ligands and receptors. Among them, the role of the CCr-1 was evaluated in the murine model of acute pancreatitis induced by cerulein associated with lung injury by developing mice lacking the CCr-1 (12). Although pancreatitis has a similar severity in wild-type and knockout mice, lung injury is significantly reduced in the mice lacking CCr-1 as evidenced by the reduction of the alveolar membrane thickening, the decreased leakage of albumin in broncho-alveolar lavage fluids, and the decreased sequestration of inflammatory cells into the lungs (12). Thus, genetic deletion of the CCr-1 reduces the severity of cerulein-induced lung injury associated with pancreatitis without influencing the severity of pancreatic injury. Thus, pancreatic injury produces and releases chemokines in the bloodstream that can activate circulating monocytes and neutrophils. Because pancreatic injury was not modified in mice lacking CCr-1 while lung injury was improved, CCr-1 plays a pivotal role in disseminating the injury from the pancreas to the lungs.

**NeuroKinin 1 Receptor (NK1R)**

The neuropeptide substance P released from nerve endings binds to NK1R and plays an important role in asthma, inflammatory bowel disease, arthritis, and other inflammatory processes. Because pancreatic acinar cells isolated from guinea pigs and mice express NK1R and because intrapancratic content of substance P increases during cerulein-induced pancreatitis, recent studies examined the role of this neuropeptide in developing the disease and its complications (13, 14). The deletion of the NK1R gene does not alter the pancreatic cell responses to cerulein. However, in NK1R-deficient mice, both pancreatitis and pancreatitis-associated lung injury are reduced in comparison to the injuries observed in wild-type mice (13). The increased lung permeability observed with cerulein is prevented by the deletion of the NK1R gene. These results indicate that neurogenic factors such as substance P play an important role in determining the severity of pancreatitis. However, the mechanisms by which substance P amplifies the severity of the pancreatitis is unclear. Substance P acts on endothelial cells via the NK1R, increasing the vascular permeability and promoting pancreatic edema (14). Substance P might act directly on the NK1R located on acinar cells; finally, substance P might directly injure pulmonary tissues.

**Intercellular Adhesion Molecule-1 (ICAM-1)**

Leukocyte sequestration within the area of injury and inflammation is a multistep process that begins with leukocyte activation, followed with the adhesion of circulating inflammatory cells to the endothelium via adhesions molecules such as ICAM-1. Under basal conditions, ICAM-1 is not constitutively expressed or is expressed only at low concentrations in most tissues, but ICAM-1 expression increases during inflammation. ICAM-1 interacts with CD11a/CD18 and CD11b/CD18 located on lymphocytes and leukocytes. The interaction between ICAM-1 and CD11/CD18 is a major determinant of leukocyte adhesion to the endothelial cells and transmigration of leukocytes into the areas of inflammation through the endothelial barrier. In two experimental models of acute pancreatitis in mice (administration of cerulein or feeding a choline-deficient, ethionine-supplemented diet), ICAM-1 concentrations in serum, pancreas, and lung increase (15). Kaufman et al. (16) also show that circulating concentrations of ICAM-1 are elevated in patients with severe pancreatitis. To confirm the pathophysiological role of the molecule, we used mice that do not express ICAM-1 and mice depleted of neutrophils by the administration of anti-neutrophil antibodies (15). The severity of acute pancreatitis and associated lung injury is partially decreased in mice deficient in ICAM-1. Neutrophil depletion also reduces the severity of pancreatic and lung injury, whereas the combination of both treatments does not further reduce the severity of the injuries. Treatment of severe pancreatitis with monoclonal antibodies against ICAM-1 also decreases both local pancreatic and lung injuries (17). These observations indicate that ICAM-1 plays an important role in pancreatitis and pancreatitis-associated lung injury.

**Cu/Zn Superoxide Dismutase and Metallothionein Transgenic Mice**

Free radical and lipid peroxide concentrations are increased in pancreatic tissues from both human and experimental acute pancreatitis (18–20). The possible sources of free radicals such as invading inflammatory cells, xanthine oxidase, cytochromes P450, and nitric oxide synthase are not yet clear. However, because O₂⁻ radical scavengers improve the severity of experimental acute pancreatitis and because prophylactic administration of antioxidants diminish edema in pancreas, free radicals play an important role in the pathogenesis of the disease (21). For that purpose, transgenic mice overexpressing Cu/Zn superoxide dismutase (an enzyme that catalyzes the dismutation of superoxide radicals to H₂O₂) were studied during acute pancreatitis. Overexpression of Cu/Zn superoxide dismutase is strongly associated with a reduction of serum amylase concentrations, pancreatic edema, and acinar cell injury in comparison to wild-type mice (22).

**Metallothionein 1 (MT-1)**

MT-1 are small, cysteine-rich, heavy metal binding proteins that protect from heavy metal toxicity, partic-
ularly for Zn homeostasis (23). MT-1 is an efficient scavenger of hydroxyl radicals (24), and cells isolated from MT-1 knockout mice are more sensitive to oxidative stress in vitro (25). During cerulein-induced pancreatitis, the pancreatic concentration of MT-1 is increased and might constitute a protective mechanism against oxidative stress. Mice overexpressing MT-1 have less severe pancreatitis than the wild-type, as evidenced by the decreased amylase concentration in serum, decreased acinar cell injury, and less pancreatic edema (26). Pancreatitis is more severe in mice deficient for MT-1 (26). Thus, genetic manipulations of MT-1 alter the outcome of cerulein-induced pancreatitis, but the exact mechanisms of MT-1 protection are not assessed in these studies.

**CATHEPSIN B**

Most researchers believe that autodigestion of the pancreas by its own digestive proteases or zymogens prematurely activated is the first event at the onset of acute pancreatitis. According to this hypothesis, activation of these digestive zymogens follows the redistribution of the lysosomal enzyme cathepsin B into a subcellular compartment containing zymogens. Activation of trypsinogen into active trypsin and subsequent activation of other zymogens then occur. A mutation of the cationic trypsinogen gene responsible for a human form of hereditary pancreatitis has recently been discovered, reinforcing the link between these early events and the disease (27). To test this hypothesis, Halangk et al. (28) developed cathepsin B-deficient mice. After induction of pancreatitis, the pancreatic trypsin activity in these mice is more than 80% lower than in wild-type mice and pancreatic injury is 50% lower. However, the prevention of trypsinogen activation by genetic deletion of cathepsin B was incomplete. Therefore, cathepsin B is not the only pathway involved in premature intra-acinar activation of trypsinogen; trypsinogen activation by other lysosomal enzymes has to be considered as an alternative. In the cathepsin B-deficient mice, although the reduction of trypsinogen activation correlates with a decrease of acinar cell necrosis, the systemic inflammatory response as well as the pancreatic leukocyte infiltration are not affected, indicating that these events are not cathepsin B-dependent.

**PERSPECTIVES**

These studies show that numerous cytokines have a major role in the pathophysiology of acute pancreatitis. They also clearly demonstrate that a single genetic deletion cannot completely prevent the occurrence of pancreatic or distant organ injury. They show numerous redundancies of ligands and receptors that characterize the families of cytokines and chemokines.

During acute pancreatitis, the activation of leukocytes and cytokine release are delayed after the primary pancreatic insult. Consequently, the genetic deletion of genes involved in the early events might have more beneficial consequences on pancreatic injury than genetic deletion of genes encoding for cytokines or chemokines that are produced later. However, although cathepsin B-deficient mice were protected against cerulein-induced pancreatitis, the protection was only partial, showing that other early pathways have to be inhibited to provide a complete protection.

**ADVANTAGES AND DISADVANTAGES OF USING THESE GENETICALLY MODIFIED ANIMALS MODELS**

The ability to remove or alter with precision a single gene of thousands in an animal is now a routine technique for creating animal models that can be used to study the pathophysiology of various diseases (29, 30). The goal of the gene targeting (knockout) method is to replace the specific gene of interest with one that is inactive, altered, or irrelevant. The deficits present in a knockout mouse can reveal or clarify the function of the mutant gene. These experimental systems are of great value in studying the pathogenesis and treatment of disorders in all fields of medicine. Consequently, a knockout mouse corresponding to a particular genetic disorder may help clarify the mechanism of the disease. The phenotype of the knockout animal can usually be anticipated by previous knowledge of the gene function but in some cases inactivation of a specific gene such as IL-2 gene results in ulcerative colitis, an unexpected mutant phenotype (31).

However, in light of recent advances, several considerations are important in evaluating the phenotype of a knockout mouse. The fact that a specific mutation has been present in the mice from the time of its conception may enable a distinction between phenotypic changes due to the mutation itself and changes caused by adaptation and compensation for the mutation. Moreover, if a gene is expressed in different tissues where it may have different functions, its alteration may induce unexpected consequences. For example, the immune system is characterized by many redundancies of ligands and receptors that complicate the understanding of the action of a single cytokine or chemokine. Mice deficient in E and P selectins, which are adhesive receptors expressed on platelets and endothelial cells, expressed higher concentrations of serum granulocyte monocyte colony-stimulating factor (GMCSF), IL-3, and peripheral leukocyte counts. Thus, one should take into consideration the role of high serum concentrations of GMCSF, IL-3, and leukocyte counts when evaluating the effect of P and E selectins. Conversely, the function of two genes may overlap, and mutation in a single gene might not reveal an abnormal phenotype. It must also be remembered that rodents have no homologue of human IL-8 and, in contrast to humans, have abundant CCR-1 on granulocytes, mak-
ing a full extrapolation of experimental data from genetically modified mice to humans difficult.

CONCLUSIONS

The use of knockout mice devoid of active pro- or anti-inflammatory mediators allows examination of the effects of a specific cytokine without any drawbacks induced by pharmacological manipulations. In acute pancreatitis, several studies clearly identify the role of these specific targeted genes. However, the immune system is characterized by redundancies of ligands and receptors, which complicates the full understanding of each report. The utility of such experimental models might have limitations, and a full extrapolation of experimental data from genetically modified mice to humans has to be made with caution.

REFERENCES


GENETICALLY MODIFIED MICE AND ACUTE PANCREATITIS