Hypothesis: Blood leukocytes play a major role in mediating local and systemic inflammation during acute pancreatitis. We hypothesize that peripheral blood mononuclear cells (PBMCs) in circulation exhibit unique changes in gene expression and could provide a "reporter" function that reflects the inflammatory response in the pancreas with acute pancreatitis.

Design: To determine specific changes in blood leukocytes during acute pancreatitis, we studied the gene transcription profile in PBMCs in a rat model of experimental pancreatitis (sodium taurocholate). Normal rats, saline controls, and a model of septic shock were used as controls. Complementary RNA obtained from PBMCs of each group (n=3 in each group) were applied to Affymetrix rat genome DNA GeneChip arrays.

Main Outcome Measure: Changes in gene expression.

Results: From the 8799 rat genes analyzed, 140 genes showed unique significant changes in their expression in PBMCs during the acute phase of pancreatitis, but not in sepsis. Among the 140 genes, 57 were up-regulated, while 69 were down-regulated. Platelet-derived growth factor receptor, prostaglandin E2 receptor, and phospholipase D were among the top up-regulated genes. Others included genes involved in G protein–coupled receptor and transforming growth factor β-mediated signaling pathways, while genes associated with apoptosis, glucocorticoid receptors, and even the cholecystokinin receptor were down-regulated.

Conclusions: Microarray analysis in transcriptional profiling of PBMCs showed that genes that are uniquely related to molecular and pancreatic function display differential expression in acute pancreatitis. Profiling genes obtained from an easily accessible source during severe pancreatitis may identify surrogate markers for disease severity.

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A CUTE PANCREATITIS IS A SEVERE inflammatory disease frequently diagnosed by acute abdominal pain associated with a concomitant rise of serum amylase and lipase concentrations. There are more than 180,000 new cases per year in the United States. While the injury and systemic manifestations are typically mild, up to 20% of the patients will exhibit a severe reaction—typified by pancreatic necrosis—and among them, the morbidity can be more than 80% and mortality, about 25%. The pathophysiologic mechanism of the disease includes the activation and release of pancreatic enzymes within the ductal system, the autodigestion of the pancreas, and multiple organ dysfunction following the release of the enzymes into the systemic circulation. A major challenge has been to identify markers of disease severity.

It likely that the synthesis and release of proinflammatory cytokines and chemokines are responsible for progression of the local injury to the pancreas and retroperitoneum. Also, inflammatory mediators produced within the gland increase the pancreatic injury and spread to distant organs, transforming a local inflammation into a severe systemic disease. The mediators involved in this systemic inflammation are similar to those encountered during sepsis.

Because it is important to predict the severity of the disease as early as possible to optimize the therapy and prevent organ dysfunction and local complications, several scores, such as Ranson criteria, Glasgow Outcome Scale score, and the Acute Physiology and Chronic Health Evaluation score, have been used. New serum markers have emerged and their ability to provide additional information on the severity of the disease has been evaluated.
The current prognostic indicators available for acute pancreatitis rely heavily on clinical markers combined with an increase in serum levels of proteins such as C-reactive protein and trypsinogen-activation peptide. Newer proteins, such as pancreatitis-associated protein, have not reached clinical utility as originally hoped.

Since pancreatitis is an immunologic response to the injury, we explored the possibility that peripheral blood mononuclear cells (PBMCs) would harbor specific markers of progression of disease. We used gene chip microarray technology to study genetic expression patterns of PBMCs obtained from rats with pancreatitis. While typically one compares these patterns with normal controls, we further compared them with PBMCs obtained from rats with intra-abdominal bacterial sepsis to elucidate unique patterns of expression in acute pancreatitis.

**METHODS**

**ANIMALS**

All animal experiments in this study were performed with the approval of the Animal Care and Use Committee of State University of New York Downstate Medical Center and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats obtained from Harlan Sprague Dawley (Indianapolis, Indiana) weighing from 225 to 250 g were used for experiments.

**EXPERIMENTAL ACUTE PANCREATITIS**

Acute pancreatitis was induced using retrograde infusion of 4% sodium taurocholate (NaT) (Sigma, St Louis, Missouri) into the pancreatic duct, as previously described. Briefly, under pentobarbital anesthesia (50 mg/kg given intraperitoneally; Abbott Laboratories, North Chicago, Illinois), a midline incision was performed. The common bile duct was identified and canulated with a 22-gauge catheter. The bile duct was then ligated to prevent the flow of bile, and 4% NaT in sterile saline was infused into the pancreas. The bile duct was further ligated with a 4-0 silk ligature. The end of the tube was beyond the ampulla of Vater in the duodenum. The cecum was then returned to the peritoneal cavity and punctured once with a 16-gauge needle proximal to the ligature. The cecum was ligated just below the ileocecal valve (2.5 cm), and the cecum was ligated just below the ileocecal valve with a 3-0 silk ligature and the antimesenteric cecal surface was punctured once with a 16-gauge needle proximal to the ligation. The cecum was then returned to the peritoneal cavity and fecal content in the ligated segment was allowed to extrude through the puncture to the peritoneum. The peritoneum and abdominal muscles were closed with silk sutures. After CLP, rats were returned to cages and allowed ad libitum access to food and water. For both the experimental model of acute pancreatitis and sepsis, control rats were anesthetized and sham operated on with a laparotomy. The pancreas or cecum was manipulated but neither pancreatitis induction nor the CLP procedure was performed.

**PBMC ISOLATION**

Twenty-four hours after pancreatitis, septic shock induction, or sham operation with saline infusion or in untouched, normal control animals who had fasted (n=3 each group, N=12), approximately 8 to 10 mL of whole blood were collected via the inferior vena cava from each rat under pentobarbital anesthesia. The PBMCs were isolated from rat whole blood by centrifugation through Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden).

**PREPARATION OF COMPLEMENTARY RNA AND GENE CHIP HYBRIDIZATION**

Preparation of complementary RNA (cRNA), hybridization, and scanning of high-density oligonucleotide microarrays were performed according to the manufacturer’s protocol (Affymetrix Inc, Santa Clara, California). Briefly, total RNA was extracted from PBMCs using TRIzol (GIBCO BRL Life Technologies, Grand Island, New York) and eluted using an RNAeasy spin column (Qiagen Inc, Valencia, California). Ten micrograms of total RNA were converted into double-stranded complementary DNA (cDNA) by reverse transcription using SuperScript Choice System (Invitrogen Corporation, Carlsbad, California) with the T7-(dT)24 primer (5’-GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG [dT24]). The double-stranded cDNA product was extracted with phenol/chloroform/isooamyl alcohol using Phase Lock Gels (Eppendorf, Westbury, New York). Double-stranded cDNA was in vitro transcribed into cRNA and nucleotides were biotinylated using the Enzo BioArray HighYield RNA Transcription Labeling Kit (Affymetrix). The in vitro transcription product was further purified using RNeasy mini columns (Qiagen) and fragmented as previously described. The fragmented in vitro transcription product was hybridized onto the rat genome U34A DNA GeneChip Array (Affymetrix), which contained approximately 7000 full-length sequences and 1000 EST (expressed sequence tag) clusters. The sequences were selected from the UniGene database. All 12 PBMC samples were subjected to RNA extraction and transcript profiling.

**DATA AND STATISTICAL ANALYSIS**

An absolute expression analysis was performed using Affymetrix MAS 5.0, and the data from genes were imported into GeneSpring software version 3.1 (Silicon Genetics, Redwood City, California) for further analyses. Differentially expressed genes were selected. Differential expression was defined as a change of at least 2-fold vs respective controls. Nonparametric tests were used, assuming nonequal means, specifically the Welch t test and Welch analysis of variance. The significance level was set at more than 2-fold change between groups, P<.05.

**RESULTS**

Rats treated with NaT showed pancreatic edema and necrosis, as we have previously observed. Those treated with CLP were not autopsied. Adequate numbers of PBMCs were obtained from each animal studied for gene array analysis.

We compared gene transcription profiles of PBMCs in normal, untouched control animals with those with acute pancreatic necrosis.
pancreatitis to identify those genes induced in pancreatitis (Figure 1). From the 8799 rat genes analyzed on the chip, using supervised cluster analysis, we identified 947 genes significantly changed by 2-fold in pancreatitis.

These 947 genes were then subjected to comparison between animals with acute pancreatitis and saline controls, which identified 170 genes that changed expression (Figure 2). Similarly, 201 unique genes were identified between rats with CLP (abdominal sepsis) and saline controls, and 409 differentially expressed genes were identified between animals with acute pancreatitis and intra-abdominal sepsis.

As shown in Figure 2, of the 170 genes that changed between pancreatitis and saline controls, 15 overlapped when compared with septic/pancreatic animals, and another 18 overlapped with septic/normal animals. In total, 140 genes were unique to PBMCs in animals with pancreatitis. Figure 3 shows a cluster analysis of the genes from the 3 groups analyzed in Figure 2.

Among the 140 genes whose expression changed in pancreatitis alone, 57 were up-regulated, while 69 were down-regulated; 25% corresponded to ESTs. Table 1 and Table 2 show the most significantly (>3-fold) up-regulated (n=14) and down-regulated genes (n=33), respectively, and their known functions.

**COMMENT**

Acute pancreatitis is a disease that begins with an insult followed by autodigestion of pancreatic tissue. Inflam-

![Figure 1](image1.png)

**Figure 1.** Scatterplot of the differentially expressed genes in peripheral blood mononuclear cells (PBMCs) isolated from rats with acute pancreatitis compared with those from normal control animals that were not operated on. The axes are arbitrary fold changes. The red points above the line are up-regulated genes, and the blue points below the line are down-regulated genes. Nine hundred forty-seven genes were identified and subjected to further analysis (see Figure 2).

![Figure 2](image2.png)

**Figure 2.** Venn diagram of differential expression of the 947 genes (identified in Figure 1) in peripheral blood mononuclear cells. Expression was compared between rats who underwent laparotomy and ductal infusion with saline, those undergoing infusion with sodium taurocholate to induce acute pancreatitis, and those with intra-abdominal sepsis induced by cecal ligation and puncture. One hundred forty genes induced specifically during sodium taurocholate acute pancreatitis were identified; the most induced and inhibited are depicted in Table 1 and Table 2, respectively.
immunologic, and cardiac systems. Ten percent to 20% of patients will develop severe disease, and of those, up to 50% can die. While patients can die from overwhelming sepsis as a result of bacterial translocation, most patients are not infected in the first few weeks of the disease. Therefore, disease severity and

Table 1. Up-regulated Genes Differentially Expressed in PBMCs in Acute Pancreatitis but Not in Septic Shock or Normal Conditionsa

<table>
<thead>
<tr>
<th>Fold Change</th>
<th>P Value</th>
<th>GenBank Accession Number</th>
<th>Name</th>
<th>Description</th>
<th>Biological Process</th>
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</thead>
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<tr>
<td>U11.9</td>
<td>.02</td>
<td>AF017251</td>
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<td>U10.7</td>
<td>.03</td>
<td>AI233279</td>
<td>Pdgfra</td>
<td>Platelet-derived growth factor receptor α</td>
<td>Protein amino acid phosphorylation, transmembrane receptor protein tyrosine kinase</td>
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<tr>
<td>U6.1</td>
<td>.03</td>
<td>Y07903</td>
<td>Adam3</td>
<td>A disintegrin and metalloprotease domain 3 (cyritestin)</td>
<td>Protein amino acid phosphorylation, transmembrane receptor protein tyrosine kinase</td>
</tr>
<tr>
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<td>.03</td>
<td>U94708</td>
<td>Piper2</td>
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<td>GPCRs class A rhodopsinlike, small-ligand GPCRs rhodopsinlike receptor</td>
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<tr>
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<td>Enoyl coenzyme A hydratase 1</td>
<td>Enzyme</td>
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<td>Phosphodiesterase 3B</td>
<td>Signal transduction</td>
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<tr>
<td>U4.6</td>
<td>.01</td>
<td>Z21935</td>
<td>Mapk4</td>
<td>Rat protein kinase rMK2</td>
<td>Protein amino acid phosphorylation ATP binding; protein serine/threonine kinase</td>
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<td>L09653</td>
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<td>Transforming growth factor β signaling pathway</td>
</tr>
<tr>
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<td>.03</td>
<td>L09653</td>
<td>Tgbr2</td>
<td>Transforming growth factor β receptor 2</td>
<td>Transforming growth factor β signaling pathway</td>
</tr>
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<td>U3.8</td>
<td>.03</td>
<td>L07736</td>
<td>Cpt1a</td>
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<td>Fatty acid degradation; mitochondrial fatty acid beta-oxidation, fatty acid metabolism, mitochondrial; membrane fraction Carn_acyltransf,acyltransferase</td>
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<td>Complement activation classical</td>
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<td>Adcy8</td>
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<td>G protein signaling; intracellular signaling cascade</td>
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<td>J04486</td>
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<td>Insulinlike growth factor binding protein 2</td>
<td>Insulinlike growth factor binding</td>
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<td>L26986</td>
<td>Adcy8</td>
<td>Adenylyl cyclase 8</td>
<td>Guanylate cyclase</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, adenosine triphosphate; GPCR, G protein–coupled receptor; PBMC, peripheral blood mononuclear cell.

aThree times over controls (n=14).
The early immunologic response leads to the severe systemic reaction and, likely, the subsequent morbidity. The immunologic response in acute pancreatitis is very different. The immunologic response leads to the severe systemic reaction and, likely, the subsequent morbidity. The immunologic response in acute pancreatitis is very different. The immunologic response leads to the severe systemic reaction and, likely, the subsequent morbidity. The immunologic response in acute pancreatitis is very different.

The early immunologic response leads to the severe systemic reaction and, likely, the subsequent morbidity. The immunologic response in acute pancreatitis is different from abdominal sepsis, since the pancreas is not invaded by bacteria at the early stages; no abscesses are seen until late, and the time lines are very different.

Predicting which patients are going to progress to severe disease has been a challenge for physicians. To date, very few serum markers have been shown to be effective predictors, and the best prognostic indicators are still the clinical parameters.2,11-15,24,25 Our laboratory has been interested in identification of new potential markers.

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ally studied the role of blood-derived inflammatory cells as markers for pancreatic inflammation. We postulate that the response to pancreatitis, and potentially the prognostic indicator of disease severity, rests in PBMCs. These are the cells that mediate the immunologic reaction to pancreatitis. While investigators have measured gene expression of the pancreas in experimental pancreatitis, and others have studied changes in the population of leukocytes in pancreatitis, to our knowledge, no one has studied the genetic map of PBMCs during disease.

We postulated that PBMCs can serve a “reporter” function as biomarkers of acute pancreatitis and, as such, would express unique genes during pancreatitis. Further, PBMCs represent an easily accessible, noninvasive source of material. We used microarray technology to assess the gene transcription profile of PBMCs in a rat model of NaT-induced acute necrotizing pancreatitis. We first compared the profile of pancreatic mice with pancreatic mice from normal controls who were not operated on. This identified 947 genes induced during pancreatitis. To determine which of these genes were uniquely expressed during pancreatitis, we compared the PBMC RNA obtained from rats induced with pancreatitis with PBMC RNA obtained from rats that underwent saline infusion alone and with rats with intra-abdominal sepsis.

We identified 140 unique genes that were induced or inhibited in PBMCs during NaT-induced (necrotizing) pancreatic disease. Not surprisingly, some of these genes highly induced were cytokines previously implicated in pancreatitis, such as receptors for platelet-derived factor, transforming growth factor β, and a variety of G protein–related signal transduction genes. The phospholipase D1 gene was involved in the intracellular modulation of cellular mitogenesis and even pancreatic organ regeneration. The prostaglandin E2 receptor was induced, which is interesting since inhibition of prostaglandin E2 by cyclooxygenase inhibitors improves survival.

Interestingly, genes associated with cell death, such as caspase 1 and BH3 interacting domain 3, and cell membrane integrity were uniquely down-regulated in PBMCs of rats with acute pancreatitis. Caspase 1, associated with cellular apoptosis, has been studied in acute pancreatitis, and its activation within the pancreas is associated with severe necrosis, but its inhibition may be protective in sepsis. BH3 domain proteins are also involved in mitochondrion-mediated cell death via BCL-2 mediated apoptosis. How these PBMC-associated apoptosis processes relate to pancreatic necrosis remains to be studied.

What is striking is that some genes associated with pancreatitis, namely the glucocorticoid receptor, cholecys
tokinin receptor, and lipase, were significantly inhibited, each by 7-fold. We have shown that glucocorticoid treatment can improve survival in animal models of pancreatitis, and others have described a relationship between glucocorticoids and the cholecystokinin receptor in the disease process. Use of either compound after the induction of the disease has not been studied.

Our data show that in acute pancreatitis, PBMCs express genes that are related to pancreatic illness and not intra-abdominal sepsis. This observation, that pancreatitis-related genes are induced in cells outside the pancreas in PBMCs during necrotizing pancreatitis, has not been previously described, to our knowledge. It should not be surprising that such genes are induced; these genes were originally identified from inflamed pancreatic tissue, which likely already had similar PBMCs infiltrating it. However, because the PBMCs are involved in the systemic inflammatory response to pancreatitis, following their expression/activation during the evolution of pancreatitis should be illuminating. The ability of easily accessible blood leukocytes to provide a reporter function for solid organ disease will likely prove useful in pancreatitis, as we have shown in other diseases. Furthermore, this approach may identify as of yet unknown genes involved in the pathogenesis of disease. Mapping the expression pattern of these genes in the clinical arena might help differentiate the patients who have mild, moderate, and severe pancreatitis, including necrosis and systemic complications. Future studies will establish a panel of these genes to differentiate between sepsis, pancreatitis, and severe necrotizing pancreatitis and determine if serum concentration of these new molecular markers, obtained from blood within the blood that modulates the inflammatory response to pancreatitis, correlate to the severity of pancreatitis. If they predict occurrence of multiple organ dysfunction, it is then conceivable that these markers will predict the outcome of the disease.

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Author Contributions: Study concept and design: Bluth, Zhang, and Zenilman. Acquisition of data: Lin and Viterbo. Analysis and interpretation of data: Bluth, Lin, and Zenilman. Drafting of the manuscript: Bluth, Zhang, and Zenilman. Critical revision of the manuscript: Bluth, Lin, Viterbo, and Zenilman. Obtained funding: Zenilman. Administrative, technical, and material support: Bluth, Lin, Viterbo, and Zenilman. Study supervision: Bluth and Zenilman.

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