ANNALS OF SURGERY
Vol. 232, No. 5, 646–652
© 2000 Lippincott Williams & Wilkins, Inc.

Comparison of *reg I* and *reg III* Levels During Acute Pancreatitis in the Rat

Michael E. Zenilman, MD, David Tuchman, MD, Qing-hu Zheng, MS, Joshua Levine, MD, and Harry Delany, MD

From the Department of Surgery, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York

Objective
To study alterations of serum levels of the pancreatic *reg* family of proteins in two models of acute pancreatitis.

Summary Background Data
The pancreatic *reg* family of proteins is expressed in the acinar pancreas. *Reg I* (pancreatic stone protein, PSP) and *reg III* (pancreatitis-associated protein, PAP) are induced after the onset of acute pancreatitis, and both have been proposed as potential markers of pancreatitis.

Methods
Pancreatitis was induced in rats by either retrograde infusion of sodium taurocholate or by direct trauma. Serum samples were obtained daily for 4 days after the procedure, and the animals were then killed. Twelve animals underwent sham procedure and six underwent daily analysis without surgery. Levels of *reg I*/PSP and *reg III*/PAP were estimated by enzyme-linked immunosorbent assay.

Results
Reg III/PAP levels increased significantly the first day after induction of both types of pancreatitis and rapidly returned to baseline in all survivors. Even animals who received retrograde infusion of saline showed a mild increase in *reg III*/PAP on the first day, whereas control animals that did not undergo surgery showed no variations. *Reg I*/PSP serum levels remained unchanged throughout all experimental periods. Postinjury *reg III*/PAP levels significantly correlated with severity of the pancreatic injury and animal survival; *reg I*/PSP levels did not.

Conclusion
After induction of pancreatitis, serum levels of *reg I* and *III* protein differ significantly. *Reg III*/PAP levels are a sensitive marker of pancreatic injury and early in the disease may be a useful prognostic indicator for disease severity.

The pancreatic *reg* family of proteins is expressed in the gastrointestinal tract. *Reg Iα*, also known as pancreatic stone protein, is normally expressed in the acinar pancreas. *Reg III*, also known as pancreatitis-associated protein 1, was originally isolated from the pancreatic juice of rats with acute pancreatitis[1] and is normally expressed at low levels in pancreas, but *reg III* levels increase 200- to 300-fold after even mild pancreatic inflammation.[1–3] It has been characterized in humans and rats[1,4] and is normally expressed in the small intestine[5,6] and not in the pancreas.[7] *Reg II* has been described only in the mouse and rat.[7]

Studies of acute pancreatitis in animals show that both *reg Iα* and *reg III* genes (mRNAs) are induced in the parenchyma,[2,8,9] and serum levels of both proteins increase.[10,11] Parenchymal levels of *reg III*/PAP-l have even been found to correlate with severity of pancreatic injury in rodents after induction by cerulein injection.[12]

The fact that the *reg* genes are induced in the pancreatic parenchyma in graded response to injury makes them a potentially sensitive marker for injury and inflammation. Although *reg III* has been described as a potential marker for pancreatic injury in both pancreatitis[13] and pancreatic transplant,[14] no controlled comparison of serum *reg Iα* and *III* levels in human or animal models of acute pancreatitis has been done.

The aim of this study was to compare the alterations of serum levels of *reg Iα* and *reg III* proteins in rat models of acute pancreatitis. Two graded models of injury were used: infusion of sodium taurocholate (NaT) retrograde into the pancreatic duct, and a model of blunt pancreatic trauma. Serum levels of *reg I* and *III* were measured by enzyme-linked immunosorbent assay (ELISA), and these were correlated with pancreatic injury and overall survival.

Presented at Digestive Disease Week, May 17, 1999, Orlando, Florida.
Supported by NIH RO1 DK 54511–01.
Correspondence: Michael E. Zenilman, MD, Dept. of Surgery, Albert Einstein College of Medicine, 1825 Eastchester Rd., Bronx, NY 10461.
E-mail: mzenilma@montefiore.org
Accepted for publication January 3, 2000.

METHODS

Models of Pancreatitis

Twenty-six rats underwent retrograde infusion of NaT into the pancreatic duct. Under pentobarbital anesthesia (50 mg/kg given intraperitoneally), the duodenum was isolated and the main pancreatic duct was cannulated with polyethylene tubing (0.51 ID, 0.94 OD; Dow Corning, Midland, MI). Five, nine, and twelve animals underwent infusion of 2%, 3%, and 4% NaT, respectively. NaT was administered in 0.9% normal saline at a rate of 1 mL/kg during a period of 2 minutes.[15] Six animals underwent infusion of saline alone, and six served as nonoperated controls.

Eighteen rats were subjected to direct pancreatic trauma using a previously published protocol.[16] Briefly, a midline laparotomy was performed under anesthesia, and the neck and body of the pancreas was placed onto a platform. Controlled injury was applied by dropping a 52-g cylindrical weight from a distance of 12″, 18″, and 24″ (n = 6 per group). This induced a graded contusion injury of 0.156, 0.234, and 0.312 Joules.[16] Six animals underwent sham procedure.

The total number of animals used for all groups was 62. All surgical procedures were approved by the Animal Institute Committee at our institution, an accredited program.

Postoperative Analysis

Serum samples was obtained daily for 4 days after the procedure from tail veins. All surviving animals were killed on postinjury day 4, and necropsies were performed on all. The severity of pancreatitis at necropsy was graded by standardized scoring of gross tissue, as previously described.[16] Briefly, a normal-appearing pancreas was scored 0, edematous pancreatitis 1, moderate hemorrhagic/necrotic pancreatitis 2, and severe hemorrhagic/necrotic pancreatitis 3. The amount of ascites present at necropsy was also quantitated and scored as 0 mL, 0 to 5 mL, and greater than 5 mL. Serum amylase was measured by a enzymatic kit (Sigma, St. Louis, MO).

ELISA

Levels of *reg Iα* (pancreatic stone protein) and *reg III* (PAP-I) were measured by direct competitive ELISA. Antibodies were produced in guinea pig to unique 10–amino acid sequences of *reg I* and *III*, each at position 54–63.[7] The sequences were *reg Iα* = YFMEDHLSWA and *reg III* = ALFQIPQTWF. Each peptide was synthesized with the N-terminus linked to KLH (Research Genetics, Huntsville, AL) and antibodies raised in guinea pigs using Freund's adjuvant. Terminal bleeds were performed under anesthesia from the superior vena cava.[17]

Stock solution of peptide were stored at 0.1 mg/mL in pH 7 solution in RPMI; for each ELISA, 0.01 mg/mL peptide was plated on 96-well plates. Plates were washed with blocking solution (Pierce, Rockford, MD) and then washed three times in phosphate-buffered saline (pH 7.0). Antibodies were used at 1:2,000 dilution for both standard curve and sample analysis. Rat serum was assayed at a 1:10 dilution and incubated overnight with antibody. After incubating each well with 100 μL sample for 1 hour at room temperature, wells were washed three times with phosphate-buffered saline. Second antibody (1:2,000, alkaline phosphatase linked goat anti-guinea pig, Accurate Chemical & Scientific Co., Westbury, NY) was added for 30 minutes, and the plates were again washed with phosphate-buffered saline three times. Development was performed with ImmunoPure PNPP (Pierce, Rockford, IL), and absorbency was read at 415 nm on a microplate reader (Biorad, Gaithersville, MD).

Western Analysis

After SDS-polyacrylamide electrophoresis (15%), 10 μg protein/well was electrophoretically transferred to nitrocellulose (Nytran; Schleicher & Schuell, Keene, NH) and polyclonal antibody was used at a 1:500 dilution. Antibody visualization was performed by alkaline phosphatase technique (NBT/BCIP, Pierce) using anti-IgG at a 1:15,000 dilution.

Statistical Analysis

Data are expressed as mean ± standard error of the mean. All data were entered into a Statistica database (Statsoft, Tulsa, OK) and analyzed by analysis of variance with repeated measures, and pooled data were compared with controls by the unpaired Student *t* test. Post hoc comparisons were performed using the Newman-Keul test. Significance was defined as $P < .05$.

RESULTS

Antibodies and ELISA Construction

Western analysis of the *reg Iα* antibody against pancreatic juice showed a band at 16 kD; Western analysis of the *reg III* antibody against small intestine showed a similar band (Fig. 1A). Both bands were inhibited by incubation with excess antigen (0.1 mg/mL, data not shown) and did not cross-react with human pancreatic juice or pure recombinant human PAP (Dynbio, Marseilles, France). The two antibodies generated reproducible ELISA standard curves, and a direct competitive ELISA was constructed. The limit of each ELISA was approximately 100 pg/mL on the standard curve and 500 pg/mL on rat serum. Standard curves for *reg Iα* and *reg III* are shown in Figure 1B.

Mean *reg Iα* and *reg III* levels for control animal serum were 2.5 ± 0.2 μg/mL and 366 ± 75 ng/mL in the trauma group, respectively, and 2.25 ± 0.98 μg/mL and 224 ± 57 ng/mL in the NaT group, respectively. The mean *reg III* level for all animals was 254 ± 49 ng/mL. By comparison, data from rat pancreatic juice revealed a level of 68 ± 11 μg/mL, and 2.8 ± 1 ng/mL, respectively.

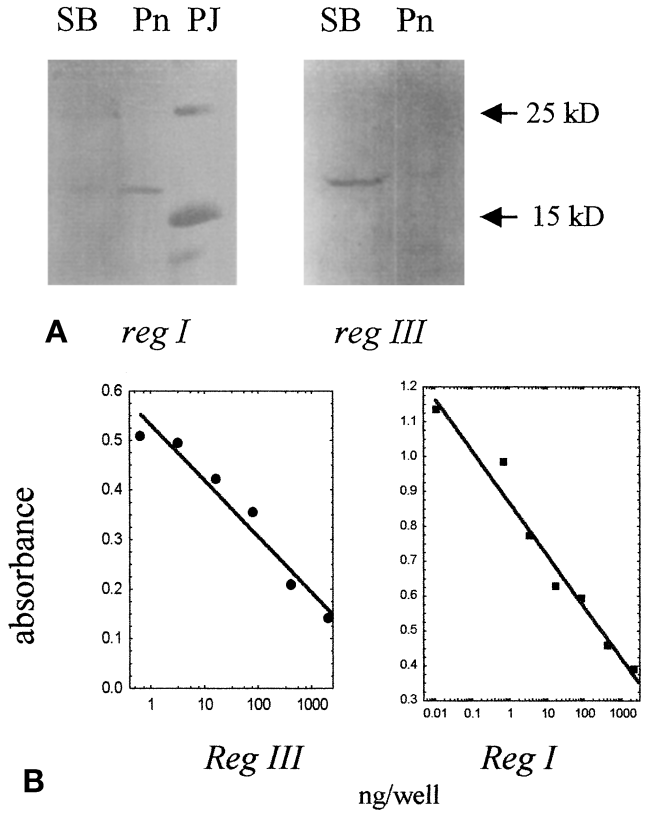

Figure 1. (**A**) Western analysis of polyclonal antibodies, created in guinea pig, to peptide fragments of *reg Iα* and *reg III*. *Reg Iα*, homologous to pancreatic stone protein, is found in pancreatic juice and not small bowel (left panel). *Reg III*, homologous to pancreatitis-associated protein, is normally found in intestine but not pancreatic juice (right panel). Both yield a band of 16 kD. This gel confirms the specificity of the antibodies. SB, small bowel; Pn, pancreas; PJ, pancreatic juice. (**B**) Standard curve of enzyme-linked immunosorbent assay for each antibody against the peptide antigen. This curve was reproducible in multiple experiments.

Pancreatitis

Animals were subjected to graded pancreatic insult (2%, 3%, or 4% NaT) or 0.156, 0.234, or 0.312 Joules of blunt trauma. Hematoxylin–eosin staining of tissue at necropsy confirmed the presence of acute necrotizing pancreatitis in those that died, and resolving pancreatitis with infiltration of polymorphonuclear cells, zymogen-depleted acinar, and acinar atrophy in those that survived. Seven animals in the NaT group died; five in the trauma group died.

An upward trend in the death rate was noted for both groups as the pancreatic insult worsened; this was significant. Specifically, by scoring the severity of pancreatic injury as 0 for no operation, 1 for sham operation, and 2, 3, and 4 for increasing NaT (2%, 3%, or 4%) or trauma (0.156, 0.234, and 0.312 Joules), the correlation between injury and death was $r = 0.32$ for the NaT group ($P = .046$), $r = 0.404$ for the trauma group ($P = .036$), and $r = 0.315$ for both groups ($P = .015$). Figure 2 shows the percentage survival plotted against each group of injury severity, and an upward trend is seen.

For all animals studied, overall survival also significantly correlated with the necropsy-based pancreatic score ($r = 0.61$, $P < .001$), fat score ($r = 0.67$, $P < .001$), and amount of ascites (mL, $r = 0.88$, $P < .001$).

All animals who died did so between day 1 and 2, allowing for determination of serum levels for those only on day 1 after surgery.

As shown in Figure 3A, serum amylase increased only with the most severe injury (from a baseline of 3,448 ± 40 to 8,905 ± 1,900 for 3% NaT on day 1, and 3,382 ± 20 to 6,677 ± 1,800 for 4% NaT, $P < .05$ vs. controls).

Reg I and *Reg III* Serum Levels in Pancreatitis

Figure 3B shows that *reg Iα* serum levels remained unchanged throughout all experimental periods. By comparison, *reg III* levels increased significantly on day 1 after induction of both types of pancreatitis (800 ± 200% for NaT and 1,200 ± 600% for trauma) and rapidly returned to baseline in all survivors (see Fig. 3C). Even animals that underwent sham retrograde infusion of the pancreatic duct with saline showed a mild increase in *reg III* on the first day (NaT group, 1,744 ± 1,200 ng/mL, p = NS), whereas sham-operated controls showed no variations (control in trauma group).

Figure 4 shows data of postinjury day 1 *reg III* levels for all groups compared with the pooled day 0 (preinjury)

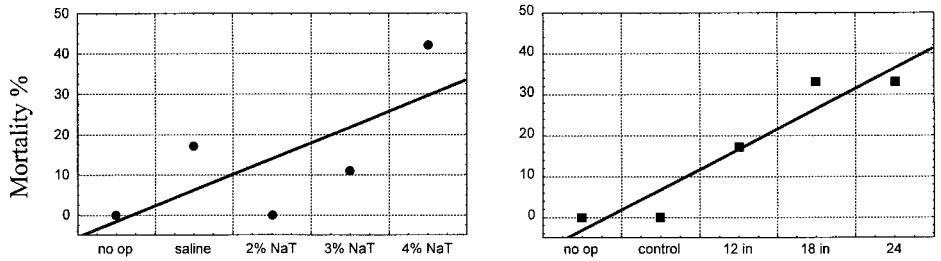

Figure 2. Scatterplot of survival vs. severity of injury for the sodium taurocholate (NaT; left panel, $r = 0.71$, $P = .15$) and trauma (right panel, $r = 0.94$, $P = .05$) experiments. Doses of NaT were 2%, 3%, and 4%; doses of trauma were 12″, 18″, and 24″ heights. Percentage survival is depicted for each group of injury dose, and an upward trend is noted. When individual animals' days of survival were plotted against dose of injury, a highly significant correlation is observed.

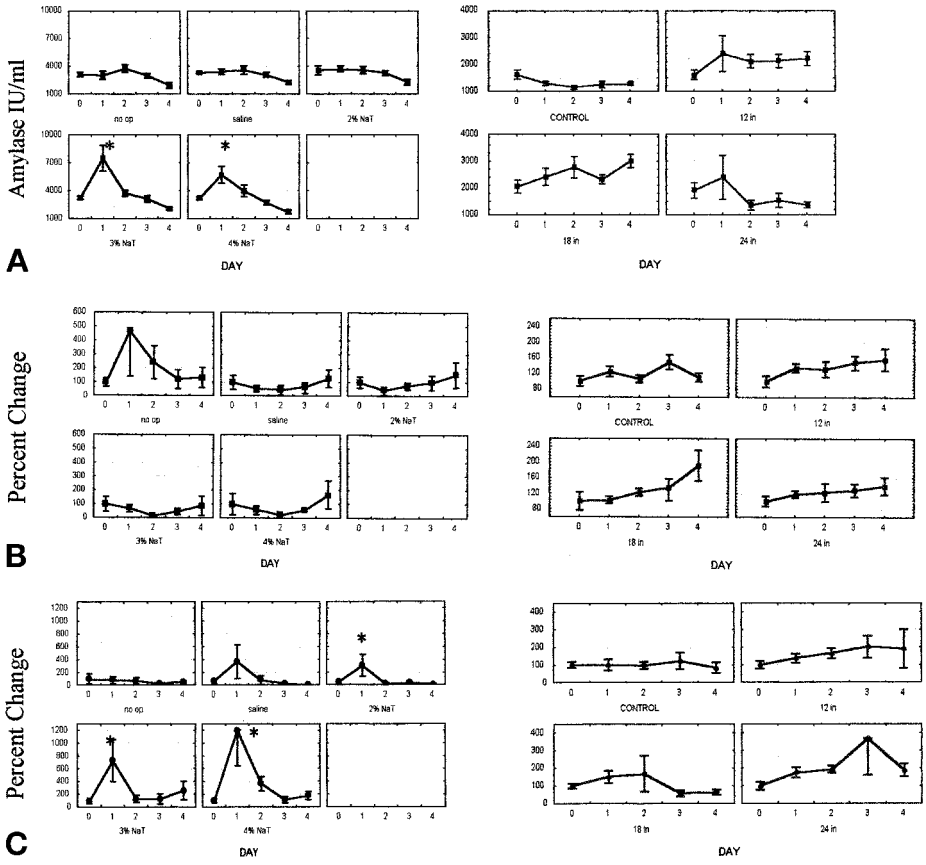

Figure 3. Serum amylase (**A**), *reg Iα*/PSP (**B**), and *reg III*/PAP (**C**) levels after induction of pancreatitis via sodium taurocholate (left panels) and trauma (right panels). Serum was collected immediately before injury (day 0) and then daily (postinjury days 1, 2, 3, 4). Surviving animals were killed on day 4. Data for *reg* proteins is shown as the percentage change from day 0. (Mean±standard error of the mean, $^*P < .05$)

levels. All postinjury day 1 levels were increased significantly for the NaT infusions, but not for increasing trauma. There were significant differences in levels in both groups between survivors and nonsurvivors. In the NaT group, nonsurvivors had a *reg III* level of 2,490 ± 1,000 ng/mL ($P < .05$ vs. baseline level and survivors on postinjury day 1, Student *t* test); in the trauma group, nonsurvivors had a *reg III* level of 2,651 ± 350 ng/mL ($P < .05$ vs. baseline level and survivors on postinjury day 1). There was no graded level of *reg III* with increasing grade of injury. Also, absolute peak *reg III* levels did not significantly correlate with severity of the pancreatic injury or animal survival. *Reg III* levels were then compared with the gross scoring of pancreatitis severity performed at necropsy. There was a significant increase in *reg III* levels, the pancreatitis severity score, and ascites in animals that did not survive (Fig. 5). There was also a significant correlation between serum *reg III* levels and severity of injury.

There was no correlation between the *reg I* and *reg III* serum levels ($r = 0.03$, $P = .58$), even if we compared levels only from day 0 and postinjury day 1. Similarly, there was no correlation between *reg I* levels and the necropsy-based scoring described above (not shown).

DISCUSSION

Reg Iα and *reg III* are proteins produced by the acinar cell of the pancreas in response to injury. *Reg I* is constitutively expressed; *reg III* is expressed only after injury. The expression of both genes increases after induction of pancreatitis, making both potential markers for severity of pancreatitis. To date, no study has measured levels of both in

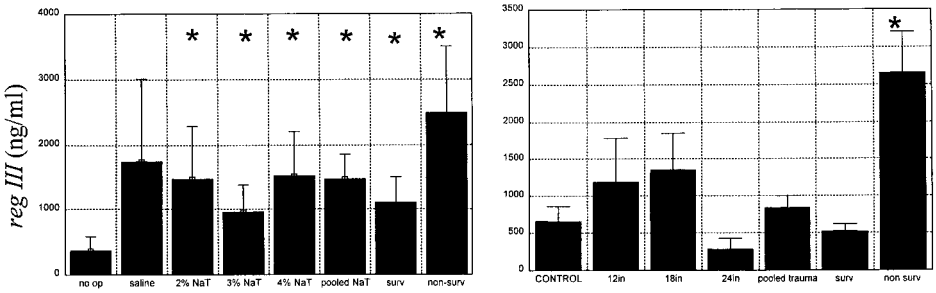

Figure 4. Serum *reg III* levels on postinjury day 1 for the sodium taurocholate (NaT; left panel, $P < .05$) and trauma (right panel, $P < .01$) groups. For comparisons, animals were grouped by injury severity, survivors and nonsurvivors, and pooled together. (Mean±standard error of the mean; * *P* value compared to control.)

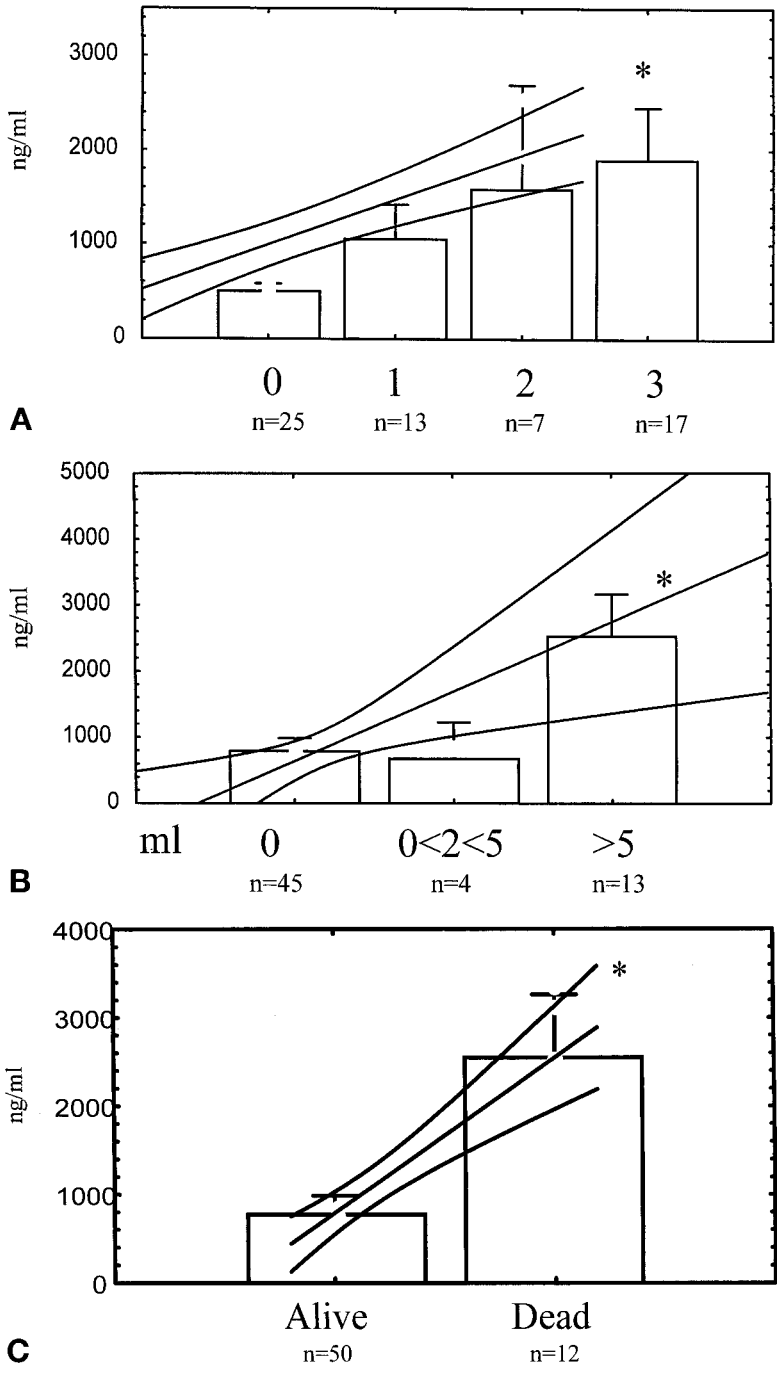

Figure 5. Serum *reg III* levels compared with (**A**) pancreatitis score ($r = 0.34$, $P < .01$), (**B**) presence/amount of ascites (mL; $r = 0.39$, $P < .01$), and (**C**) survival ($r = 0.40$, $P < .01$) of all animals on postinjury day 1. Number of animals in each group is noted. There was a significant correlation between all measures of pancreatic injury and the level of *reg III*. This suggests that serum levels of the protein are a useful marker of pancreatic injury. *Reg Iα* levels, however, did not correlate with severity of injury. (Mean±standard error of the mean, $P < .05$ vs. zero score or survivors.)

serum under controlled conditions in an animal model of pancreatitis. Also, no study has compared the utility of each as a marker of pancreatic injury.

Although the *reg* family of proteins has been cloned and sequenced in rat, mouse, cow, and human, its function has yet to be determined. This family of secreted proteins has a strong resemblance to calcium-dependent lectins.[7] *Reg Iα* was originally described[18] as "pancreatic stone protein", a protein that is a major component of pancreatic stones in patients with chronic calcific pancreatitis. Protein chemistry studies have shown that *reg Iα* is a major exocrine product and its presence prevents calcium carbonate stone formation in normal pancreatic juice.[19,20] This physiologic property has been localized to the amino-(N)-terminal 11 amino acids of the 166-amino acid molecule. As a result of this characteristic, the *reg Iα* protein has also been named lithostatine.[21] In rat, *reg Iα* has also been demonstrated in pancreatic juice, and its amino acid sequence is 68% identical to human.[7] It has been shown that *reg Iα* is important in islet function[22] and β-cell regeneration. We have observed that *reg Iα* gene expression correlates with β-cell function in models of aging, islet hyperplasia, and chronic

pancreatitis.[23–26] We have shown that human and rat *reg Iα* proteins are mitogens to ductal and β-cells, implicating *reg* as a growth factor.[27] *Reg III*, 44% homologous to *reg Iα*, is not normally expressed in the pancreas but is constitutively expressed in small intestine. It is also known as pancreatitis-associated protein because its levels increase 200- to 300-fold after the induction of pancreatitis.[1,2,4,5,8] Immediately after the induction of pancreatitis, the *reg III* protein localizes to the rough endoplasmic reticulum and fibrous material in the acinar lumen.[28] Although its function is unknown, *reg III* has been described as an acute phase reactant.[1] Iovanna et al[8] showed that *reg III* protein can promote bacterial aggregation, suggesting that it may be important in preventing bacterial growth after pancreatitis. Ortiz et al[29] recently reported that *reg III* may be a free radical scavenger that prevents apoptosis of acinar cells during pancreatitis. Our laboratory has shown that the bovine pancreatic thread protein, 65% homologous to rat *reg III*, is mitogenic to pancreatic-derived cells,[27] suggesting a function in the regeneration of the injured pancreas.

After the induction of acute pancreatitis, both *reg Iα* and *reg III* genes are induced in the pancreatic parenchyma.[2,8,9] Their graded induction to graded injury makes them a potentially sensitive marker for pancreatic injury and inflammation. Although *reg III* has been described as a potential marker for pancreatic injury in both pancreatitis[10,11,13] and pancreatic transplant,[14] no controlled comparison of serum *reg Iα* and *III* levels in human or animal models of acute pancreatitis has been done.

We used two models of graded pancreatic injury to determine the use of serum *reg* levels in acute pancreatitis. Using peptides unique to *reg Iα* and *reg III*, we produced polyclonal antibodies in guinea pig and developed an ELISA for both *reg Iα* and *reg III* in rat. Each antibody reacted with extract from tissue that normally expresses it (rat pancreatic juice for *reg Iα* and intestine for *reg III*). Baseline serum levels of *reg Iα* and *III* protein differ significantly. Because the amino acid sequences of each peptide differed significantly from human (by five amino acids for *reg Iα* and by three for *reg III*), neither antibody cross-reacted with human tissue (not shown).

We then studied the level of *reg Iα* and *reg III* in the serum of animals after graded pancreatic injury, either chemical by retrograde infusion of NaT into the pancreatic duct or traumatic using a calibrated weight. Both models yielded increasing death rates with increased injury. A gross scoring system was used to compare severity of injury with *reg Iα* and *III* levels. We observed that serum levels of *reg III* levels increased significantly after induction of pancreatitis, but *reg Iα* levels did not.

After induction of pancreatitis, survival correlated well with severity of injury as well as severity of pancreatitis. Serum levels of *reg III* increased significantly even after mild injury, indicating that *reg III* was a sensitive marker of pancreatic injury. In fact, in control animals in which retrograde infusion of saline was given, a slight increase in *reg III* levels was noted. Light microscopic examination showed that these animals had mild pancreatitis, and they exhibited no elevation in serum amylase.

By contrast, serum levels of *reg Iα* did not increase even after the most severe injury. This implies that *reg III* alone is a potential marker of the severity of pancreatitis.

Serum *reg III* levels peaked on postinjury day 1, which probably corresponds temporally to the peak expression of its gene and protein product. Further, postinjury day 1 levels of *reg III* correlated well with the overall death rate, the necropsy-observed pancreatic severity score, and the volume of ascitic fluid in the peritoneal cavity.

In both models, we expected postinjury deaths to be spread out over a period of 3 or 4 days, corresponding with increasing pancreatic injury. Unfortunately, we noted that most deaths occurred at postinjury day 1, and in most survivors *reg III* levels rapidly returned to normal.

We can therefore conclude that serum *reg III* levels are a useful marker of disease severity early (in the first 24 hours) in the disease. A better model would be to compare serum *reg III* levels with the pancreatic scores on postinjury day 1, and this is under way. We have compared the *reg III* levels on date of necropsy with pancreatic injury and noted a significant correlation (not shown).

A recent study in humans suggested that daily serum *reg III* levels could be used as a dynamic assessment of the severity of pancreatitis.[13] Those investigators noted that in patients with mild pancreatitis, *reg III* levels increase and rapidly decrease. In those with severe pancreatitis and subsequent complications, levels stay elevated. We could not confirm this observation experimentally, because complications and late death did not occur. We therefore cannot confirm the utility of *reg III* as a dynamic prognostic indicator of severity.

A reproducible model of complications or late death after pancreatitis is needed for better understanding of the predictive value of *reg III* serum levels. The choline-deficient diet plus ethionine model in mouse has a predictable rate of death and complications and may be a better model. The mouse sequences of *reg Iα* and *III* are known,[7] so formulating a similar ELISA should be straightforward.

We conclude that serum levels of *reg III* are a sensitive marker of pancreatic injury, but *reg I* is not. This is the first study to examine the serum levels of two different members of the *reg* family simultaneously under experimental conditions of pancreatitis. *Reg III* is indeed a useful marker of pancreatitis and correlates well with severity and even survival. *Reg III* has potential as a prognostic indicator of severity of this devastating disease.

Acknowledgments

The authors thank Amelia Blisset, MD, and Eutiqio Teh for their technical help.

References

1. Keim V, Iovanna JL, Rohr G, Usadel KH, Dagorn JC. Characterization of a rat pancreatic secretory protein associated with pancreatitis. Gastroenterology 1991; 100:775–782.
2. Iovanna JL, Keim V, Michel R, Dagorn JC. Pancreatic gene expression is altered during acute experimental pancreatitis in the rat Am J Physiol 1991; 261:G485–G489.
3. Stephanova E, Tissir F, Dusetti N, Iovanna J, Szpirer C. The rat genes encoding the pancreatitis-associated proteins I, II and III (Pap1, Pap2 Pap3) and the lithostatin/pancreatic stone protein/regeneration protein (*reg*) colocalize at 4q33–4q34. Cytogenet Cell Genet 1996; 72:83–85.
4. Orelle B, Keim V, Dagorn JC, Iovanna JL. Human pancreatitis-associated protein: messenger RNA cloning and expression in pancreatic diseases. J Clin Invest 1992; 90:2284–2291.
5. Iovanna JL, Keim V, Bosshard A, et al. PAP, a pancreatic secretory protein induced during acute pancreatitis, is expressed in rat intestine. Am J Physiol 1993; 265:G611–G618.
6. Masciotra L, de la Porte PL, Frigerio JM, Dusetti NJ, Dagorn JC, Iovanna JL. Immunocytochemical localization of pancreatitis-associated protein in human small intestine. Dig Dis Sci 1995; 40:519–524.
7. Unno M, Yonekura H, Nakagawara K, et al. Structure, chromosomal localization and expression of mouse *reg* genes *reg* I and *reg* II. J Biol Chem 1993; 268:15974–15982.
8. Iovanna J, Orelle B, Keim V, Dagorn JC. Messenger RNA sequence and expression of rat pancreatitis-associated protein, a lectin-related protein overexpressed during acute experimental pancreatitis. J Biol Chem 1991; 266:24664–24669.
9. Roquier S, Verdier JM, Iovanna J, Dagorn JC, Giorgi D. Rat pancreatic stone protein messenger RNA. J Biol Chem 1991; 266:786–791.
10. Schmiegel W, Burchert M, Kalthoff H, et al. Immunochemical characterization and quantitative distribution of pancreatic stone protein in sera and pancreatic secretions in pancreatic disorders. Gastroenterology 1990; 99:1421–1430.
11. Satomura Y, Sawabu N, Mouri I, et al. Measurement of serum PSP/*reg* protein concentration in various diseases with a newly developed enzyme-linked immunosorbent assay. J Gastroenterol 1995; 30:643–650.
12. Keim V, Willemer S, Iovanna JL, Adler G, Dagorn JC. Rat pancreatitis-associated protein is expressed in relation to severity of experimental pancreatitis. Pancreas 1994; 9:606–612.
13. Iovanna JL, Keim V, Nordback I, et al. Serum levels of pancreatitis-associated protein as indicators of the course of acute pancreatitis. Multicentric Study Group on Acute Pancreatitis. Gastroenterology 1994; 106:728–734.
14. van der Pijl JW, Boonstra JG, Barthellemy S, et al. Pancreatitis-associated protein: a putative marker for pancreas graft rejection. Transplantation 1997; 63:995–1003.
15. Lankisch PG, Goke B, Folsch UR, Winkler K, Otto J, Creutzfeldt W. Influence of secretin on the course of experimental pancreatitis in the rat. Digestion 1983; 26:187–191.
16. Delany HM, Ali KB, Trocino AA, Teh EL, Steinberg JJ, Levenson SM. Traumatic pancreatitis: method and effects of IV fluids and Sandostatin. J Surg Res 1996; 60:41–48.
17. Whorton JA. Collecting blood from the anterior vena cava of the guinea pig. Lab Anim 1990;66.
18. de Caro A, Lohse J, Sarles H. Characterization of a protein isolated from pancreatic calculi of men suffering from chronic calcifying pancreatitis. Biochem Biophys Res Comm 1979; 87:1176–1182.
19. Bernard JP, Adrich Z, Montalto G, et al. Inhibition of nucleation and crystal growth of calcium carbonate by human lithostatine. Gastroenterology 1992; 103:1277–1284.
20. Giorgi D, Bernard JP, Rouquier S, Iovanna J, Sarles H, Dagorn JC. Secretory pancreatic stone protein messenger RNA. J Clin Invest 1989; 84:100–106.
21. Sarles H, Dagorn JC, Giorgi D, Bernard JP. Renaming pancreatic stone protein "lithostatine." Gastroenterology 1990; 99:900–901.
22. Watanabe T, Yonemura Y, Yonekura H, et al. Pancreatic beta-cell replication and amelioration of surgical diabetes by Reg protein. Proc Natl Acad Sci USA 1994; 91:3589–3592.
23. Zenilman ME, Perfetti R, Swinson K, Magnuson TH, Shuldiner AR. Pancreatic regeneration (*reg*) gene expression in a rat model of islet hyperplasia. Surgery 1996; 119:576–584.
24. Perfetti R, Egan J, Zenilman ME, Shuldiner AR. Differential expression of *reg-I* and *reg-II* genes during aging in the normal mouse. J Gerontol Biol Sci 1996; 51A:B308–B315.
25. Zenilman ME, Levine JL, Zheng Q. Effect of pancreatic duct ligation on rat pancreatic *reg I* and insulin levels. Gastroenterology 1999; 116:A658.
26. Levine JL, Zenilman ME, Zheng Q. Longitudinal effect of rat pancreatic duct ligation on serum *reg I*, glucose and insulin levels. Gastroenterology 1999; 116:A622.
27. Zenilman ME, Magnuson TH, Swinson K, Egan J, Perfetti R, Shuldiner AR. Pancreatic thread protein is mitogenic to pancreatic-derived cells in culture. Gastroenterology 1996; 110:1208–1214.
28. Morriset J, Iovanna J, Grondin G. Localization of rat pancreatitis-associated protein during bile salt-induced pancreatitis. Gastroenterology 1997; 112:543–550.
29. Ortiz EM, Dusetti NJ, Vasseur S, et al. The pancreatitis-associated protein is induced by free radicals in AR4–2J cells and confers cells resistance to apoptosis. Gastroenterology 1998; 114:808–816.