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# Technetium-99m-Labeled White Blood Cells

## A New Method to Define the Local and Systemic Role of Leukocytes in Acute Experimental Pancreatitis

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**Objective**
We developed a new method to quantitate leukocyte accumulation in tissues and used it to examine the time course and severity of acute experimental pancreatitis.

**Background**
Leukocyte activation and infiltration are believed to be critical steps in the progression from mild to severe pancreatitis and responsible for many of its systemic complications.

**Methods**
Pancreatitis of graded severity was induced in Sprague-Dawley rats with a combination of caerulein and controlled intraductal infusion. Technetium-99m ($^{99m}$Tc)-labeled leukocytes were quantified in pancreas, lung, liver, spleen, and kidney and compared with myeloperoxidase activity. The severity of pancreatitis was ascertained by wet/dry weight ratio, plasma amylase, and trypsinogen activation peptide in the pancreas. The time course of leukocyte accumulation was determined over 24 hours.

**Results**
Pancreatic leukocyte infiltration correlated well with tissue myeloperoxidase concentrations. In mild pancreatitis, leukocytes accumulated only in the pancreas. Moderate and severe pancreatitis were characterized by much greater leukocyte infiltration in the pancreas than in mild disease ($p < 0.01$), and increased $^{99m}$Tc radioactivity was detectable in the lung as early as 3 hours. $^{99m}$Tc radioactivity correlated directly with the three levels of pancreatitis.

**Conclusions**
Mild pancreatitis is characterized by low-level leukocyte activation and accumulation in the pancreas without recruitment of other organs; marked leukocyte accumulation was found in

the pancreas and in the lung in more severe grades of pancreatitis. These findings provide a basis for the pathophysiologic production of cytokines and oxygen free radicals, which potentiate organ injury in severe pancreatitis. This study validates a new tool to study local and systemic effects of leukocytes in pancreatitis as well as new therapeutic hypotheses.

There is extensive evidence that activated leukocytes and their products, including reactive oxygen species, lysosomal hydrolases, and cytokines, play an early and essential role in the progression from mild to severe pancreatitis and in the development of its systemic complications.[1-3] Quantitation of leukocytes in tissues is therefore likely to provide insights into the local and systemic roles of leukocytes in the evaluation of pancreatitis and for monitoring the effect of various treatment regimens. The method, aside from histologic assessment, most frequently used to evaluate leukocyte infiltration of organs in experimental pancreatitis uses myeloperoxidase, an enzyme stored in the azurophilic granules of polymorphonuclear leukocytes. However, measurement of myeloperoxidase activity has its drawbacks because the amount of enzyme stored in individual white blood cells varies,[4] and its activity can also be affected by drugs, including heparin.[5,6] A technique that directly labels leukocytes would be preferable for quantitative experimental studies.

Clinical scintigraphy with technetium-99m ($^{99m}$Tc)-labeled leukocytes has been used to localize sites of leukocyte infiltration and inflamed tissues in abdominal infections,[7] ulcerative colitis,[8] and even acute pancreatitis.[9] Because the pancreas cannot be visualized in whole-body scintigraphy of rodents due to its proximity to the liver,[10] and because we desired more quantitative information, we excised the organs of interest and measured the radioactivity in tissues as a direct measurement of leukocyte infiltration. To validate this technique in experimental pancreatitis, we used well-characterized models representing the full range of severity observed in the clinical situation[11] and compared the radiolabeled leukocyte counts with myeloperoxidase activity and other markers of pancreatic injury.

## MATERIALS AND METHODS

### Animals

Experiments were performed in 42 male Sprague-Dawley rats weighing 275 to 350 g. Animals were fasted overnight before the experiment, with free access to water. Care was provided in accordance with the procedure outlined in the *Guide for Care and Use of Laboratory Animals* (NIH Publication #85-23, 1985). Surgical anesthesia was induced with vaporized ether and maintained by an intramuscular injection of pentobarbital (20 mg/kg) and ketamine (40 mg/kg). A polyethylene catheter (ID, 0.5 mm) was inserted into the left carotid artery of each animal, subcutaneously tunneled to the back, and exited through a steel tether that allowed the animals free movement and access to water during the experiment.

### Model

Animals were randomly allocated to a control group (n = 6) or three grades of acute pancreatitis—mild edematous (n = 6), intermediate necrotizing (n = 6), and severe necrotizing (n = 6)—and then killed after 6 hours. Mild pancreatitis was induced by continuous intra-arterial infusion of caerulein (5 μg/kg/h) for 6 hours.[11] Caerulein was reconstituted in normal saline and infused at 3 mL/kg/h. For induction of necrotizing pancreatitis, the same infusion of caerulein was preceded by a volume (1.2 mL/kg)-, time (10 minutes)-, and pressure (10 mmHg)-controlled retrograde infusion of glycodeoxycholic acid (GDOC) into the biliopancreatic duct. Moderate and severe necrotizing pancreatitis were induced with 2.5 mmol/L (GDOC 2.5) and 10 mmol/L (GDOC 10), respectively, as described by Schmidt et al.[11] To elucidate the time course of injury, additional animals with intermediate-severity pancreatitis were evaluated at 3, 12, and 24 hours (n = 6 per group).

Plasma for amylase concentration was obtained at baseline and immediately before the animals were killed using an intra-arterial pentobarbital overdose (200 mg/kg). The pancreas of each animal was used to evaluate pancreatic edema, trypsinogen activation in pancreatic tissue, and leukocyte accumulation as evaluated by myeloperoxidase and $^{99m}$Tc radioactivity measurements. $^{99m}$Tc radioactivity was also measured in the lung, liver, spleen, kidney, ascites, and blood.

### Edema Assessment

Pancreatic edema was evaluated by measuring water content. A portion of the pancreas was removed immediately after sacrifice, trimmed of fat, and weighed. Pancreatic water content was determined by calculating the wet/dry weight ratio from the initial weight of the pancreas

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(wet weight) and its weight after incubation at 160°C for 24 hours (dry weight) as described previously.[12]

## Trypsinogen Activation Peptide Measurements

Ectopic trypsinogen activation has been demonstrated within the pancreas in a variety of models of experimental pancreatic injury.[13,14] The amount of activation, as documented by quantitation of trypsinogen activation peptide (TAP), increases with the severity of pancreatic injury[15] and predicts the outcome of pancreatitis.[16] For measurement of TAP concentrations in pancreatic tissue, two samples of tissue (0.1–0.3 g) were excised from the pancreas. The specimens were then immersed in 0.2 M Tris–HCl buffer (pH 7.3) containing 20 mM EDTA, immediately boiled (100°C) for 10 minutes to denature residual protease activity, and homogenized with a Brinkman Polytron (Brinkman Instruments, Westbury, NY) for 30 seconds. After centrifugation (580 g, 10 minutes, 4°C), the resulting supernatant was stored at −20°C until assayed. TAP was measured by an enzyme-linked immunosorbent assay,[17] and results were expressed as nmol TAP per milligram of pancreas. Synthetic TAP (YD4K), a conjugate of rabbit serum albumin with YD4K, and rabbit anti-TAP antiserum containing calcium-independent anti-TAP antibodies were provided by Professor J. Hermon-Taylor (St. George's Hospital Medical School, London, UK). Biotin-goat ant-rabbit IgG antibody and alkaline phosphatase-labeled extravidin were purchased from Sigma Chemical Company (St. Louis, MO). This assay correlates well with the previously reported radioimmunoassay described by Hurley et al.[18]

## Amylase Measurement

Amylase activity in serum was determined according to the method of Ceska et al.[19] using the Phadebas Amylase Test (Pharmacia Diagnostics, Uppsala, Sweden).

## $^{99m}$Tc-Labeled Leukocytes

The lipophilic ligand $^{99m}$Tc-Exametazime ($^{99m}$Tc-HMPAO, Ceretec, Amersham Healthcare, Arlington Heights, IL) is taken up by leukocytes and selectively retained by neutrophils. In this study, rat white blood cells were radiolabeled with this reagent by previously described methods[20] with minor modifications. $^{99m}$Tc-Exametazime kits (Amersham Healthcare, Arlington Heights, IL) were radiolabeled with $^{99m}$Tc-pertechnectate (DuPont, Billerica, MA) in accordance with the manufacturer's instructions. Whole blood was harvested from donor rats (male Sprague-Dawley) with 20% v/v anticoagulant citrates dextrose solution as an anticoagulant. The anticoagulated blood was diluted (1:1) with a 6% solution of hydroxyethylstarch (Hespan; DuPont, Wilmington, DE) and leukocyte-rich plasma. A leukocyte pellet was obtained by centrifuging the leukocyte-rich plasma at 150 g for 5 minutes, and the leukocyte-poor supernatant was centrifuged at 1500 g for 15 minutes to produce a platelet-poor plasma (PPP). The leukocyte pellet was resuspended in PPP and $^{99m}$Tc-Exametazime was added. After incubating for 15 minutes at room temperature, the mixture was centrifuged and the $^{99m}$Tc leukocyte pellet was resuspended in fresh PPP. Labeling efficiency was determined by comparing the radioactivity in the $^{99m}$Tc-labeled leukocyte pellet with the free radioactivity in the labeling mixture supernatant. Typical labeling efficiencies were 25% to 60%.

$^{99m}$Tc-labeled leukocytes were injected into the rats before the induction of pancreatitis. After the animals were killed at the end of the study (3, 6, 12, or 24 hours), two samples of pancreatic tissue (0.1–0.3 g), one from the head and one from the tail, and samples of the lung, liver, spleen, and kidney (0.2–0.5 g) were excised, rinsed with saline, blotted dry, and weighed before radioactivity was measured. Radioactivity in samples of blood and ascites fluid was also measured.

$^{99m}$Tc radioactivity was measured within 2 hours after the animals were killed using a well-type gamma counter (LKB model #1282, Wallac Oy, Finland). The data are presented as the percentage of the injected dose per gram of tissue (%ID/g).

## Myeloperoxidase Activity

Excised pancreatic tissues were rinsed with saline, blotted dry, shock-frozen in liquid nitrogen, and stored frozen at −80°C until thawing for determination of myeloperoxidase activity using methods previously described[21,22] with minor modifications. Briefly, pancreatic tissue was homogenized in 0.1 M sodium phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (Sigma H5882) and 5% soybean trypsin inhibitor (both from Sigma Chemical Co.) before sonication and was then frozen directly on dry ice. The specimens were freeze–thawed three times, and after each cycle sonication was repeated. Suspensions were then centrifuged at 20,000 g for 15 minutes, and the resulting supernatant was assayed. Myeloperoxidase activity was measured with a spectrophotometer (UV-160, Shimazdu, Japan) at 470 nm by mixing an aliquot (25 $\mu$L) of the supernatant with 1.0 mL of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.0016 mL guaiacol (Sigma G5502, Sigma Chemical Co.) and 0.0005% hydrogen peroxide (Sigma T9003, Sigma Chemical Co.) as substrates. To assess the possibility of heterogeneous distribution of leukocyte infiltration in the animals with necrotizing pancreatitis, values in tissues

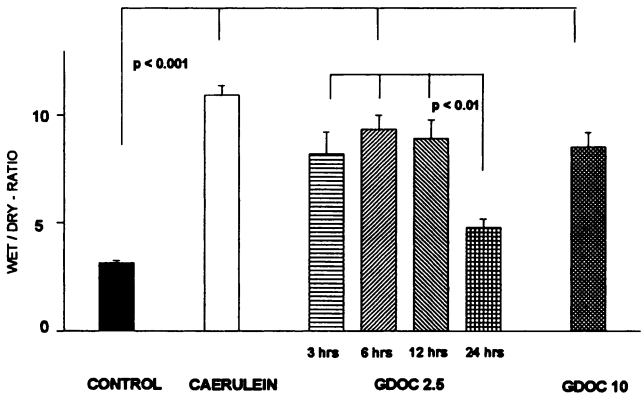


**Figure 1.** Pancreatic edema as assessed by wet/dry weight ratio. Controls and animals with mild pancreatitis (caerulein) and severe necrotizing pancreatitis (GDOC 10) were evaluated at 6 hours. Animals with moderate necrotizing pancreatitis (GDOC 2.5) were assessed at 3, 6, 12, and 24 hours. Data are presented as mean ± standard error of the mean.

obtained from the head and tail of the pancreas were compared.

## Statistical Analysis

Results are presented as mean ± standard error of the mean. Differences between groups were compared by the Student's t test. When a normal distribution was not present, data are presented as median and 25th and 75th percentile and the Mann-Whitney rank sum test was used for statistical analysis. A 5% probability of type I experimental error ($p < 0.05$) was considered to be of statistical significance. Regression analysis was used to evaluate the correlation between $^{99m}$Tc radioactivity measurements and other parameters of pancreatic injury.

## RESULTS

Pancreatic edema was present in all animals with pancreatitis compared with controls ($p < 0.001$). Caerulein-induced mild pancreatitis was characterized by maximal pancreatic water content; edema was less prominent with increasing severity of necrotizing disease ($p < 0.02$, caerulein *vs.* GDOC 10; Fig. 1). Edema formation was already maximally present 3 hours after induction of pancreatitis of intermediate severity and remained unchanged up to 12 hours before decreasing significantly at 24 hours after induction of pancreatitis ($p = 0.01$, GDOC 2.5 at 24 hours *vs.* 3, 6, and 12 hours).

The level of TAP was significantly increased in pancreatic tissues of animals with mild pancreatitis ($p < 0.01$ *vs.* control), and there was a considerably greater increase in necrotizing pancreatitis ($p < 0.001$, GDOC 2.5 and GDOC 10 *vs.* control; $p < 0.05$, GDOC 10 *vs.* caerulein; Fig. 2). The time course of ectopic trypsinogen activation in necrotizing pancreatitis of intermediate severity shows maximal levels at 3 and 6 hours after induction of pancreatitis and a significant fall-off by 24 hours (see Fig. 2).

Amylase in plasma did not change significantly in control animals receiving saline but was significantly increased in all animals in which either mild or severe pancreatitis was induced in comparison with control animals (Table 1). There was no difference in amylase activity between the different severities of pancreatitis at 6 hours after induction of pancreatitis. The time course of the plasma amylase in the GDOC 2.5 group demonstrates a maximum at 3 hours and then a progressive decrease even before the secretory stimulus by caerulein was stopped at 6 hours.

The level of $^{99m}$Tc-labeled leukocytes (Fig. 3) significantly increased in the pancreas in mild edematous pancreatitis ($p < 0.001$ *vs.* control) and increased still further in proportion to the severity of the injury ($p < 0.001$, caerulein *vs.* GDOC 2.5; $p < 0.05$, GDOC 2.5 *vs.* GDOC 10). Leukocyte accumulation in the tail of the pancreas compared with the head was not different in control animals or in those with mild pancreatitis, but there were fewer leukocytes infiltrating in the tail than in the head in both grades of necrotizing pancreatitis (data not shown). There was also a significant progressive increase in leuko-

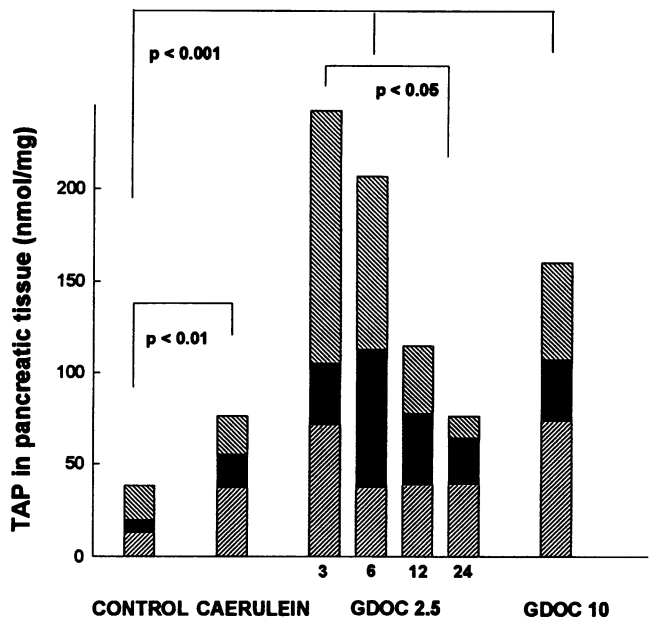


**Figure 2.** Ectopic trypsinogen activation as evaluated by trypsinogen activation peptides (TAP) in pancreatic tissue. Controls and animals with mild pancreatitis (caerulein) and severe necrotizing pancreatitis (GDOC 10) were evaluated at 6 hours. Animals with moderate necrotizing pancreatitis (GDOC 2.5) were assessed at 3, 6, 12, and 24 hours. Data are presented as 25th percentile (bottom of black portion of the bars), median (top of black portion of the bars), and 75th percentile (top of the bars).

**Table 1. AMYLASE IN PLASMA**

| | Delta Amylase (U/L)* |
|---|---|
| Control† | 6.04 ± 3.72 |
| Caerulein‡ | 498.48 ± 51.81¶ |
| GDOC 2.5§ | |
| 3 hr | 653.16 ± 55.76¶ |
| 6 hr | 453.25 ± 34.60¶,# |
| 12 hr | 375.17 ± 32.63¶,** |
| 24 hr | 80.80 ± 19.40††,‡‡ |
| GDOC 10‖ | 495.00 ± 44.08¶ |

Data are mean ± SEM.
* Delta of Amylase in plasma at endpoint (3, 6, 12, or 24 hr) and baseline (0 hr).
† Control animals, which received saline, and were killed at 6 h.
‡ Animals with caerulein-induced mild pancreatitis, which were killed at 6 hr.
§ Animals with intermediate necrotizing pancreatitis, which was induced with intraductal glycodeoxycholic acid (2.5 nmol/L) and subsequent caerulein infusion, and killed at 3, 6, 12, or 24 hr.
‖ Animals with severe necrotizing pancreatitis, which was induced with intraductal glycodeoxycholic acid (10 nmol/L) and subsequent caerulein infusion, and killed at 6 hr.
¶ $p < 0.001$ *vs.* control.
# $p < 0.05$ *vs.* GDOC 2.5, at 3 hr.
** $p < 0.01$ *vs.* GDOC 2.5, at 3 hr.
†† $p < 0.01$ *vs.* control
‡‡ $p < 0.001$ *vs.* GDOC 2.5, at 3, 6, and 12 hr.

cyte accumulation over the whole observation time; the level continued to increase even after the caerulein infusion was stopped at 6 hours (see Fig. 3).

Myeloperoxidase activity in pancreatic tissue also demonstrated a stepwise increase of leukocyte infiltration with worsening of pancreatitis (Fig. 4). Although there was

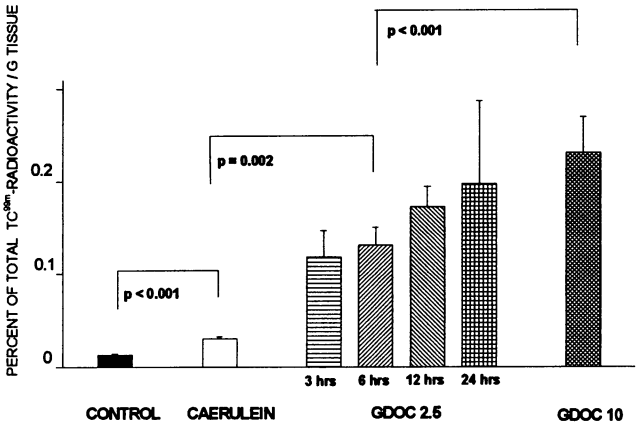


**Figure 3.** Leukocyte infiltration of the pancreatic head as assessed by technetium-99m-labeled leukocytes is presented as the percentage of the total injected technetium-99m radioactivity per gram of pancreas. Controls and animals with mild pancreatitis (caerulein) and severe necrotizing pancreatitis (GDOC 10) were evaluated at 6 hours. Animals with moderate necrotizing pancreatitis (GDOC 2.5) were assessed at 3, 6, 12. and 24 hours. Data are presented as mean ± standard error of the mean.

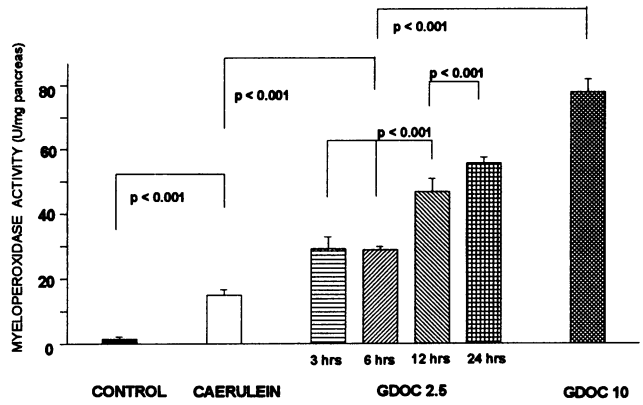


**Figure 4.** Leukocyte infiltration of the pancreatic head as evaluated by myeloperoxidase activity in tissue. Controls and animals with mild pancreatitis (caerulein) and severe necrotizing pancreatitis (GDOC 10) were evaluated at 6 hours. Animals with moderate necrotizing pancreatitis (GDOC 2.5) were assessed at 3, 6, 12, and 24 hours. Data are presented as mean ± standard error of the mean.

evidence of minor leukocyte accumulation in mild pancreatitis, the two grades of necrotizing pancreatitis were characterized by much higher myeloperoxidase levels ($p < 0.001$, GDOC 2.5 *vs.* caerulein and GDOC 10 *vs.* GDOC 2.5). The time course of myeloperoxidase activity further confirmed that shown by the $^{99m}$Tc-labeled leukocytes. Also, as indicated with the labeled leukocytes, the inflammatory response appeared to be less pronounced in the tail of the pancreas than in the head of the pancreas in intermediate and severe necrotizing disease (data not shown).

Figure 5 demonstrates a very significant positive correlation ($r = 0.8111$) between measurements of myeloperoxidase activity and $^{99m}$Tc radioactivity detected in the pancreas.

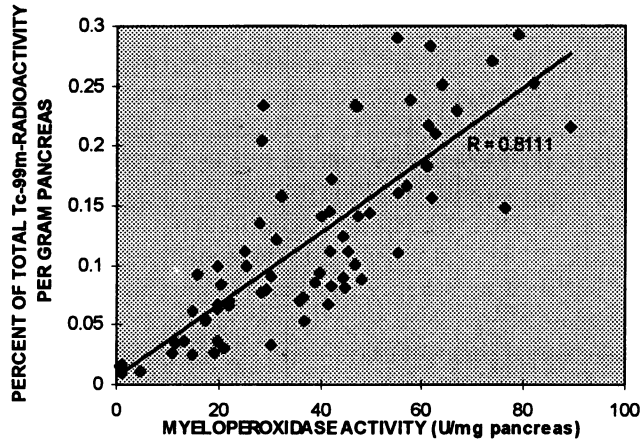


**Figure 5.** Comparison of two methods for quantitating leukocyte infiltration of the pancreas: technetium-99m radioactivity (presented as percentage of the total injected dose per gram of tissue) *versus* leukocyte enzyme activity as assessed by myeloperoxidase.

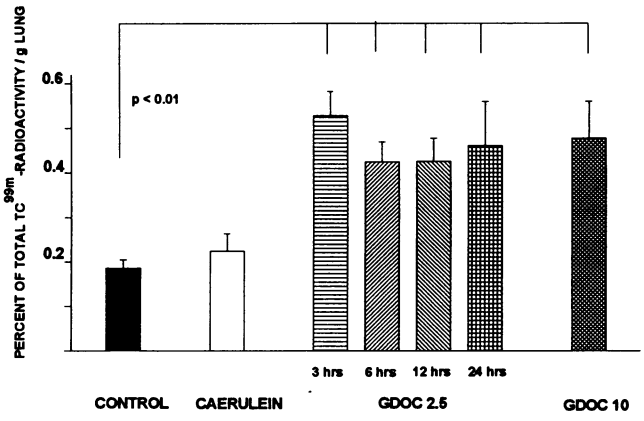


**Figure 6.** Leukocyte infiltration of the lung as assessed by technetium-99m-labeled leukocytes, presented as the percentage of total injected technetium-99m radioactivity per gram of tissue. Controls and animals with mild pancreatitis (caerulein) and severe necrotizing pancreatitis (GDOC 10) were evaluated at 6 hours. Animals with moderate necrotizing pancreatitis (GDOC 2.5) were assessed at 3, 6, 12, and 24 hours. Data are presented as mean ± standard error of the mean.

Recruitment of organs other than the pancreas was assessed by measuring $^{99m}$Tc radioactivity in these organs. Necrotizing pancreatitis ($p < 0.01$, control *vs.* GDOC 2.5 and GDOC 10) but not mild pancreatitis led to increased leukocyte accumulation in the lungs (Fig. 6). There was no difference in leukocyte infiltration of the lung between the two grades of severity of necrotizing pancreatitis, and also no difference over the 24-hour experimental period, although at the same time pancreatic injury was increasing (see Figs. 3 and 4). There was no increase in radioactivity in any of the other solid organs or in the blood (Table 2). Ascites, found only in necrotizing pancreatitis, contained higher levels of radioactivity in severe than in intermediate injury ($p < 0.038$).

## DISCUSSION

The standard techniques of evaluating leukocyte infiltration of organs are histologic assessment and measurement of myeloperoxidase activity, both of which are indirect measurements. Myeloperoxidase measurement is further hampered by the fact that some drugs, including heparin, can influence its activity[5,6]; therefore, the measured myeloperoxidase levels may be misleading as an index of leukocyte accumulation, especially for studies of new treatment regimens. This caution is particularly pertinent because some treatment strategies for pancreatitis have been directed at antagonizing mediators leading to recruitment of white blood cells, including cytokines,[23] oxygen free radicals,[24] and platelet-activating factor.[25] Using the direct labeling of leukocytes with $^{99m}$Tc-Exametazime, a method routinely used in the clinical setting for the scintigraphic detection of inflammatory sites in several diseases,[7-9] we have been able to quantitate directly the white blood cells infiltrating the pancreas and other organs in acute pancreatitis of varying severity in rats. The added advantage of using a radioactive label is that any organ and even fluids such as ascites and blood can be assessed immediately without further manipulation. Future development of small gamma emission probes should allow accurate quantitative segmental *in situ* measurements of labeled leukocytes in individual organs.

The comparison of the two methods used in this study for quantitating leukocyte infiltration shows a very good correlation between myeloperoxidase activity and

**Table 2. DISTRIBUTION OF TOTAL TECHNETIUM-99m-RADIOACTIVITY BY ORGAN**

| | Liver | Spleen | Kidney | Blood | Ascites |
|---|---|---|---|---|---|
| Control* | 0.309 ± 0.066 | 2.243 ± 0.482 | 0.436 ± 0.067 | 0.569 ± 0.080 | — |
| Caerulein† | 0.338 ± 0.045 | 2.954 ± 0.434 | 0.475 ± 0.094 | 0.701 ± 0.076 | — |
| GDOC 2.5‡ | | | | | |
| 3 hr | 0.285 ± 0.045 | 1.185 ± 0.148 | 0.313 ± 0.045 | 0.886 ± 0.069 | 0.031 ± 0.007 |
| 6 hr | 0.368 ± 0.096 | 1.219 ± 0.309 | 0.367 ± 0.0463 | 0.954 ± 0.072 | 0.023 ± 0.004 |
| 12 hr | 0.195 ± 0.023 | 1.727 ± 0.074 | 0.289 ± 0.044 | 0.669 ± 0.081 | 0.023 ± 0.003 |
| 24 hr | 0.187 ± 0.0441 | 1.165 ± 0.248 | 0.361 ± 0.067 | 0.572 ± 0.083 | — |
| GDOC 10§ | 0.465 ± 0.085 | 2.043 ± 0.485 | 0.447 ± 0.051 | 0.524 ± 0.095 | 0.045 ± 0.009‖ |

Data are percent of total administered Technetium-99m-counts per gram organ weight (mean ± SEM).

* Control animals, which received saline, and were killed at 6 hr.

† Animals with caerulein-induced mild pancreatitis, which were killed at 6 hr.

‡ Animals with intermediate necrotizing pancreatitis, which was induced with intraductal glycodeoxycholic acid (2.5 nmol/L) and subsequent caerulein infusion, and killed at 3, 6, 12, or 24 hr.

§ Animals with severe necrotizing pancreatitis, which was induced with intraductal glycodeoxycholic acid (10 nmol/L) and subsequent caerulein infusion, and killed at 6 hr.

‖ $p < 0.05$ *vs.* GDOC 2.5, at 6 hr.

$^{99m}$Tc-labeled leukocytes (see Fig. 5), and therefore between the absolute number of leukocytes as assessed by $^{99m}$Tc labeling and leukocyte function as evaluated by activity of the enzyme myeloperoxidase. Whereas the histologic assessment of pancreatic inflammation showed great homogeneity in this model of severe necrotizing disease,[26] the measurement of $^{99m}$Tc-labeled leukocytes revealed significant differences in leukocyte accumulation between the tail and head of the pancreas, as did the measurement of myeloperoxidase. Not surprisingly, both methods are more capable of demonstrating regional differences in leukocyte infiltration than histologic assessment alone. It may be advantageous in some experimental circumstances to use both methods for confirmation or to bring out functional alterations.

In these studies, we have examined the entire clinical spectrum of acute pancreatitis. Mild pancreatitis is a self-limiting disorder characterized by interstitial edema, hyperamylasemia, and disturbances of acinar cell morphology and the secretory process.[27,28] In our experimental model, which morphologically mimics mild human pancreatitis, there was the expected pancreatic edema, a moderate increase of TAP in pancreatic tissue, and marked hyperamylasemia 6 hours after induction of pancreatitis. The present studies add observations of moderate leukocyte infiltration confined to the pancreas without involvement of other organs.

In contrast, our models of necrotizing pancreatitis[11,26] produced a relative homogeneous regional distribution of inflammation and acinar necrosis as assessed by histology. Pancreatic edema was significantly increased but was less pronounced than in mild edematous pancreatitis, as had been demonstrated previously.[12] The amylase activity in serum was not different from mild edematous disease, which shows again that amylase is not a reliable marker of severity in experimental pancreatitis.[11,12]. TAP levels in pancreatic tissue were higher in necrotizing than in mild edematous disease, again demonstrating that the TAP level correlates with the severity of pancreatitis.[12,15,16] The catastrophic consequences of trypsinogen activation with further activation of pancreatic enzymes and autodigestion of the gland may be an explanation for the difference between mild, self-limiting and necrotizing disease with high morbidity and mortality,[11,29] although trypsinogen activation seems to be principally important early in the pathogenesis of pancreatic necrosis; TAP levels peaked at 6 hours and decreased significantly thereafter.

Necrotizing pancreatitis, unlike milder forms of the disease, is characterized by decreased pancreatic microcirculatory perfusion and ischemia.[30,31] This is attributed in part to excessive leukocyte–endothelium interaction in postcapillary venules, leading to increased venular resistance by obstruction of small vessels.[32–34] Leukocyte–endothelium interaction has been shown to increase with time and to correlate with the severity of pancreatitis.[32] Adherent leukocytes are known to cause endothelial cell detachment by neutrophil-derived proteases[35] and elastase and to release noxious agents such as phospholipases, DNAses, RNAses, lysosomal hydrolases, platelet-activating factor, myeloperoxidases, and an enormous quantity of oxygen free radicals.[2,35,36] In our studies, we quantitated pancreatic leukocyte infiltration by $^{99m}$Tc labeling and by myeloperoxidase measurements, both of which document the relation between pancreatic leukocyte content and the severity and time course of pancreatitis. We did not address the mechanism of leukocyte recruitment in necrotizing disease, but the dissociation between ongoing trypsinogen activation and progressive leukocyte accumulation seen in our study suggests that ectopic trypsinogen activation, which has been proposed as the possible initiating event of pancreatitis,[17,27,37] may not be necessary for the further evolution of severe disease.

In addition to the local pancreatic injury, we assessed systemic complications of acute pancreatitis. Leukocyte infiltration, as evaluated by $^{99m}$Tc radioactivity measurements, was not increased in any organ studied in mild edematous pancreatitis compared with control animals, whereas the lung was significantly inflamed in necrotizing pancreatitis, regardless of the degree of severity. This was observed as early as 3 hours and persisted for 24 hours. These findings are consistent with the clinical observation that respiratory complications, mainly adult respiratory distress syndrome, and other systemic complications occur only in severe pancreatitis.[1,2] However, these results differ from several previous experimental studies that reported increased leukocyte activity in the lung in the first 12 hours of caerulein-induced pancreatitis.[38,39] Our findings are also supported by studies performed in this and other laboratories that show no deterioration of arterial blood gas levels in mild disease.[12,31] The white blood cell accumulation in the lungs in severe disease, however, is well described in experimental[40,41] and clinical studies[42,43] and is thought to be secondary to proinflammatory cytokine release by leukocytes[23,44,45] and altered polymorphonuclear leukocyte function.[3,46] It was recently shown that the cytokines interleukin-1 and tumor necrosis factor increase endothelial permeability even in absence of leukocytes and, more importantly, increase the expression of adhesion molecules.[47] Circulatory cytokines may therefore play a pathogenic role in the lung injury associated with severe pancreatitis by increasing endothelial adhesion molecule expression in the pulmonary vascular bed. Further leukocyte accumulation and activation in the pulmonary microcirculation might then potentiate the lung injury. The apparent specificity of this process is interesting: increased leukocyte accumulation was not noted in

any other organ in our study, except for the inflammatory exudate in the peritoneal cavity.

In conclusion, we have developed a novel approach to quantitating leukocytes in tissues and used the method to investigate inflammatory changes in pathologic states. We exemplified gradations of abnormal leukocyte accumulation that are organ-specific and correlate with severity in our experimental models of pancreatitis. The method was validated by comparison with myeloperoxidase levels but has the advantage of being free of the limitations imposed by the inhibitory or stimulating actions of certain drugs on myeloperoxidase activation. There is potential for adaptation to *in situ* analysis, which would allow sequential observations.

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