

Peritoneal acidosis mediates immunoprotection in laparoscopic surgery

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Background. We have shown previously that abdominal insufflation with CO₂ increases serum levels of IL-10 and TNF α and increases survival among animals with lipopolysaccharide (LPS)-induced sepsis, even after a laparotomy. We demonstrated previously that the effect of CO₂ is not from changes in systemic pH, although the peritoneum is locally acidotic during abdominal insufflation with CO₂ even when systemic pH is corrected. We hypothesized that acidification of the peritoneum via means other than CO₂ insufflation would produce alterations in the inflammatory response similar to those associated with CO₂ pneumoperitoneum.

Methods. In total, 42 rats were randomized into 7 groups (n = 6): 1) LPS only, 2) anesthesia control, 3) helium pneumoperitoneum, 4) CO₂ pneumoperitoneum, 5) buffered mild acid lavage, 6) buffered strong acid lavage, and 7) buffered strong acid lavage + helium pneumoperitoneum. Animals received anesthesia with vaporized isoflurane (except the LPS-only group) and their respective abdominal treatment (pneumoperitoneum and/or lavage) for 30 min followed immediately by stimulation with systemic LPS (1 mg/kg, IV). Blood was harvested via cardiac puncture 60 min after LPS injection, and serum levels of IL-10 and TNF α levels were determined by enzyme-linked immunosorbent assay.

Results. Mean peritoneal pH decreased (P < .05) after CO₂ pneumoperitoneum, buffered strong acid lavage, and buffered strong acid lavage + helium pneumoperitoneum, and it decreased (P = .1) after helium pneumoperitoneum alone and buffered mild acid lavage. IL-10 levels were increased (P < .01), and TNF α levels decreased (P < .001) among animals with acidic peritoneal cavities compared with animals with pH-normal peritoneal cavities. Decreasing peritoneal pH correlated with both increasing IL-10 levels (r = -.465, P < .01) and decreasing TNF α levels (r = 0.448, P < .01). Among animals with peritoneal acidosis, there were no differences in levels of IL-10 or TNF α regardless of insufflation status (P > .05 for both cytokines).

Conclusions. Acidification of the peritoneal cavity whether by abdominal insufflation or by peritoneal acid lavage increases serum IL-10 and decreases serum TNF α levels in response to systemic LPS challenge. The degree of peritoneal acidification correlates with the degree of inflammatory response reduction. These results support the hypothesis that pneumoperitoneum-mediated attenuation of the inflammatory response after laparoscopic surgery occurs via a mechanism of peritoneal cell acidification. (Surgery 2007;142:357-64.)

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THE LAPAROSCOPIC APPROACH TO SURGERY OF THE INTRA-ABDOMINAL VISCERA is now well accepted for appropriate procedures. Patients undergoing minimally invasive abdominal surgery have less postoperative pain, shorter postoperative ileus, shorter hospital stays, a more rapid return to preoperative activity, and superior cosmesis compared with their counterparts who underwent operation through a laparotomy.¹⁻⁴ Because clinical data have shown that the release of inflammatory mediators is less after laparoscopy than after conventional open surgery,^{5,6} the molecular mechanisms underlying the improved results observed after laparoscopic surgery have become an area of active investigation.

Our group has shown that peritoneal insufflation with CO₂ blunts the hepatic expression of acute phase genes in laparoscopic models of perioperative sepsis.^{7,8} We also demonstrated that abdominal insufflation with CO₂ (and to a lesser degree helium and air) increases plasma levels of IL-10 and attenuates TNF α production, increasing survival among animals with lipopolysaccharide (LPS)-induced sepsis. The protective effect of CO₂ pneumoperitoneum is even capable of “rescuing” animals from abdominal sepsis (increasing survival) that have already undergone a laparotomy.⁹ Furthermore, the mechanism of CO₂-insufflation-specific reduction of the inflammatory response involves IL-10-mediated downregulation of TNF α .^{9,10} With regard to the physiologic “trigger” that initiates the modulation of the immune response by pneumoperitoneum, we have shown that the inflammation-attenuating effects of CO₂ pneumoperitoneum during laparoscopy are not from changes in systemic pH.¹¹ A significant local peritoneal acidosis, however, occurs during laparoscopy with CO₂, that this effect is transient—occurring only during active abdominal insufflation—and that the effect is specifically attributable to the use of carbon dioxide.¹²

Therefore, we hypothesized that acidification of the peritoneum via means other than CO₂ insufflation would produce alterations in the inflammatory response similar to those associated with CO₂ pneumoperitoneum and the degree of attenuation of the inflammatory response (upregulation of anti-inflammatory cytokines such as IL-10 and downregulation of proinflammatory cytokines such as TNF α) would correlate with the degree of peritoneal acidification.

MATERIAL AND METHODS

General procedures. All procedures were part of an animal protocol reviewed and approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass), 10 to 12 weeks old, weighing between 250 and 300 g were housed in cages where standard chow and water were available ad libitum. The animal housing environment was maintained at a temperature of 22°C with a 12-h light/dark cycle. The rats were acclimatized to their environment for 3 to 5 days on arrival and then fasted overnight before intervention. All procedures were performed under aseptic conditions. Anesthesia was induced in an isoflurane chamber and maintained using vaporized isoflurane delivered through a nosecone. Catheters for arterial blood sampling made from polyethyl-

ene tubing with an outer diameter of 0.965 mm and an internal diameter of 0.58 mm flushed with heparinized saline were placed in the right femoral arteries and left femoral veins under direct vision through 1-cm groin incisions as described previously.¹³ Arterial blood was analyzed using a portable handheld blood gas analyzer (iStat; Abbott, East Windsor, NJ). Pneumoperitoneum was achieved by delivering either carbon dioxide (CO₂) or helium through an 18-gauge angiocatheter placed percutaneously through the abdominal wall. Insufflation pressure was maintained at 4 mmHg using a laparoscopic insufflator (Olympus America Inc., Melville, NY). Peritoneal pH was measured using an Accumet AB15 Basic benchtop pH meter (Fisher Scientific International Inc., Hampton, NH). An MI-508 esophageal pH microelectrode (Microelectrodes Inc., Bedford, NH) was placed in a dependent portion of the peritoneal cavity posterior to the liver such that the tip of the catheter was bathed constantly in the small amount of peritoneal fluid present there. The electrode was positioned through the abdominal wall via a percutaneously placed 14-gauge angiocatheter. An MI-402 reference electrode (Microelectrodes Inc., Bedford, NH) was inserted transanally into the rectum. The system was calibrated before use with each animal by immersing the tips of the pH and reference electrodes in sterile, commercially prepared buffer solutions (Fisher Scientific, Fair Lawn, NJ) of pH 7.0 and pH 4.0. All animals were euthanized via anesthetic overdose at the end of the experiments. Buffered acidic peritoneal lavage solutions (20 mL) were introduced and removed through a 14-gauge fenestrated angiocatheter placed through the abdominal wall. Buffered lavage solutions were made by formulating a 280 osmol solution of 90-mmol NaCl, 0.5-mmol MgCl₂, 10-mmol Na₂HPO₄, and 20-mmol NaC₃H₅O₃ titrated to the desired pH with HCl. Figure 1 shows the overall setup for all animal procedures.

pH pilot experiment. Anesthetized rats had acidic buffered lavage solutions of varying pH instilled into their peritoneal cavities for 30 min. Peritoneal pH was measured at 0, 15, 30, 45, and 60 min (the last 2 measurements were made after the acidic solutions were removed) and was compared with peritoneal pH measurements from animals receiving CO₂ and He abdominal insufflation as well as He abdominal insufflation with acid peritoneal lavage. The strong acid solution described above had the pH characteristics that approximated most closely the conditions present over time during CO₂ pneumoperitoneum.

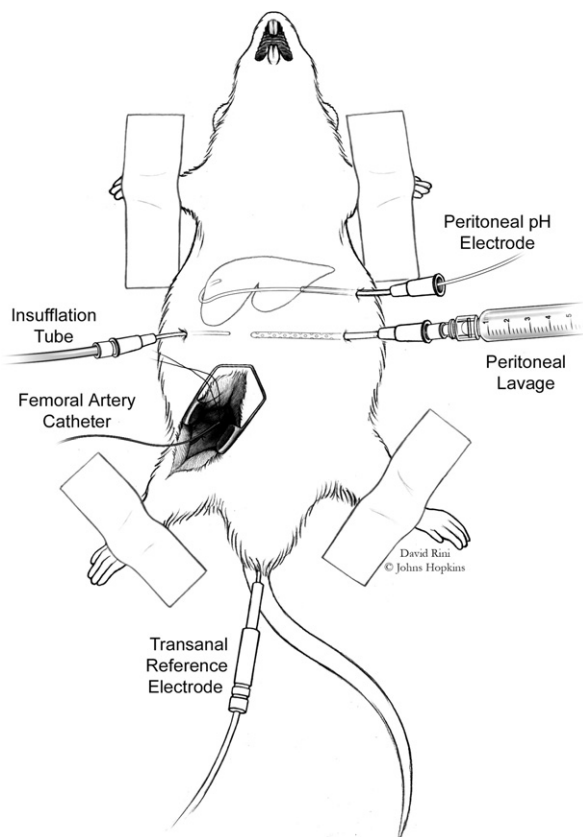


Fig 1. Set up for rodent pneumoperitoneum, arterial blood sampling, peritoneal pH monitoring, and peritoneal lavage.

Experimental design. Forty-two rats were randomized into 7 groups ($n = 6$): (1) LPS only, (2) anesthesia control, (3) helium pneumoperitoneum, (4) CO₂ pneumoperitoneum, (5) buffered mild acid lavage, (6) buffered strong acid lavage, and (7) buffered strong acid lavage + helium pneumoperitoneum. Animals were anesthetized (except the LPS-only group) and their respective abdominal treatment (pneumoperitoneum and/or lavage) administered for 30 min. At the end of 30 min, the lavage groups had as much as possible of the acidic solutions aspirated from their peritoneal cavities, and a pH neutral solution was instilled in place to assure normalization of the peritoneal pH. Systemic lipopolysaccharide (LPS, 1 mg/kg from *Escherichia coli* serotype 026:B6; Sigma-Aldrich, St. Louis, Mo) was administered via intravenous injection into the penile vein immediately after peritoneal treatment. Blood for cytokine assay was harvested via cardiac puncture 60 min after LPS injection. Plasma levels of IL-10 and TNF α protein were determined by enzyme-linked immunosorbent assay using commercially available kits (Biosource, Camarillo, Calif).

Statistical analysis. Mean peritoneal pH measurements after 30 min of peritoneal insufflation or lavage (or both) were compared with average baseline parameters from the same group using the Student t test. Cytokine data are expressed as mean \pm standard error of the mean. The Student t test was used to compare cytokine levels between the LPS-only and anesthesia control groups (groups 1 and 2, respectively). Cytokine levels among animals with pH-normal peritoneal cavities (groups 1 and 2) were compared with levels among animals with acidic peritoneal cavities (groups 3-7) using the Student t test. One-way analysis of variance test was used to detect general differences in serum cytokine levels among the acidic groups. Correlation (expressed as r) and significance of correlation between pH and cytokine levels were determined using the Pearson product moment correlation method. Differences and correlation were considered significant when $P < .05$. Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Seattle, Wash) and SigmaStat (SPSS Incorporated, Chicago, Ill) software.

RESULTS

pH pilot experiment. In an effort to replicate the intra-abdominal pH conditions present during and after abdominal insufflation via a mechanism other than gaseous insufflation, rats had acidic buffered lavage solutions of 2 different pHs ("mild" and "strong") instilled into their peritoneal cavities for 30 min. Figure 2 shows the effect on peritoneal and arterial pH of insufflation with either CO₂ or He, or of abdominal installation of mildly acidic or strongly acidic solutions with or without He peritoneal insufflation. The peritoneal pH curves for CO₂ pneumoperitoneum, strong acid lavage, and strong acid lavage + He pneumoperitoneum were similar over time—reaching a nadir between 15 min and 30 min, and returning to near-baseline levels approximately 15 min after desufflation or acidic fluid removal. The peritoneal pH curves for He pneumoperitoneum and mild acid lavage were also similar, but they decreased by much less than the other 3 groups.

Effects of pH on cytokine expression. To determine the effects of peritoneal acidification on cytokine production by both gaseous insufflation and acid peritoneal lavage, rats were randomized into the following groups: (1) LPS-only, (2) anesthesia control, (3) helium pneumoperitoneum, (4) CO₂ pneumoperitoneum, (5) mild acid peritoneal lavage, (6) strong acid peritoneal lavage, and (7) strong acid peritoneal lavage + helium pneumoperitoneum. Mean peritoneal pH did not change

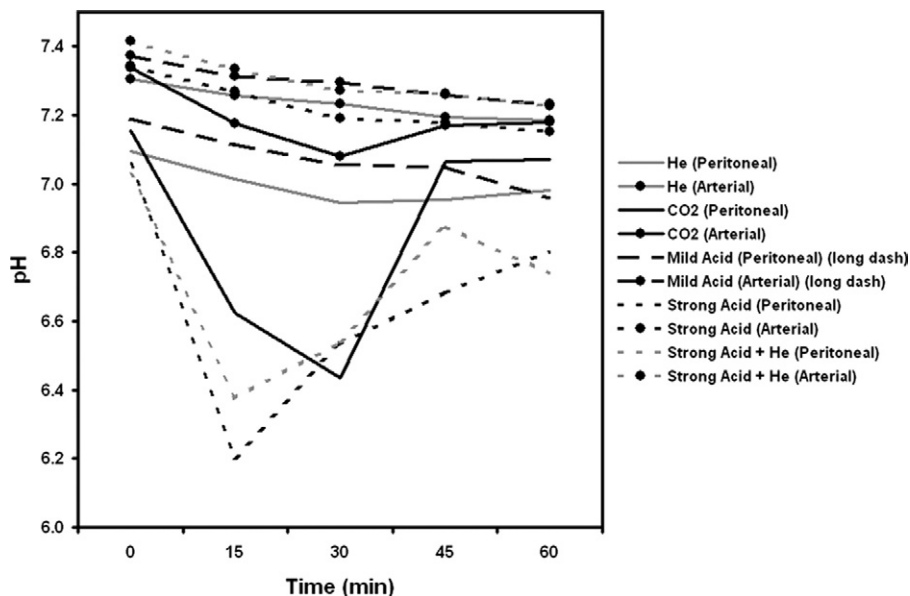


Fig 2. Arterial (filled circle data points included) and peritoneal (no data points included) pH before, during, and after 30 min of abdominal helium insufflation (solid gray), CO₂ insufflation (solid black), mild acid lavage (long dashed black), strong acid lavage (dotted black), and strong acid lavage + helium insufflation (dotted gray).

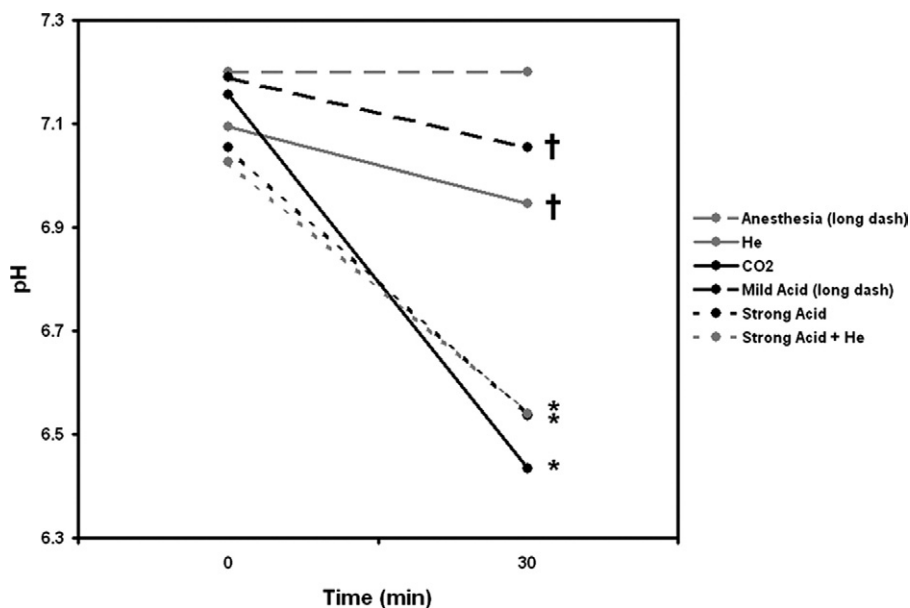


Fig 3. Peritoneal pH before and after 30 min of anesthesia (long dashed gray), abdominal helium insufflation (solid gray), abdominal CO₂ insufflation (solid black), mild acid peritoneal lavage (long dashed black), strong acid peritoneal lavage (dotted black), and strong acid peritoneal lavage + abdominal helium insufflation (dotted gray). * $P < .05$ for peritoneal pH after 30 min compared with baseline values. † $P = .1$ for peritoneal pH after 30 min compared with baseline values.

in the group that received anesthesia only (and could not be measured in the LPS-only group); however, peritoneal pH decreased after 30 min of CO₂ pneumoperitoneum, buffered strong acid lavage, and buffered strong acid lavage + helium

pneumoperitoneum ($P < .05$ for all 3, Fig 3). Peritoneal pH decreased after 30 min of helium pneumoperitoneum alone and after buffered mild acid lavage, but these differences did not reach statistical significance ($P = .1$ for both).

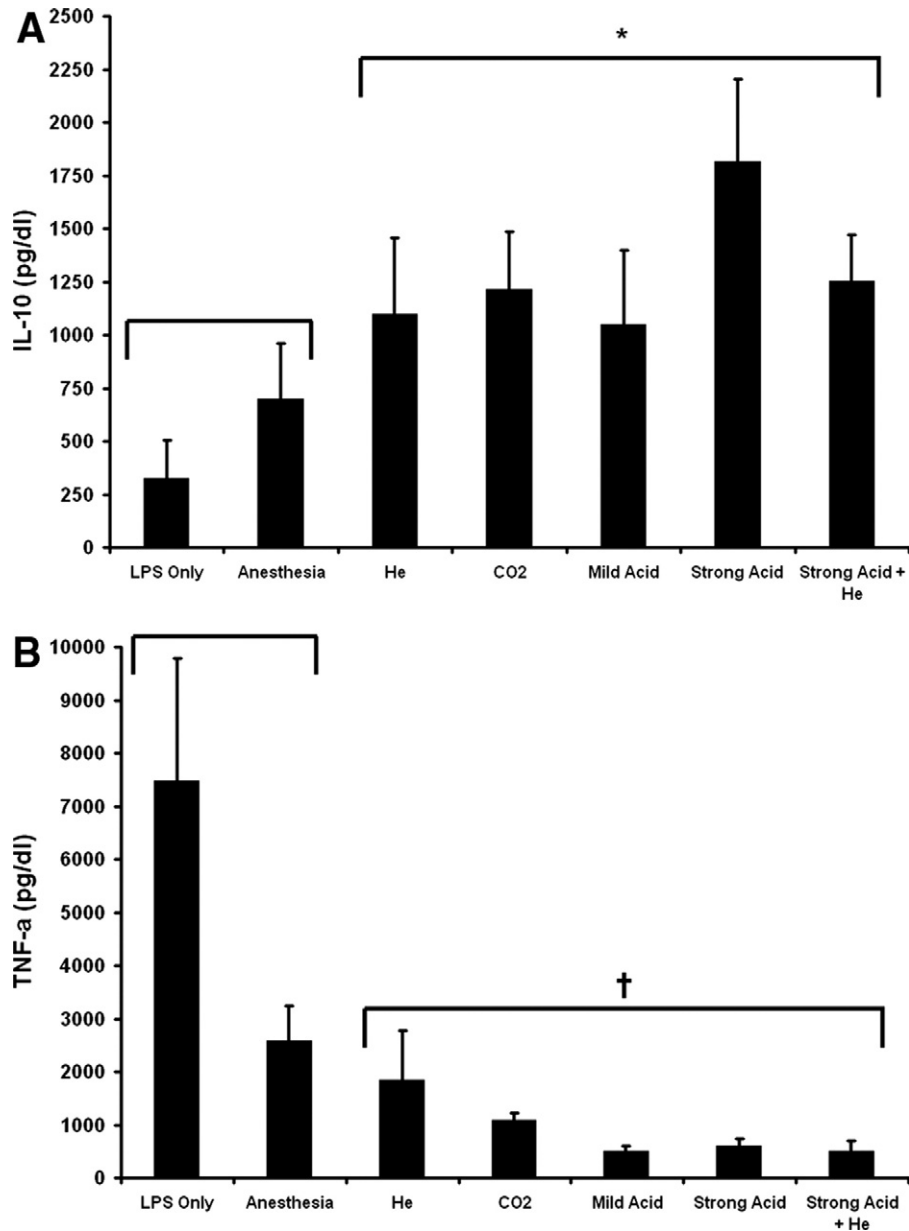


Fig 4. Serum cytokine levels 60 min after LPS injection among rats (n = 42) treated with (1) LPS-only, (2) anesthesia-only, (3) helium pneumoperitoneum, (4) CO₂ pneumoperitoneum, (5) mild acid peritoneal lavage, (6) strong acid peritoneal lavage, and (7) strong acid peritoneal lavage + helium pneumoperitoneum. IL-10 levels (top graph) were increased (* $P < .01$), and TNF α levels (bottom graph) were decreased ($\dagger P < .001$) among animals with acidic peritoneal cavities (groups 3-7) compared with animals with pH-normal peritoneal cavities (groups 1 and 2).

Serum levels of both a proinflammatory (TNF α) and an anti-inflammatory (IL-10) cytokine were measured 60 min after LPS injection in all 7 groups. IL-10 and TNF α levels from animals that received LPS only were not different than from animals that also received anesthesia alone ($P > .05$ for both cytokines). IL-10 levels from animals with decreased peritoneal pH (groups 3-7) were

compared with levels from animals with normal peritoneal pH (groups 1 and 2), and they were found to be increased overall ($P < .01$, Fig 4). The same analysis was made for TNF α , serum levels of which were found to be significantly depressed among the peritoneally acidotic animals compared with animals with pH-normal peritoneal cavities ($P < .001$). Among the groups

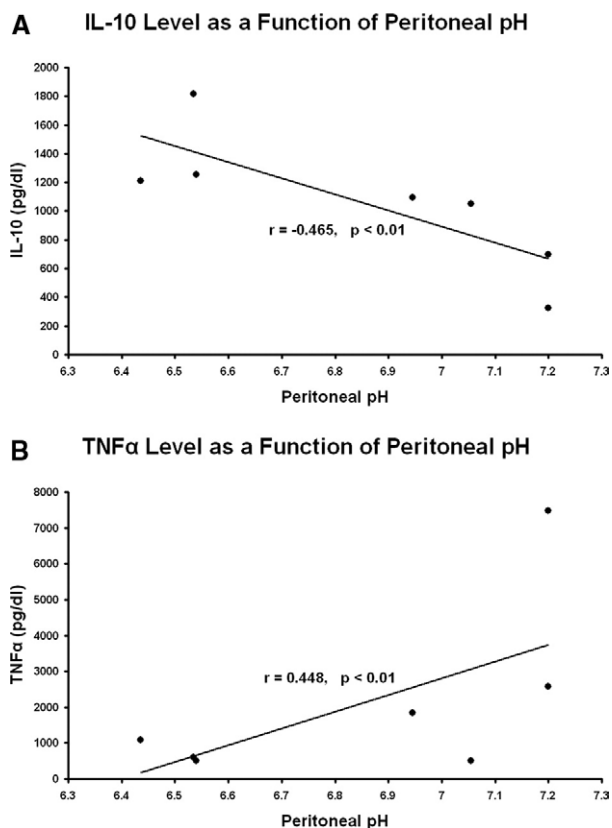


Fig 5. Correlation between peritoneal pH and serum IL-10 (top graph) and TNF α (bottom graph) serum levels. The degree of peritoneal acidification correlated directly to the degree of IL-10 production ($r = -0.465$, $P < .01$), and inversely to the degree of TNF α production ($r = 0.448$, $P < .01$).

of animals with peritoneal acidosis (groups 3-7), no differences were found in levels of either IL-10 or TNF α with respect to insufflation status ($P > .05$ for both cytokines).

The potential correlation between the degree of peritoneal acidification and the level of cytokine modulation was evaluated by plotting IL-10 and TNF α levels against peritoneal pH (Fig 5). IL-10 levels correlated in an inverse relationship to peritoneal pH ($r = -0.465$, $P < .01$), and TNF α levels correlated in a direct relationship to peritoneal pH ($r = 0.448$, $P < .01$).

DISCUSSION

More than a quarter of a million laparoscopic cholecystectomies are performed annually in the United States alone.¹⁴ Millions more patients worldwide undergo laparoscopic surgery each year under the care of surgeons from virtually every surgical subspecialty.

Despite the pervasiveness of the minimally invasive paradigm, disproportionately little is known about the basic mechanism underlying the benefits of the laparoscopic approach. A substantial body of evidence now refutes the once generally accepted notion that smaller incisions alone account for the observed differences between laparoscopic and conventional surgery. In addition to altered host physiology through the mechanical effects of abdominal distension/pressure,^{10,15-19} we proposed that a major advantage of laparoscopic surgery relates to the effects of CO₂ pneumoperitoneum on the immune/inflammatory response.^{7-11,20-23}

In the current study, we hypothesized that peritoneal acidity secondary to CO₂ insufflation is responsible for the decreased inflammatory response observed after the administration of LPS. Specifically, we hypothesized that acidification of the peritoneum via means other than CO₂ insufflation would produce alterations in the inflammatory response similar to those associated with CO₂ pneumoperitoneum. We have shown that not only is this the case, but that even modest changes in peritoneal pH have effects on the humoral response to LPS stimulation. We further hypothesized that the degree of inflammatory response attenuation would correlate with the degree of peritoneal acidification. Again, this proved to be true as evidenced by the inverse correlation between peritoneal pH and production of the anti-inflammatory cytokine IL-10, and the direct correlation between pH and production of the pro-inflammatory cytokine TNF α . We employ systemic endotoxemia (LPS) as a model of operative stress, because the degree of insult can be titrated, because the model is consistently reproducible, and because LPS activates known pathways of the inflammatory cascade.

Although we had originally intended to include a pH-normal peritoneal lavage group to serve as a pH control for the "strong" acid lavage group, a buffered solution that could be instilled into the abdomen and that would keep the original peritoneal pH from changing proved illusive. The degree of peritoneal acidosis, which occurred in the "mild" acid group, however, correlated closely to the mild degree of acidosis produced by helium pneumoperitoneum. The similar cytokine release patterns associated with these 2 groups suggests that the modest inflammation-attenuating effects of helium pneumoperitoneum observed in many of our experiments may, in fact, be from a mild acid-producing effect rather than a neurally mediated mechanical effect such as we and others have long supposed. Although it is well established that cellular absorption of CO₂ leads to increased carbonic acid production,^{21,24-32}

how helium pneumoperitoneum causes mild local acidosis is unknown. Because helium is generally believed to be a biologically inert gas, it is likely that the acidosis occurs as a consequence of a mechanical effect of abdominal distension. It is possible that the pressure of pneumoperitoneum with any gas is enough to impede nutrient flow on a cellular level such that the most superficial layer of the peritoneum and the cellular components adherent thereto (such as peritoneal macrophages) trend more toward anaerobic metabolism during abdominal insufflation.

West et al³³ have shown that macrophages acidified with CO₂ both in vitro (in culture) and in vivo (during pneumoperitoneum) produce less TNF in response to in vitro LPS stimulation compared with exposure to air or helium, that maintenance of normal intracellular pH is required for LPS-stimulated macrophage TNF release, and that the effects of CO₂ on macrophages endure for a period of hours.³⁴ Collectively, these data suggest strongly that transient acidification of the peritoneal macrophage is fundamental to the basic science of laparoscopic surgery.

The current study lends support to the notion that acidification of the peritoneum—and therefore acidification of the peritoneal macrophage—by CO₂ plays an important role in the mechanism underlying laparoscopic-surgery-associated inflammatory response reduction. Future work investigating the effects of CO₂ pneumoperitoneum (and peritoneal acidosis in general) in animals whose peritoneal macrophages have been depleted would further confirm or refute the presupposition that it is through the peritoneal macrophage that acidosis mediates its effect. The next step toward understanding the mechanism of pH-mediated modulation of inflammatory cell activation would involve in-depth investigation of the specific intracellular mechanism(s) involved.

In summary, we have shown that the peritoneal pH conditions during and following pneumoperitoneum with CO₂ and with helium can be modeled by the instillation of acidic peritoneal lavage solutions, that acidification of the peritoneal cavity whether by either abdominal insufflation or peritoneal acid lavage increases serum IL-10 and decreases serum TNF α in response to systemic LPS challenge, and that the degree of peritoneal acidification correlates directly with the degree of IL-10 production and inversely with the degree of TNF α production. These results support the hypothesis that pneumoperitoneum-mediated attenuation of the inflammatory response following laparoscopic

surgery occurs via a mechanism of peritoneal cell acidification.

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