

# Carbon dioxide pneumoperitoneum-mediated attenuation of the inflammatory response is independent of systemic acidosis

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**Background.** The purpose of this study was to determine if systemic acidosis induced by peritoneal absorption of carbon dioxide (CO<sub>2</sub>) during laparoscopy plays a role in CO<sub>2</sub> pneumoperitoneum-mediated attenuation of the acute phase inflammatory response associated with perioperative sepsis. The influence of hepatic polymorphonuclear (PMN) leukocyte infiltration on this phenomenon was also investigated.

**Methods.** Forty-five rats were randomized into 5 groups: anesthesia control, open cecal ligation and puncture (OCLP), laparoscopic cecal ligation and puncture using helium for insufflation (He LCLP), LCLP using CO<sub>2</sub> with continued spontaneous ventilation (LCLP-SV), and LCLP using CO<sub>2</sub> with intubation and positive pressure ventilation (LCLP-PPV).

**Results.** After 30 minutes, arterial blood gas parameters remained normal in control, OCLP rats, and He LCLP rats, while CO<sub>2</sub> LCLP-SV rats developed significant hypercarbic acidosis. This acidosis was corrected in CO<sub>2</sub> LCLP-PPV rats (P < .0001 vs CO<sub>2</sub> LCLP-SV for both). Expression of the rat acute phase gene  $\alpha_2$ -macroglobulin was greater after OCLP and He LCLP than after either CO<sub>2</sub> LCLP-SV or CO<sub>2</sub> LCLP-PPV (P < .0001 vs either CO<sub>2</sub> OCLP-SV for both). However, levels of  $\alpha_2$ -macroglobulin were not significantly different between the acidotic (LCLP-SV) and normocarbic (LCLP-PPV) CO<sub>2</sub> groups. Infiltration of the hepatic parenchyma by PMNs did not differ significantly between groups.

**Conclusions.** CO<sub>2</sub> insufflation-induced systemic acidosis is not responsible for the reduction in the acute phase inflammatory response observed in laparoscopic animal models of sepsis. Hepatic PMN infiltration also does not appear to mediate this effect. (*Surgery* 2005;137:559-66.)

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THE ADVANTAGES OF MINIMALLY INVASIVE SURGERY for appropriate procedures are now well accepted. Decreased postoperative pain, shorter hospital

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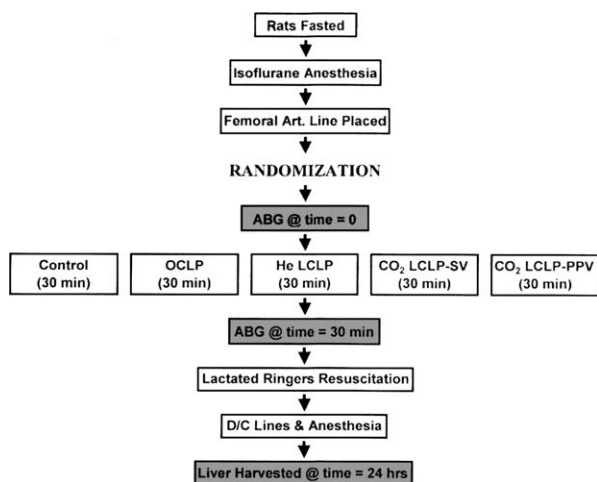
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stays, a more rapid return to preoperative activity, decreased postoperative ileus, preserved immune function, and superior cosmesis are among the benefits of the laparoscopic approach.<sup>1-6</sup> However, the mechanisms underpinning the improved results observed after laparoscopic surgery are still poorly understood.

Recently published work from our group demonstrates that peritoneal insufflation with carbon dioxide (CO<sub>2</sub>) blunts the hepatic expression of the rat acute phase genes  $\alpha_2$ -macroglobulin and  $\beta$ -fibrinogen in laparoscopic models of perioperative sepsis.<sup>7,8</sup> These data and those from other researchers<sup>9,10</sup> challenge the once generally accepted notion that smaller incisions alone account for the observed benefits of laparoscopy, but they do not delineate how CO<sub>2</sub> pneumoperitoneum exerts its effect. Given the centrality of CO<sub>2</sub> to the primary buffer in biologic systems (the bicarbonate buffer system: CO<sub>2</sub> + H<sub>2</sub>O  $\leftrightarrow$  H<sub>2</sub>CO<sub>3</sub>  $\leftrightarrow$  HCO<sub>3</sub><sup>-</sup> + H<sup>+</sup>), it is



**Fig 1.** Schematic description of experimental design. Forty-five rats were randomized into 5 groups: anesthesia-only (Control); open cecal ligation and puncture (OCLP); laparoscopic CLP using helium for insufflation (He LCLP); LCLP using carbon dioxide (CO<sub>2</sub>) with continued spontaneous ventilation (CO<sub>2</sub> LCLP-SV); and LCLP using carbon dioxide with positive pressure ventilation (CO<sub>2</sub> LCLP-PPV). ABG parameters were measured before (time = 0) and after (time = 30 min) experimental procedures. Livers were harvested 24 hours postoperatively for hepatic acute phase gene expression and PMN leukocyte infiltration analysis. ABG, Arterial blood gas; D/C, discontinue.

reasonable to propose that such effects might be mediated through changes in acid-base chemistry.

In addition to the proliferation of cytokines and other effects, the inflammatory response is characterized by the expression of cell surface receptors on activated endothelial cells that guide the adhesion and extravasation of polymorphonuclear (PMN) leukocytes.<sup>11</sup> The liver responds to PMN tissue infiltration by expressing hepatic acute-phase genes, which are beneficial to the individual in the early stages of injury but can be deleterious if gene expression is exaggerated or left unchecked.<sup>12</sup> Because  $\alpha_2$ -macroglobulin is an important acute phase reactant in rats that parallels the behavior of human hepatic acute phase reactants (C-reactive protein, prealbumin, transferrin,  $\alpha_1$ -trypsin inhibitor, retinol-binding protein, haptoglobin, ceruloplasmin, etc),<sup>13</sup> it is crucial to determine if its variable expression during laparoscopy is mediated through PMN infiltration of the liver.

The purposes of this study were (1) to determine if systemic acidosis induced by peritoneal absorption of CO<sub>2</sub> during laparoscopy plays a role in CO<sub>2</sub> pneumoperitoneum-mediated attenuation of the acute phase inflammatory response associ-

ated with perioperative sepsis and (2) to investigate the influence of hepatic PMN leukocyte infiltration on this phenomenon.

## 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. A schematic description of our experimental design is shown in Fig 1. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass), 10- to 12-weeks-old and weighing between 250 and 350 g, were housed in cages in which standard chow and water were available ad libitum. The rats were acclimatized to their environment for 3 to 5 days upon arrival and then fasted overnight before intervention. All surgical procedures were performed under aseptic conditions. Induction and maintenance anesthesia was achieved with the use of vaporized isoflurane delivered through a nose cone and, eventually, through an endotracheal tube in the intubation and positive pressure ventilation group (CO<sub>2</sub> LCLP-PPV). Catheters for arterial blood sampling, made from polyethylene tubing with an outer diameter of 0.965 mm and an internal diameter of 0.58 mm, were flushed with heparinized saline and then placed in the right femoral artery of each rat under direct vision through a 1-cm groin incision. Blood was analyzed with the use of an automated arterial blood gas (ABG) analyzer (Chiron Diagnostics, East Walpole, Mass). Rats randomized to receive mechanical ventilation (CO<sub>2</sub> LCLP-PPV) were intubated with a 14-gauge angiocatheter under endoscopic vision with the use of a 3-mm laparoscope as a laryngoscope.<sup>14</sup> Ventilator settings for this group were a tidal volume of 2.5 mL and a respiratory rate of 100 breaths per minute (minute ventilation, 250 mL/min or approximately 900 mL/kg/min).

**Experimental groups.** Baseline ABG samples were drawn from each rat (ABG @ time = 0). For the next 30 minutes, rats underwent the following procedures according to their randomly chosen group assignment: continued anesthesia only (Control, n = 5); open cecal ligation and puncture (CLP) through a 5-cm midline laparotomy (OCLP, n = 10); laparoscopic CLP using helium for insufflation (He LCLP, n = 10); LCLP using CO<sub>2</sub> with continued spontaneous ventilation (CO<sub>2</sub> LCLP-SV, n = 10); or LCLP using CO<sub>2</sub> with positive pressure ventilation (CO<sub>2</sub> LCLP-PPV, n = 10). After 30 minutes of pneumoperitoneum, laparotomy, or control, a second ABG sample was obtained (ABG @ time = 30 min).

**Cecal ligation and puncture.** Pneumoperitoneum was achieved by introducing a 22-gauge angiocatheter into the peritoneal cavity and insufflating (insufflator; Olympus, Melville, NY) the abdomen with 3 to 4 mm Hg gas.<sup>14</sup> Laparoscopic procedures were performed with the use of a 3-mm, 30° angled, rigid laparoscope (Olympus) and 1-mm instruments (Storz, Tuttlingen, Germany). CLP consisted of dissection of the cecum, ligation midway between the ileocecal valve and the terminal cecum with the use of a 3-0 silk tie, and double puncture of the isolated cecum with a hollow 16-gauge needle introduced through the abdominal wall. To ensure equal tension on the silk ligatures between groups, we tied all cecal ligation knots were tied extracorporally.

**Postoperative handling.** At the conclusion of the 30-minute intervention, all lines and tubes were removed, and all incisions were sutured closed with interrupted 3-0 silk. Animals were resuscitated with a subcutaneous injection of lactated Ringer's solution (30 mL/kg) and were again housed in cages in which water was available ad libitum. Animals were euthanized 24 hours postoperatively, at which time livers were harvested for analysis of hepatic acute phase gene expression and PMN leukocyte infiltration. At the time of necropsy, the peritoneal cavity was visually inspected for evidence of effective CLP (ie, cecal necrosis and cloudy peritoneal fluid).

**Hepatic gene expression.** Harvested liver tissue was flash-frozen in liquid nitrogen and stored at -80°C. RNA was isolated from liver samples with the use of Trizol (Gibco, Gaithersburg, Md). Total RNA (10 µg) was electrophoresed in formaldehyde-agarose gels and visualized by staining of the gel with ethidium bromide. Samples of RNA that appeared degraded were discarded. RNA samples were immobilized onto nylon-modified membranes (GeneScreen Plus; NEN Life Science Products, Inc, Boston, Mass) by Northern blotting for the  $\alpha_2$ -macroglobulin blot and by slot blotting for the  $\beta$ -fibrinogen, metallothionein, and 28S rRNA blots. Blots were hybridized with radiolabeled complementary DNA probes for  $\alpha_2$ -macroglobulin (rat, full-length),  $\beta$ -fibrinogen (pig, fragment), metallothionein (pig, full-length), and the 28S rRNA subunit. Radioactive probes were prepared by the random primer method<sup>15</sup> with the use of [ $\alpha$ -32P]dATP and [ $\alpha$ -32P]dCTP (ICN Pharmaceuticals, Irvine, Calif) as previously described.<sup>16</sup> Blots were hybridized in 50% formamide, 75 mmol/L sodium citrate (pH 7.0), 0.75 mol/L sodium chloride, 1% sodium dodecyl sulfate (SDS), 2.5× Denhardt's solution, 100 g/mL denatured salmon

sperm DNA, 1 mmol/L EDTA, and 20 mmol/L sodium phosphate (pH 6.5) for 16 hours at 42°C. Blots were washed with 50 mmol/L TRIS (hydroxymethyl) aminomethane (TRIS pH, 8.6), 1 mol/L NaCl, 2 mmol/L EDTA, and 1% SDS at 42°C for 1 hour. Later, blots were washed with 2× saline-sodium citrate buffer (0.015 mol/L sodium citrate [pH 7.0] and 0.15 mol/L NaCl) containing 0.1% SDS at 42°C for 30 minutes. The resultant hybridization signals were detected with the use of a phosphorimager (Storm 820; Molecular Dynamics, Buckinghamshire, England). Autoradiograms in the linear range of exposure were quantified by scanning laser densitometry. Data are expressed in normalized messenger RNA (mRNA)-expression units representing the individual gene densitometry signal normalized to the corresponding 28S rRNA subunit signal (the latter is an indirect measurement of the total amount of RNA in a sample) and scaled to the expression of the anesthesia-only control group.

**Hepatic PMN leukocyte infiltration.** Harvested liver tissue was preserved in formalin for 1 week. Tissue slices were then fixed to glass slides and stained with hematoxylin and eosin. The number of PMN leukocytes was determined for each slide by averaging the cell counts from 10 high-powered fields.

**Statistical analysis.** ABG parameters at the 30-minute time point from the 2 CO<sub>2</sub> groups (CO<sub>2</sub> LCLP-SV and CO<sub>2</sub> LCLP-PPV) were compared by using the Student *t* test. The 1-way ANOVA test was used to detect general differences in mRNA expression among all groups. To elucidate specific significances in these parameters between groups, we performed multiple pair-wise comparisons using Tukey's test. Differences between groups were considered significant when *P* < .05. Analysis was performed by using Microsoft Excel (Microsoft Corp, Redmond, Wash) and SigmaStat (SPSS Inc, Chicago, Ill) software.

## RESULTS

**ABG parameters.** The range of respiratory rates was normal at baseline for all anesthetized animals (range over the first 5 minutes of anesthesia, 80-100 per minute). After 30 minutes of anesthesia with or without laparotomy or pneumoperitoneum, respiratory rate decreased slightly for the anesthesia control, OCLP, and He LCLP groups (range over the last 5 minutes of anesthesia, 68-92). In contrast, respiratory rate increased slightly among the rats that underwent LCLP using CO<sub>2</sub> with spontaneous ventilation (CO<sub>2</sub> LCLP-SV;

**Table I.** ABG parameters\*

	Control	OCLP	He LCLP	CO <sub>2</sub> LCLP-SV	CO <sub>2</sub> LCLP-PPV
pH @ time = 0	7.43 (±0.02)	7.43 (±0.01)	7.42 (±0.01)	7.43 (±0.01)	7.41 (±0.01)
pH @ time = 30 min	7.38 (±0.02)	7.38 (±0.01)	7.38 (±0.01)	7.30 (±0.01)†	7.40 (±0.01)
pCO <sub>2</sub> @ time = 0	38.3 (±2.2)	38.2 (±1.1)	38.5 (±1.1)	39.3 (±0.9)	38.2 (±2.5)
pCO <sub>2</sub> @ time = 30 min	41.5 (±1.0)	42.1 (±1.0)	42.5 (±1.1)	53.2 (±1.9)‡	36.8 (±1.5)

ABG, Arterial blood gas; OCLP, open CLP; CLP, cecal ligation and puncture; LCLP, laparoscopic cecal ligation and puncture; He LCLP, laparoscopic CLP using helium (He); CO<sub>2</sub> LCLP-SV, laparoscopic CLP using carbon dioxide (CO<sub>2</sub>) with continued spontaneous ventilation; CO<sub>2</sub> LCLP-PPV, LCLP using carbon dioxide with positive pressure ventilation.

\*Blood samples were obtained through a femoral artery catheter placed immediately after induction of anesthesia. Blood was analyzed with the use of an automated ABG analyzer (Chiron Diagnostics). Data are mean ± SEM. pCO<sub>2</sub> data are expressed in millimeters of mercury (mm Hg).

†*P* < .0001 vs LCLP-PPV for pH @ time = 30 min.

‡*P* < .0001 vs LCLP-PPV for pCO<sub>2</sub> @ time = 30 min (Student *t* test).

range, 84-108), while respiratory rate among the rats that underwent LCLP using CO<sub>2</sub> with intubation and positive pressure ventilation (CO<sub>2</sub> LCLP-PPV) was kept constant at 100. Baseline ABG parameters were normal for all rats (Table I). After 30 minutes, ABG parameters remained normal in rats from the anesthesia control, OCLP, and He LCLP groups. Rats in the CO<sub>2</sub> LCLP-SV group developed significant hypercarbic acidosis with mean pH of 7.30 and pCO<sub>2</sub> of 53.2 after 30 minutes. This acidosis was corrected in rats that underwent LCLP using CO<sub>2</sub> with intubation and positive pressure ventilation (CO<sub>2</sub> LCLP-PPV) with mean pH of 7.40 and pCO<sub>2</sub> of 36.8 at 30 minutes (*P* < .0001 vs CO<sub>2</sub> LCLP-SV for pH and pCO<sub>2</sub>).

#### Postoperative evaluation and necropsy findings.

Rats in the anesthesia-only control group exhibited normal activity and had no piloerection during the 24 hours after intervention. In contrast, all rats that underwent CLP exhibited decreased activity and significant piloerection. At necropsy, rats from the anesthesia control group were found to have only a small amount of clear peritoneal fluid and no cecal necrosis. After CLP, all rats were found to have foul-smelling abdominal cavities and significant cloudy peritoneal fluid consistent with fecal contamination. Furthermore, the gross appearance of the ligated and punctured ceca was consistent with necrosis (friable and discolored) in all rats that received CLP.

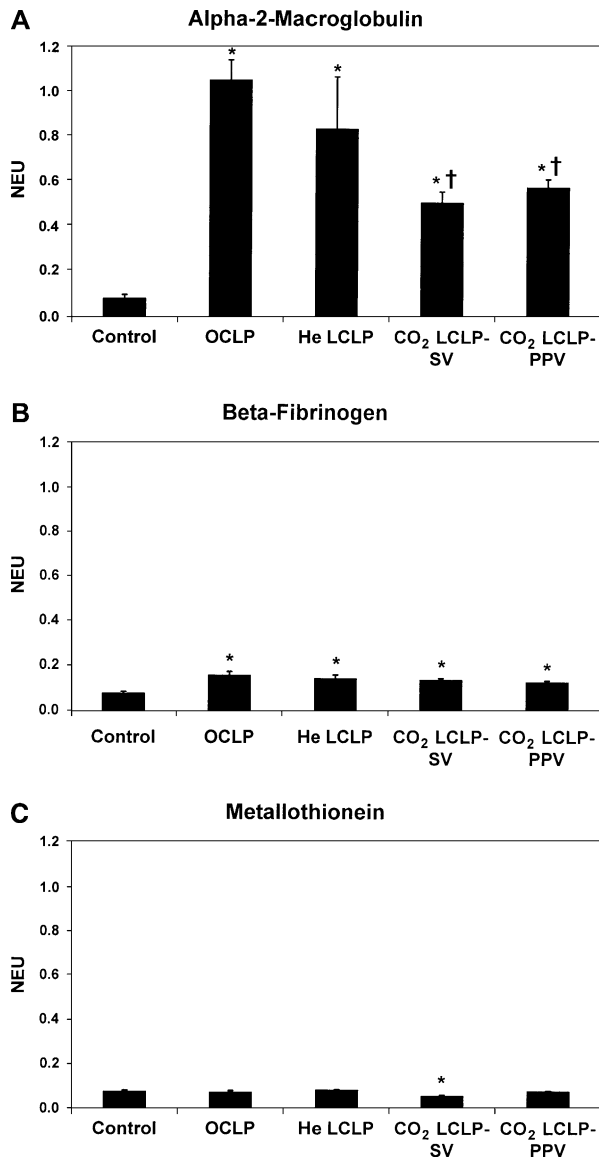
**Hepatic acute phase gene response.** Expression of the rat acute phase genes  $\alpha_2$ -macroglobulin and  $\beta$ -fibrinogen was analyzed by blotting and hybridization of RNA isolated from liver samples obtained 24 hours after either CLP or control procedure. Levels of hepatic  $\alpha_2$ -macroglobulin and  $\beta$ -fibrinogen mRNA were low in the absence of CLP-induced sepsis (anesthesia control) (Fig 2). All methods of CLP resulted in increased levels of  $\alpha_2$ -macroglobulin, compared with controls (*P* < .01). Furthermore, the levels of  $\alpha_2$ -macroglobulin mRNA detected

after both OCLP and He LCLP were higher than those detected after either CO<sub>2</sub> LCLP-SV or CO<sub>2</sub> LCLP-PPV (*P* < .0001 vs OCLP). However, levels of  $\alpha_2$ -macroglobulin were similar between the 2 laparoscopic groups using CO<sub>2</sub> as the insufflation gas (CO<sub>2</sub> LCLP-SV and CO<sub>2</sub> LCLP-PPV). While increased levels of  $\beta$ -fibrinogen mRNA among the CLP animals were also significant (*P* < .01), the relative degree to which  $\beta$ -fibrinogen increased was less, compared with  $\alpha_2$ -macroglobulin. Hepatic levels of  $\beta$ -fibrinogen mRNA between CLP groups were not significantly different. The level of mRNA coding for the free-radical scavenger protein metallothionein, while low overall, was less after CO<sub>2</sub> LCLP-SV than after all other experimental and control procedures (*P* < .01 for all).

**Hepatic PMN leukocyte infiltration.** The degree of hepatic parenchymal infiltration by PMN leukocytes was determined by histologic analysis of liver samples harvested 24 hours after either CLP or control procedure. The number of PMNs per high-powered field of liver tissue was not significantly different among any of the experimental or control groups (Fig 3).

## DISCUSSION

Data emerging from the study of the basic science of laparoscopy expose the relevance of the unique biologic activity of CO<sub>2</sub> to the mechanisms underlying the advantages of laparoscopy.<sup>10,17-24</sup> The immune-modifying affects of pneumoperitoneum appear to represent a final common pathway that contributes to favorable outcomes in laparoscopy from both sepsis-inflammation and tumor biology perspectives. Given the ever increasing volume of animal and clinical studies suggesting that the gas of insufflation influences the outcome of laparoscopy, it would be imprudent to attribute the advantages of laparoscopy solely to reduced tissue injury secondary to smaller incisions. Furthermore, as surgeons continue to



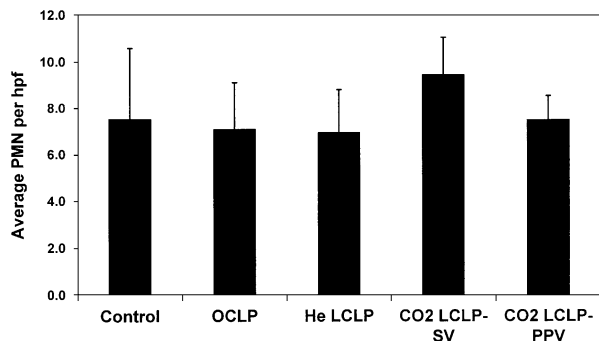
**Fig 2.** Hepatic gene expression in rats 24 hours after either CLP or control procedure. **A and B**, All methods of CLP resulted in significantly increased expression of  $\alpha_2$ -macroglobulin and  $\beta$ -fibrinogen compared with the control group. Expression of these rat acute phase genes was greater after open cecal ligation and puncture (OCLP) and laparoscopic CLP using helium (He LCLP) than after either LCLP using carbon dioxide (CO<sub>2</sub>) with spontaneous ventilation (CO<sub>2</sub> LCLP-SV) or LCLP using carbon dioxide with positive pressure ventilation (CO<sub>2</sub> LCLP-PPV). Levels of  $\alpha_2$ -macroglobulin and  $\beta$ -fibrinogen were not significantly different between the 2 CO<sub>2</sub> LCLP groups. **C**, Expression of metallothionein (a gene coding for a free-radical scavenger protein) was similar to that of the control group for all groups except the CO<sub>2</sub> LCLP-SV group (in which it was less). RNA was isolated from hepatic tissue, fixed to membranes, hybridized with radiolabeled probes, and visualized with the use of a phosphorimager. Autoradiograms in

perform increasingly longer, more-complex operations with extensive operative dissection and internal tissue manipulation, the degree of surgical insult attributable to the size of the incision(s) becomes relatively less important. As the magnitude of the operation begins to outweigh the magnitude of the incision, the predominant difference between laparoscopic and open surgery becomes the unique physiology of CO<sub>2</sub> pneumoperitoneum.

Recent work from our group demonstrates that peritoneal insufflation with CO<sub>2</sub> blunts the hepatic expression of the rat acute phase gene  $\alpha_2$ -macroglobulin in multiple laparoscopic models of perioperative sepsis.<sup>7,8</sup> Because CO<sub>2</sub> absorbed during laparoscopic surgery can affect clinical acid-base status,<sup>25,26</sup> and because most rodent models of laparoscopic surgery employ anesthetic delivery systems that do not require mechanical ventilation,<sup>27</sup> we felt it important to determine if our findings, and the findings of other researchers, could be attributed to systemic changes in acid-base equilibrium (Fig 4). Our decision to use the LCLP model as opposed to other models of stress (eg, lipopolysaccharide injection) in these and previous<sup>7</sup> experiments was based on our desire to produce clinically relevant stress that has unique bearing on laparoscopy. The cumulative stress of laparoscopic access to the celom and bacterial contamination of the peritoneum provided in the LCLP model affords a physiologic milieu analogous to the environments present during laparoscopic colon surgery or diagnostic laparoscopy in abdominal sepsis. The validity of our model has been previously described.<sup>7</sup>

We investigated the influence of CO<sub>2</sub> pneumoperitoneum-induced systemic acidosis on the inflammatory response induced by CLP in rats. Criticisms regarding the use of animal models of laparoscopy that do not include an extrinsic mechanism for controlling hypercarbia prompted us to measure acid-base status in our animals. As might be expected, our data show that when animals

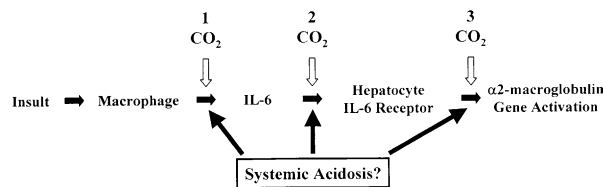
the linear range of exposure were quantified by scanning laser densitometry. Data are means, expressed in normalized mRNA-expression units representing the individual gene densitometry signal normalized to the corresponding 28S rRNA subunit signal (the latter is an indirect measurement of the total amount of RNA in a sample) and scaled to the expression of the anesthesia-only control group. \**P* < .01 vs Control; †*P* < .0001 vs OCLP, by 1-way ANOVA and multiple pair-wise comparisons using Tukey's test. Error bars represent  $\pm$  SEM. NEU, Normalized mRNA-expression units.



**Fig 3.** Level of hepatic PMN leukocyte infiltration in rats 24 hours after either CLP via various methods or control procedure. The small variation in the average number of PMNs counted per high-powered field between groups was not statistically significant and suggests that CLP via any method did not induce a significant degree of hepatic PMN infiltration. *Error bars* represent  $\pm$  SEM. *PMN*, Polymorphonuclear; *hpf*, high-powered field; *CLP*, cecal ligation and puncture; *CO<sub>2</sub>*, carbon dioxide; *OCLP*, open cecal ligation and puncture; *He LCLP*, laparoscopic CLP using helium (He); *CO<sub>2</sub> LCLP-SV*, LCLP using carbon dioxide (CO<sub>2</sub>) with spontaneous ventilation; *CO<sub>2</sub> LCLP-PPV*, LCLP using carbon dioxide with positive pressure ventilation.

reliant on spontaneous respiration for ventilation are anesthetized and subjected to CO<sub>2</sub> pneumoperitoneum they accumulate dissolved CO<sub>2</sub> in the blood and develop substantial hypercarbic acidosis, which is refractory to their small compensatory increase in spontaneous respiratory rate. In animals that are intubated and mechanically ventilated with increased minute ventilation (ie, by increasing tidal volume and maintaining respiratory rate), our data also show that the acidemia can be completely compensated.

The inflammatory response was evaluated at the level of acute phase gene expression in the liver. The gene coding for  $\alpha_2$ -macroglobulin protein was chosen in our study because  $\alpha_2$ -macroglobulin is an important acute phase reactant in rats, and its behavior parallels that of human hepatic acute phase reactants.<sup>13</sup> We also measured hepatic levels of  $\beta$ -fibrinogen mRNA because previously we have shown  $\beta$ -fibrinogen gene expression to parallel that of  $\alpha_2$ -macroglobulin in septic rats.<sup>7</sup> The gene coding for metallothionein was chosen as a control because its expression is not significantly affected by CLP or insufflation with different gases.<sup>7,28</sup> Hepatic gene expression was analyzed 24 hours after either CLP or control procedure on the basis of previously published data showing maximal expression of  $\alpha_2$ -macroglobulin at that time point after similar insult.<sup>29</sup> We justified inclusion of only a single non-CLP control group (anesthesia-only)



**Fig 4.** Mechanism of hepatic acute phase gene activation after intra-abdominal insult. CO<sub>2</sub> pneumoperitoneum may attenuate this inflammatory response pathway by acting at any of 3 well-defined steps: CO<sub>2</sub> may alter the release of cytokines (1); CO<sub>2</sub> may influence the clearance of circulating inflammatory mediators or alter cytokine-cytokine receptor interactions (2); or hepatocytes may be affected directly by CO<sub>2</sub> (3). Our study sought to address whether or not the blunting effect of CO<sub>2</sub> pneumoperitoneum on the hepatic acute phase response is mediated through systemic acidosis. *CO<sub>2</sub>*, Carbon dioxide; *IL-6*, interleukin 6.

because we have previously demonstrated that pneumoperitoneum alone (with CO<sub>2</sub>, helium, or air) and sham laparotomy do not induce expression of any of the 3 genes of interest more than does anesthesia alone.<sup>7,28</sup>

In the current study, CLP induced significant expression of  $\alpha_2$ -macroglobulin, compared with anesthesia-only controls. Consistent with our previous work,<sup>7</sup>  $\alpha_2$ -macroglobulin was additionally expressed more after OCLP and after He LCLP than after LCLP using CO<sub>2</sub> in both spontaneously ventilated (CO<sub>2</sub> LCLP-SV) and mechanically ventilated (CO<sub>2</sub> LCLP-PPV) rats. The most crucial finding in the current study was that the expression of  $\alpha_2$ -macroglobulin was similar among the non-intubated (ie, acidotic) and the mechanically ventilated (ie, normocarbic) rats who underwent LCLP using CO<sub>2</sub> for insufflation. Thus, we observed attenuation of hepatic  $\alpha_2$ -macroglobulin gene expression after laparoscopic CLP that is attributable to the presence of CO<sub>2</sub> pneumoperitoneum but is independent of the influence of absorbed CO<sub>2</sub> on systemic acid-base physiology.

While the focus of our group's investigation is on the effects of CO<sub>2</sub> pneumoperitoneum on the  $\alpha_2$ -macroglobulin pathway (Fig 4), the results for  $\beta$ -fibrinogen and metallothionein in the current study warrant explanation. Although our previous work showed  $\beta$ -fibrinogen to be relatively less strongly expressed in the liver 24 hours after CLP (~50% less), compared with  $\alpha_2$ -macroglobulin,<sup>7</sup> the current study showed that relative  $\beta$ -fibrinogen levels were as much as 86% lower, compared with  $\alpha_2$ -macroglobulin, thus making any differences between CLP groups difficult to detect. A number of explanations for this inconsistency with our

previous results are possible: (1) Seasonal variation may have affected the  $\beta$ -fibrinogen response to CLP (the current study was performed during the early winter while the previous study was conducted in the late summer). (2) Gender-dependent responses to stress related to cyclic hormonal variation have been noted<sup>30</sup> (the current study used male rats, while the previous study used female rats). (3) Recent data from our laboratory and others have shown that anesthesia can have a profound impact on the response to sepsis<sup>31,32</sup> (the now-discontinued methoxyflurane was replaced in the current study by readily available isoflurane). While the relative expression of metallothionein in our previous work was similar to that in the current study,<sup>7</sup> we did find a significant decrease in the amount of mRNA coding for this free-radical scavenger protein in the CO<sub>2</sub> LCLP-SV group. However, this difference is probably of little clinical importance given both the overall low expression of this gene in all groups and the small scale of the difference.

Because our group has previously shown that neutrophil infiltration in the liver plays a role in variable inflammation after lipopolysaccharide (LPS) challenge among genetically distinct strains of mice,<sup>12</sup> we felt it important to measure the amount of hepatic PMN leukocyte infiltration among the groups in our study. Attenuation of the hepatic acute phase response could be caused by CO<sub>2</sub>-mediated downregulation of the expression of activated endothelial cell surface receptors in the liver, thus reducing the adhesion and extravasation of PMNs. However, the degree of neutrophil infiltration found in our study did not differ among CLP groups. In fact, no increase in hepatic PMN infiltration was observed in our study after CLP by any method, compared with the anesthesia-only group. This finding suggests that not only is variable neutrophil infiltration of the liver not responsible for decreased hepatic acute phase gene activation after LCLP using CO<sub>2</sub> for insufflation, but it also underscores the vast mechanistic differences between the inflammatory responses created in the CLP and LPS models.

This study confirms that laparoscopy downregulates the hepatic expression of  $\alpha_2$ -macroglobulin, and that this effect is caused neither by systemic acidosis nor by variable hepatic neutrophil infiltration. Delineation of the exact mechanism whereby CO<sub>2</sub> influences hepatic gene expression remains a challenge. CO<sub>2</sub> pneumoperitoneum may attenuate the inflammatory response cascade by acting at any of 3 well-defined steps: (1) CO<sub>2</sub> may block the release of cytokines from Kupffer

cells and resident peritoneal macrophages through local peritoneal acidosis or other mechanisms. (2) CO<sub>2</sub> may influence the clearance of circulating inflammatory mediators or alter cytokine-cytokine receptor interactions in the liver and elsewhere. (3) Hepatocytes may be affected directly by CO<sub>2</sub> via alterations in second messenger system function or transcription factor action (Fig 4).<sup>33-35</sup> Because we and others have shown that levels of circulating inflammatory cytokines are different after laparoscopic and conventional surgery,<sup>17,36,37</sup> and because *in vivo* intracellular acidification of peritoneal macrophages during laparoscopy has been shown to inhibit the *in vitro* LPS-mediated release of cytokines from these cells when harvested,<sup>10</sup> the majority of active research in this field is focused on this first step. In future experiments, we plan to evaluate the effect on hepatic acute phase gene expression of local acidification of the peritoneal fluid via means other than CO<sub>2</sub> insufflation, and we plan to perform inter-animal peritoneal macrophage transfer experiments to more clearly define the role of these cells in this process. Certainly, as the penetration of laparoscopy in surgical practice continues to increase, and as the scope of patients to whom the laparoscopic paradigm is applied broadens, the biology of pneumoperitoneum will become an ever more important area of investigation.

## CONCLUSION

Our study shows that CO<sub>2</sub> insufflation induces systemic acidosis in anesthetized, nonventilated rats, that CO<sub>2</sub> pneumoperitoneum-induced hypercarbic acidosis is reversible via mechanical ventilation, and that systemic acidosis is not responsible for the attenuation of hepatic  $\alpha_2$ -macroglobulin gene expression observed in laparoscopic animal models of sepsis. We have further demonstrated that the reduction in inflammation associated with laparoscopy is not mediated through variable hepatic PMN infiltration. CO<sub>2</sub> pneumoperitoneum remains a clinically relevant attenuator of the inflammatory response during laparoscopic surgery, independent of systemic effects on acid-base physiology.

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