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SURGICAL ENDOSCOPY
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# Laparoscopic surgery and the parasympathetic nervous system

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**Abstract**
*Background:* Laparoscopic surgery preserves the immune system and has anti-inflammatory properties. $CO_2$ pneumoperitoneum attenuates lipopolysaccharide (LPS)-induced cytokine production and increases survival. We tested the hypothesis that $CO_2$ pneumoperitoneum mediates its immunomodulatory properties via stimulation of the cholinergic pathway.
*Method:* In the first experiment, rats ($n = 68$) received atropine 1 mg/kg or saline injection 10 min prior to LPS injection and were randomization into four 30-min treatment subgroups: LPS only control, anesthesia control, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum. In a second experiment, rats ($n = 40$) received atropine 2 mg/kg or saline 10 min prior to randomization into the same four subgroups described previously. In a third experiment, rats ($n = 96$) received atropine 2 mg/kg or saline 10 min prior to randomization into eight 30-min treatment subgroups followed by LPS injection: LPS only control; anesthesia control; and $CO_2$ or helium pneumoperitoneum at 4, 8, and 12 mmHg. In a fourth experiment, rats ($n = 58$) were subjected to bilateral subdiaphragmatic truncal vagotomy or sham operation. Two weeks postoperatively, animals were randomized into four 30-min treatment subgroups followed by LPS injection: LPS only control, anesthesia control, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum. Blood samples were collected from all animals 1.5 h after LPS injection, and cytokine levels were determined by enzyme-linked immunosorbent assay.
*Results:* Serum tumor necrosis factor-α (TNF-α) levels were consistently suppressed among the saline–$CO_2$ pneumoperitoneum groups compared to saline–LPS only control groups ($p < 0.05$ for all four experiments). All chemically vagotomized animals had significantly reduced TNF-α levels compared to their saline-treated counterparts ($p < 0.05$ for all), except among the $CO_2$ pneumoperitoneum-treated animals. Increasing insufflation pressure with helium eliminated differences ($p < 0.05$) in TNF-α production between saline- and atropine-treated groups but had no effect among $CO_2$ pneumoperitoneum-treated animals. Finally, vagotomy (whether chemical or surgical) independently decreased LPS-stimulated TNF-α production in all four experiments.
*Conclusion:* $CO_2$ pneumoperitoneum modulates the immune system independent of the vagus nerve and the cholinergic pathway.

**Key words:** Carbon dioxide — Vagus nerve — Cholinergic — Sepsis — Pneumoperitoneum — Minimally invasive surgery

Laparoscopic surgery has gained increased popularity during the past 20 years due to decreased postoperative pain, decreased tissue trauma, shorter length of stay, faster recovery period, more rapid return to oral intake, and the reduced likelihood of adhesion formation following minimally invasive surgery [4, 16]. Carbon dioxide is the ideal gas for laparoscopic surgery because it is colorless, stable, buffered in the blood, eliminated by the lungs, there is a low risk of venous gas embolism, and it is nonflammable/nonexplosive [17, 22]. Because $CO_2$ pneumoperitoneum can cause acid–base changes [10] that have the potential to produce deleterious physiological side effects on the cardiopulmonary system, helium has been proposed as an alternative gas [18]. However, because helium pneumoperitoneum carries an increased risk of gas embolism [20], and because most of the acid–base changes seen with $CO_2$ are ameliorated with increased minute ventilation [11, 12], helium has fallen out of favor in clinical practice.

Evidence suggests that pneumoperitoneum is protective via two mechanisms. Evidence for a specific biologic effect of $CO_2$ pneumoperitoneum includes the

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ability of $CO_2$ to attenuate the lipopolysaccharide (LPS) and cecal ligation and puncture-induced acute phase inflammatory response [2, 3, 12, 14]. Moreover, we have shown that $CO_2$ pneumoperitoneum increases survival in an animal model of sepsis [13]. The mechanical distension of pneumoperitoneum also has some immunoprotective properties because insufflation with any gas has been shown to decrease production of proinflammatory cytokines following LPS stimulation [13]. The goal of this study was to characterize the mechanical portion of these immune-attenuating effects.

Abdominal insufflation leads to distention of the peritoneal cavity and increased intraabdominal pressure, which can produce hemodynamic alterations [1, 5, 9, 15]. Electrical stimulation of the vagus nerve has been shown to decrease LPS-stimulated tumor necrosis factor-α (TNF-α) production and prevent the development of endotoxic shock [6]. The protection offered through electrical stimulation of the vagus nerve has been coined the "cholinergic anti-inflammatory pathway" and entails stimulation of the vagus nerve (efferent signal) causing tissue release of acetylcholine, which binds specifically to nicotinic $\alpha_7$ subunit receptors on macrophages, which in turn inhibit cytokine production [23]. Communication to the central nervous system through the vagus nerve is bidirectional, and both efferent and afferent signals play a major role in maintaining equilibrium during stress and/or inflammation [19, 23, 24].

In this study, our goal was to investigate the mechanical mechanism of pneumoperitoneum-mediated attenuation of the inflammatory response and determine if the insufflation of laparoscopic surgery creates sufficient intraabdominal distention to stimulate the abdominal vagus branches and activate the cholinergic anti-inflammatory pathway.

## Materials and methods

### *General procedures*

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing approximately 250–350 g were housed in plastic cages in which standard chow and water were available ad libitum. Animals were acclimatized to their environment for 3–5 days following arrival and were then fasted for 16 h prior to procedures. The animal housing environment maintained a 12-h light/dark cycle, a temperature of 72°F, and humidity between 30 and 70%. All surgical procedures were performed under aseptic conditions. Prior to surgery, rats were preanesthetized rapidly in a 15 × 15-in. glass jar using vaporized isoflurane (IsoFlo, Abbot Laboratories, North Chicago, IL, USA). Spontaneously breathing anesthetized animals were placed supine and restrained with adhesive tape. Anesthesia was maintained by delivering 2.5% vaporized isoflurane through a nose cone. Following the completion of the experiment, all rats were killed via anesthetic overdose. All animal procedures were performed using a protocol approved by Johns Hopkins Medical Institution Animal Care and Use Committee.

### *Chemical vagotomy experiments*

For all protocols described here, LPS *Escherichia coli* serotype 026 (Sigma–Aldrich, St. Louis, MO, USA) was used. Atropine (Sigma–Aldrich) was dissolved in sterile saline and a bolus dose was administered intraperitoneally. Pneumoperitoneum pressure was 4 mmHg unless otherwise indicated. In all protocols, blood samples were collected via cardiac puncture 1.5 h after LPS injection, and plasma was isolated for the detection of cytokines using a rat enzyme-linked immunosorbent assay kit (Biosource, Camarillo, CA, USA). Figure 1 conceptualizes the master design of the entire study.

In experiment 1, rats ($n = 68$) were randomized to receive atropine 1 mg/kg or saline injection via a bolus intraperitoneal injection 10 min prior to randomization into four subgroups: LPS only control, anesthesia control, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum. The LPS only control group received anesthesia for just long enough to administer LPS ( < 5 min). The remaining three groups all received anesthesia for a full 30 min, and the two pneumoperitoneum groups simultaneously underwent insufflation with their respective gases for 30 min. The dose of LPS was 8 mg/kg and was administered as an intraperitoneal injection to all rats.

In experiment 2, rats ($n = 40$) were randomized to receive atropine 2 mg/kg or saline injection via a bolus intraperitoneal injection 10 min prior to randomization into the same four subgroups described in experiment 1. In experiment 2, LPS was administered intravenously through the penile vein (1 mg/kg) at the end of the 30-min treatment, simultaneous to an additional 2 mg/kg dose of atropine.

In experiment 3, rats ($n = 96$) received atropine 2 mg/kg or saline 10 min prior to randomization into eight 30-min treatment subgroups: LPS only control; anesthesia control; $CO_2$ pneumoperitoneum at 4, 8, and 12 mmHg; and helium pneumoperitoneum at 4, 8, and 12 mmHg. LPS (1 mg/kg intravenous via the penile vein) was administered at the end of the 30-min treatment.

### *Surgical vagotomy*

Rats were subjected to bilateral subdiaphragmatic truncal vagotomy ($n = 30$) or sham operation ($n = 28$). The abdominal area of the rat was prepped with 70% isopropyl alcohol. The abdominal cavity was opened by a midline laparotomy. The lower esophagus and stomach were retracted and covered with saline-moistened sterile gauze. Careful dissection with fine microsurgical instruments (Roboz Surgical Instruments, Rockville, MD, USA) was carried out to reach both vagus trunks and minimize unnecessary injury to surrounding tissue and vessels. Once identified with the aid of an operating microscope (Olympus), the anterior and posterior vagus branches were ligated with 4–0 silk and divided with Castroviejo microscissors. In the sham-operated animals, the vagus trunks were identified but not ligated or divided. Skin incisions were sutured with continuous 4–0 polysorb (Vicryl). Postoperatively, animals were housed in plastic cages in which water and standard chow were available ad libitum. Two weeks postoperatively, both vagotomized and sham-operated animals were randomized into four 30-min treatment subgroups: LPS only control, anesthesia control, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum. LPS (1 mg/kg intravenous) was administered at the end of the 30-min treatment.

### *Statistical analysis*

Cytokine data shown are mean ± standard error of the mean (SEM). General differences in cytokine levels among groups were determined using one-way analysis of variance (ANOVA), with multiple pairwise comparisons using the Student–Newman–Keuls method being used to elucidate specific significances in these parameters between groups. Statistical differences between group pairs (e.g., between an atropine-treated animal and its respective saline control) were determined by Student's *t*-test. Differences were considered significant when $p < 0.05$. Analysis was performed using Microsoft Excel (Microsoft, Seattle, WA, USA) and Sigma Stat (SPSS, Chicago, IL, USA) software.

## Results

### *Chemical vagotomy experiments*

#### Experiment 1

Among the saline control animals, the serum TNF-α level of the $CO_2$ pneumoperitoneum subgroup was re-

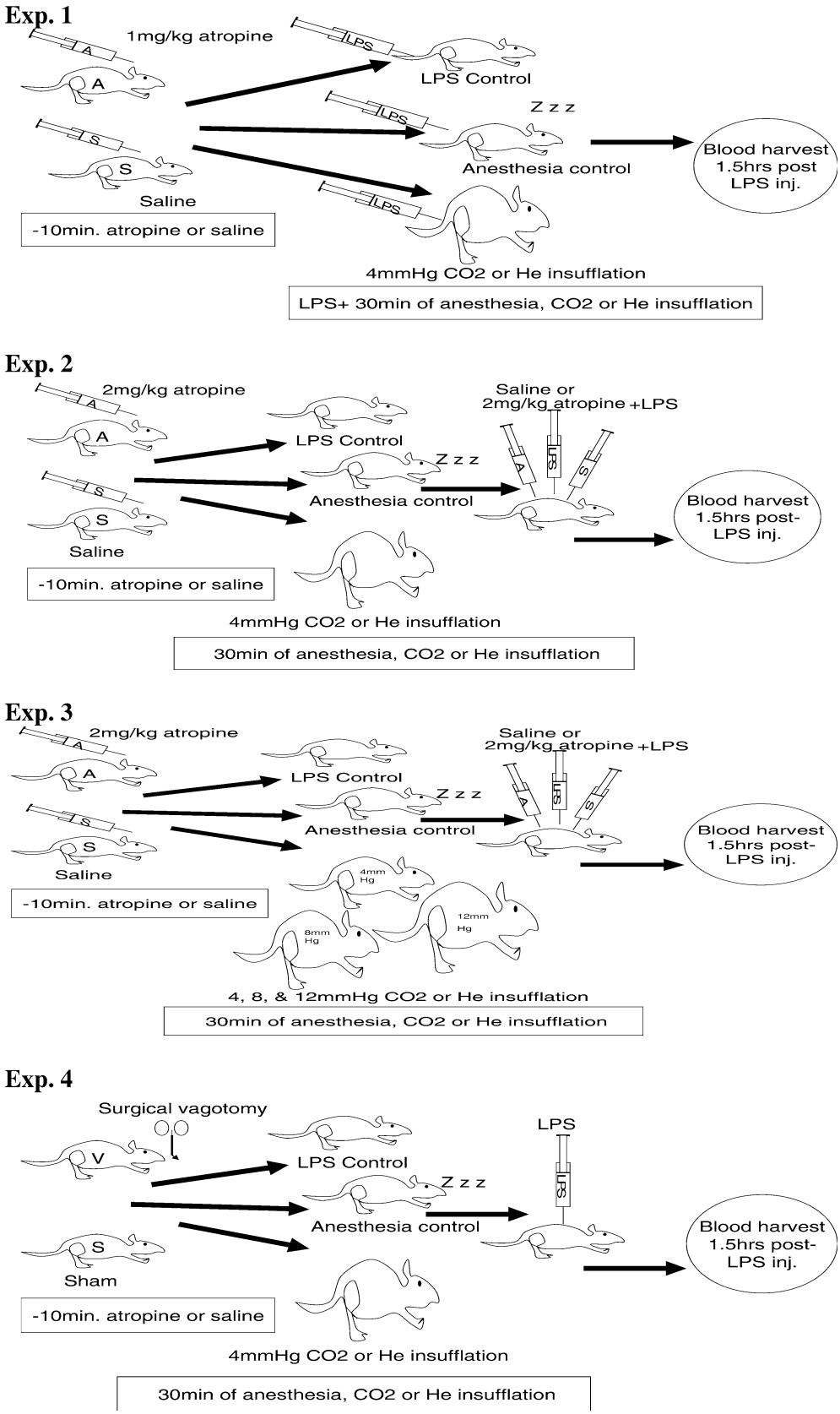


**Fig. 1.** Flow diagram of experiments.

duced by 56.7, 76.5, and 58.5% compared to helium pneumoperitoneum, anesthesia, and the LPS control group, respectively ($p < 0.05$ vs anesthesia and LPS control groups; Fig. 2A). There were no statistically significant differences in the TNF-$\alpha$ levels among the atropine-blocked subgroups; however, except for $CO_2$,

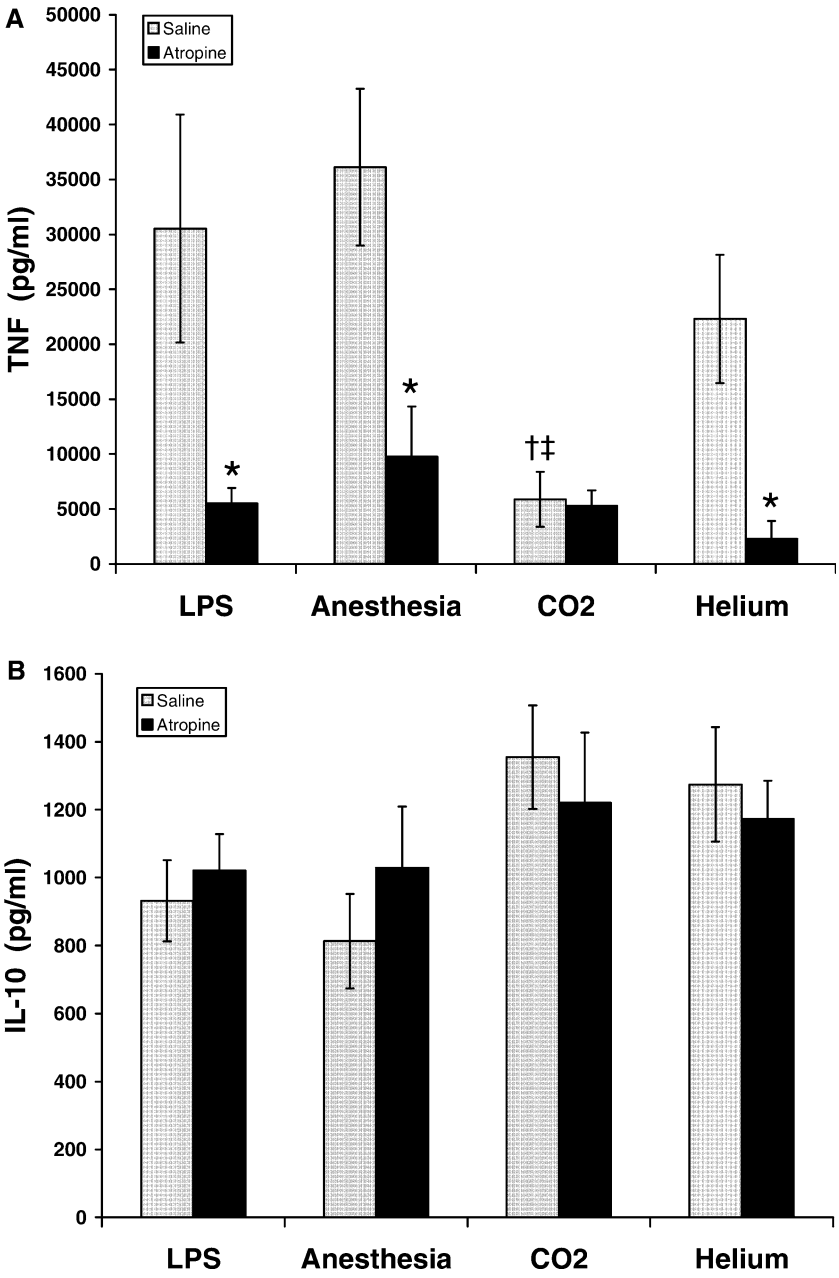


**Fig. 2.** Serum TNF-α (A) and IL-10 (B) levels 90 min following LPS injection among rats ($n = 68$) treated with atropine sulfate or saline 10 min prior to LPS injection. LPS only, anesthesia, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum received an LPS injection simultaneous with 30 min of their respective experimental condition. Error bars represent ± SEM. *$p < 0.05$ versus respective saline counterpart by $t$-test; †$p < 0.05$ versus saline LPS control and ‡$p < 0.05$ versus saline anesthesia by ANOVA.

each was lower than its respective saline counterpart ($p < 0.05$). Only among the $CO_2$-treated animals were the TNF-α levels similarly depressed for both atropine- and saline-treated animals. Interleukin (IL)-10 levels were similar between treatment groups and between atropine- and saline-treated pairs (Fig. 2B).

## Experiment 2

To ensure adequate atropine-mediated vagal blockade in our model, we performed a similar experiment using a higher dose and redosing of atropine. Serum TNF-α levels of the saline-treated $CO_2$ pneumoperitoneum animals were reduced by 81, 78, and 91% compared to saline-treated helium pneumoperitoneum, anesthesia, and LPS control groups, respectively (Fig. 3). Among the saline-treated animals, the anesthesia, $CO_2$, and helium pneumoperitoneum groups had significantly lower TNF-α levels compared to LPS only ($p < 0.05$ for all). Again, all of the atropine-treated subgroups had TNF-α levels significantly lower than their respective saline-treated counterparts ($p < 0.05$), except for the $CO_2$ pneumoperitoneum pair.

## Experiment 3

To ensure adequate vagal stimulation in our model, we tested different intraabdominal pressures. Among saline-treated animals, $CO_2$ pneumoperitoneum at any pressure significantly decreased TNF-α levels compared to both anesthesia and LPS only ($p < 0.05$ for all; Fig. 4). Among $CO_2$-insufflated animals, levels of

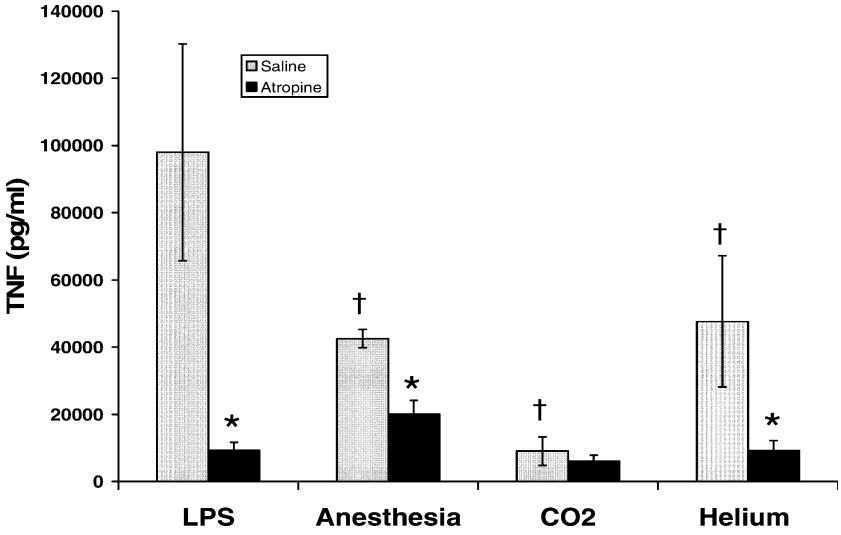


**Fig. 3.** Serum TNF-$\alpha$ levels 90 min following LPS injection among rats ($n = 40$) treated with atropine sulfate or saline 10 min prior to randomization into LPS only, anesthesia, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum, with the former three receiving 30 min of their respective experimental condition. After 30 min, the latter three received LPS and a second dose of atropine. Error bars represent ± SEM. *$p < 0.05$ versus respective saline counterpart by $t$-test; †$p < 0.05$ versus saline LPS control by ANOVA.

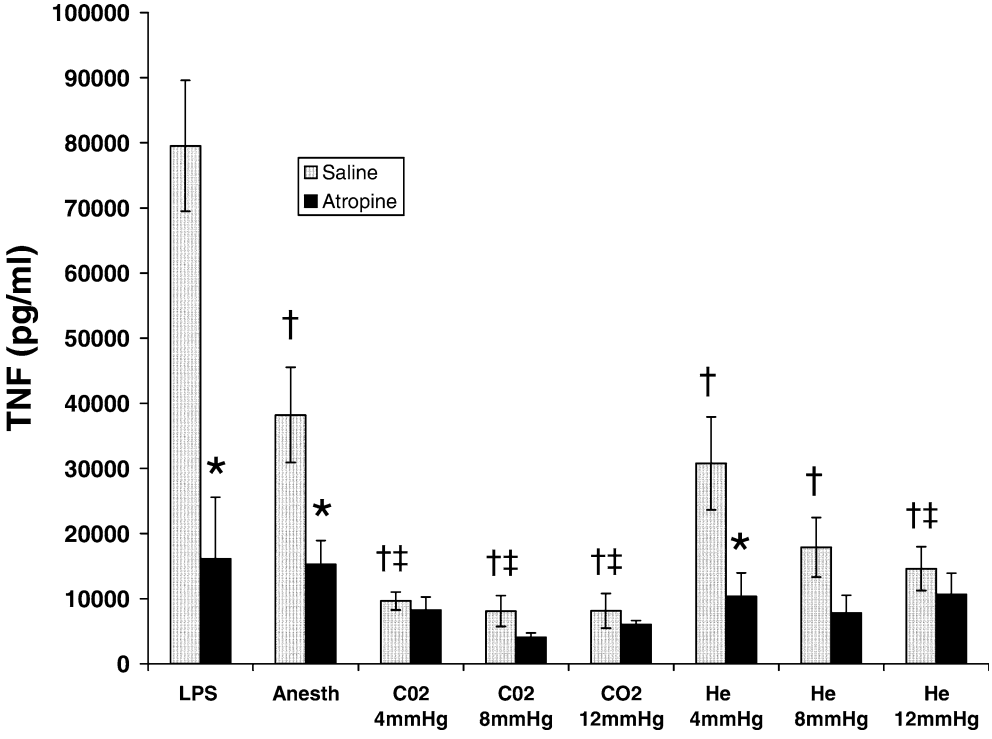


**Fig. 4.** Serum TNF-$\alpha$ levels 90 min following LPS injection among rats ($n = 96$) treated with atropine sulfate or saline 10 min prior to randomization into LPS only, anesthesia for 30 min, 4, 8, and 12 mmHg of $CO_2$ pneumoperitoneum, and 4, 8, and 12 mmHg of helium pneumoperitoneum for 30 min, after which all animals received LPS. Error bars represent ± SEM. *$p < 0.05$ versus respective saline counterpart by $t$-test; $^{\dagger}p < 0.05$ versus saline LPS control and $^{\ddagger}p < 0.05$ versus saline anesthesia by ANOVA.

TNF-$\alpha$ were not significantly different compared to their saline counterparts, even with increasing insufflation pressures. However, among the helium pneumoperitoneum-treated animals, the difference between the saline- and atropine-treated subgroups that was present at 4 mmHg (with the saline-treated level being higher; $p < 0.05$) was abolished at 8 and 12 mmHg. Furthermore, among saline-treated animals, insufflation with 12 mmHg helium pneumoperitoneum produced TNF-$\alpha$ levels significantly lower than anesthesia alone. Significant differences were again found between the saline-treated LPS control and anesthesia control groups compared to their atropine-treated counterparts ($p < 0.05$ for both).

*Surgical vagotomy*

To validate our findings in chemically vagotomized animals, we repeated our experiment in a model of surgical vagotomy. Serum TNF-$\alpha$ levels of the saline-treated $CO_2$ pneumperitoneum subgroup were reduced by 72, 82, and 75% compared to helium pneumoperitoneum, anesthesia, and the LPS control group, respectively (trend only, $p = 0.06$; Fig. 5A). No difference between the saline- and atropine-treated animals was found for any of the groups. Furthermore, IL-10 levels were also similar among the saline- and atropine-treated counterparts (Fig. 5B), except among the LPS only control animals, in which the atropine-treated level was higher ($p < 0.05$).

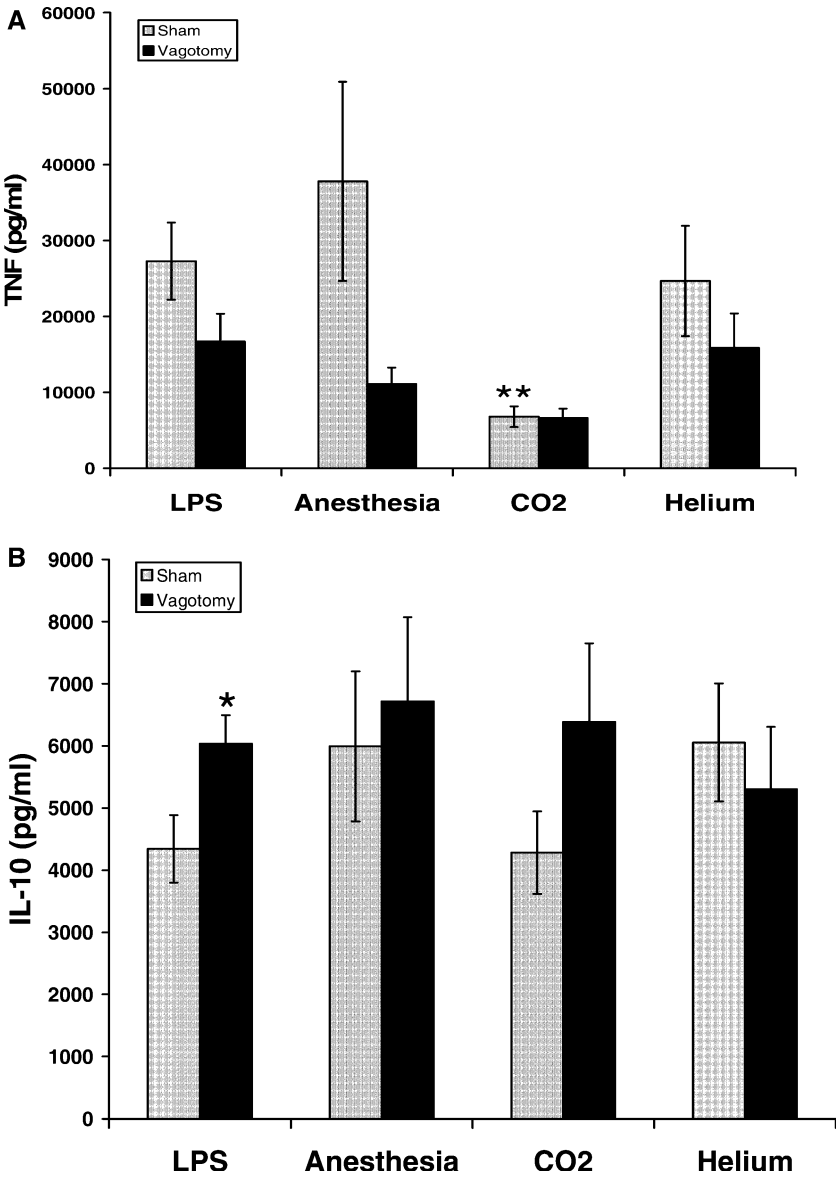


**Fig. 5.** LPS-stimulated serum TNF-α (A) and IL-10 (B) levels among surgically vagotomized or sham-operated rats ($n = 58$). Two weeks postoperatively, animals were randomized into LPS only, anesthesia, $CO_2$ pneumoperitoneum, and helium pneumoperitoneum, with the latter three receiving 30 min of their respective experimental condition, followed immediately by LPS administration. Error bars represent ± SEM. *$p < 0.05$ versus respective saline counterpart by *t*-test; **$p < 0.06$ versus saline LPS only by ANOVA.

## Discussion

Our results reject our original hypothesis that laparoscopic pneumoperitoneum distention stimulates abdominal vagus nerve branches and therein attenuates the inflammatory response via activation of the cholinergic anti-inflammatory pathway [23]. Through this mechanism, we had hypothesized that laparoscopic surgery provides protection against sepsis and injury, specifically by decreasing TNF-α production. The idea was that blocking the cholinergic pathway with atropine sulfate or surgical vagotomy would no longer enable the mechanical distention caused with gas insufflation to stimulate vagal branches. On the contrary, we have shown that in both a chemical and a surgical model, no difference in TNF-α or IL-10 production occurs among the $CO_2$ pneumoperitoneum animals between the vagotomized and nonvagotomized subgroups (Figs. 2–5). In contrast, the atropine-treated LPS control, anesthesia, and helium pneumoperitoneum (at 4 mmHg) groups had significantly decreased TNF-α production compared to their saline counterparts. However, at pressures $>4$ mmHg, the difference in TNF-α production between the vagotomized and nonvagotomized animals was eliminated. When comparing only atropine- or surgical vagotomy-treated animals, no differences in TNF-α production were found in any of the experiments conducted.

We have previously shown that $CO_2$ insufflation attenuates TNF-α production and increases survival, whereas helium pneumoperitoneum has modest attenuating effects on TNF-α levels, but without increasing survival in a rat model of sepsis [13]. The decrease in TNF-α levels seen with $CO_2$ and helium pneumoperitoneum suggests that the common denominator between both is the pressure exerted in the peritoneal cavity.

In our study, cholinergic blockade with 1 mg/kg of atropine sulfate and an even greater vagal blockade with up to 4 mg/kg inhibited TNF-α production in all groups regardless of other experimental conditions (e.g., anes-

thesia and helium pneumoperitoneum). Furthermore, our surgically vagotomized animals also uniformly yielded low TNF-α levels. Thus, we have shown that vagal blockade through two different mechanisms—chemically with atropine and surgically with vagotomy—uniformally suppresses LPS-induced TNF-α production.

The anti-inflammatory effect of chemical vagotomy with atropine is a novel finding. The fact that this effect was consistent with different means of vagotomy makes the results even more convincing. However, it should be noted that our results with respect to surgical vagotomy are somewhat inconsistent with previous reports in the literature. Borovikova et al. [6] have shown that surgical vagotomy increases LPS-stimulated TNF-α production compared to sham-operated animals. The discrepancy could be due to different experimental conditions, such as different surgical vagotomy techniques, different postvagotomy recovery time prior to experimentation, different timing in blood harvesting, and differences in the dose of LPS used.

Regarding intraabdominal pressure, 4 mmHg helium pneumoperitoneum in saline-treated animals yielded consistently higher TNF-α levels in atropine-treated animals. However, at pressures of 8 and 12 mmHg, the TNF-α levels from the saline-treated helium pneumoperitoneum animals were reduced to such a degree that they became similar to those of their atropine-treated counterparts. These findings suggest that vagally mediated attenuation of the inflammatory response may occur among helium-insufflated animals at higher pressures.

With respect to $CO_2$-mediated attenuation of TNF-α production, our findings are consistent with those of our previous work [3, 13]. Insufflation with $CO_2$ was the only experimental group that consistently yielded similar TNF-α levels between saline- and atropine-treated animals. The strong anti-inflammatory properties of $CO_2$ suppressed TNF-α by such a great degree that any additional inhibitory effect of atropine or surgical vagotomy was unappreciable. Furthermore, any increasing suppression of TNF-α production that might be expected with greater intraabdominal pressure was also undetectable given the strong suppression by $CO_2$.

One of the limitations of our study was that we did not measure actual efferent vagal activity. Future work should be conducted to confirm that pneumoperitoneum—possibly in synergy of certain tissue manipulation maneuvers—does actually stimulate the intraabdominal vagal branches. Gastric fundoplication and other interventions in which vagal branches are actively manipulated should be studied and efferent vagal activity measured. Furthermore, the role of the sympathetic system in laparoscopic surgery should be explored. Additional research will focus on the potential role of $CO_2$ pneumoperitoneum as a treatment modality in surgical patients critically ill with sepsis.

Since its introduction, many of the benefits of laparoscopy have been attributed to decreased tissue trauma as a result of smaller incisions [21]. A number of studies have been conducted to establish which gas is ideal for pneumoperitoneum [7, 8, 17, 18, 22]. In general, $CO_2$ has been accepted as the ideal gas; however, many of the advantages of gas $CO_2$ pneumoperitoneum have only recently been appreciated. In addition to facilitating visualization of the operative field in a closed surgical procedure, $CO_2$ pneumoperitoneum has the added benefit of being a potent beneficial immunomodulatory agent.

In conclusion, our data suggest that the pneumoperitoneum of laparoscopic surgery has anti-inflammatory properties in sepsis that are independent of vagal stimulation. Furthermore, we have confirmed that the $CO_2$ of pneumoperitoneum is, by itself, a potent anti-inflammatory agent. Finally, blocking muscarinic receptors via atropine treatment profoundly suppresses TNF-α production regardless of other experimental variables. Our results support the broad use of laparoscopy in general surgery, specifically in trauma and septic patients. Laparoscopy may be the preferred approach to surgical treatment and diagnosis in critically ill patients.

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