Immune Response to Laparoscopic Bowel Injury

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ABSTRACT

Background and Purpose: Laparoscopic bowel injuries are rare but potentially fatal if recognition is delayed. Unlike the situation after open surgery, patients with unrecognized bowel injury after laparoscopy do not present with the typical “acute surgical abdomen.” We investigated monocyte, neutrophil, and lymphocyte apoptosis as indicators of the immune response and whether this response is stimulated or suppressed by laparoscopic bowel injury compared with bowel injury induced during open surgery.

Materials and Methods: After an animal protocol was approved, laparoscopy was performed in a rabbit model. A total of 44 animals were divided into four groups of 11 rabbits each. Laparoscopic bowel injury was created using 30-W electrocautery at 0 (control), 1, and 5 hours after induction of pneumoperitoneum. Bowel injury was created in the fourth group during open laparotomy. Animals were euthanized at 0, 1 day, 1 week, or 2 weeks after surgery. Apoptosis was assessed by staining the nuclei of blood cells with H-33342 dye.

Results: At 1 week, neutrophil, monocyte, and lymphocyte apoptosis levels were 2.4- to 5-fold lower after laparoscopy (1-hour pneumoperitoneum) compared with open surgery. However, at 2 weeks, the percentage of apoptosis had equalized in the two groups. Interestingly, with longer laparoscopic procedures (5 hours), the percentage of apoptosis at 0 and 1 day more closely approached that seen after open surgery. At 2 weeks, there was a significant difference in apoptosis levels in all cell types between the experimental groups compared with controls (P < 0.001). No animals undergoing a 5-hour open procedure survived to 2 weeks after bowel injury.

Conclusions: Open surgery resulted in a significant increase in programmed cell death compared with controls in the immediate postoperative period following bowel injury. Laparoscopic surgery produced a delayed response and after 2 weeks with bowel perforation approached open surgery levels. The difference in the degree of cellular death may be secondary to a smaller degree of stimulation of the immune response in laparoscopic surgery.

INTRODUCTION

OVER THE PAST DECADE, laparoscopic surgery has been used increasingly and is becoming the preferred approach for treatment of several abdominal diseases. There is a significant learning curve with laparoscopic surgery. With increasingly complex procedures being performed, there are more complications. Although laparoscopic bowel injuries are rare, with an incidence of 0.07% to 0.7%, they are potentially fatal when their recognition is delayed.1 Bowel injury is a potential complication of any abdominal or retroperitoneal surgical procedure. Many of these complications involve inadvertent contact of energy sources with organs in the pelvis.

In laparoscopic cases, the traditional signs and symptoms of bowel injury can manifest differently from those observed after open surgery.1 Patients with unrecognized laparoscopic bowel injury present with low fever and leukopenia (white blood cell counts of 1000 to 4000). Furthermore, ileus, abdominal pain, and nausea and vomiting are uncommon complaints. Whereas patients have generalized discomfort with open surgery, the consistent initial presenting complaint with unrecognized bowel injury after laparoscopy is extreme pain.
near the trocar site closest to the injury, although no purulence or erythema is noted at the trocar site. This presentation differs from the typical “acute surgical abdomen” in which the patient presents with abdominal distention, abdominal pain, and peritoneal signs. One abnormal blood test in addition to the decreased white blood cell count is the elevation in immature polymorphonuclear cells (PMN). While this bandemia represents an elevation in the total number of cells, the function of these cells may be compromised because of their immaturity. Thus, they may be unable to fight infection or manifest an appropriate response to injury. Evidence from animal studies has demonstrated that macrophage apoptosis, as well as factors such as chronic treatment with morphine, can inhibit macrophage migration. Likewise, laparoscopic surgery may result in insufficient activation of macrophages that would fail to counter an inadvertent bowel injury.

We hypothesized that one potential cause for the difference, and less clinically obvious, presentation of laparoscopic bowel injury is the blunting of the stress-related immune response associated with laparoscopy, as opposed to the more vigorous immune response induced by open surgery. The severity of surgical injury is known to be a major determinant of the intensity of the postoperative immune reaction. Evidence for a diminished immune response has come mainly from several studies showing lower serum concentrations of the cytokine interleukin-6 (IL-6) in patients undergoing laparoscopic surgery compared with those having open surgery. However, not all studies on postlaparoscopy IL-6 have found such a difference. Thus, there is a need to investigate the effect of laparoscopy on activation of the immune response on a cellular level, rather than simply looking at comparative cytokine concentrations.

Neutrophil apoptosis is one measure of the degree of immune reaction at the cellular level. Neutrophils are involved in the immune response after traumatic injury as well as in the acute inflammatory reaction to infection. These cells migrate to the area of insult and degranulate, releasing oxygen-derived free radicals that destroy the offending agent. Apoptosis, or programmed cell death, provides a mechanism for deactivation of neutrophils and is postulated to be a key mechanism in resolution of the inflammatory response. The role of macrophages in this scenario is to clear apoptotic neutrophils, allowing their membranes to remain intact and preventing leakage of proinflammatory mediators, which would further promulgate the immune cascade.

The basic aims of this study were to investigate neutrophil, monocyte, and lymphocyte apoptosis and to determine the extent to which these processes occur in laparoscopic compared with open surgical bowel injury. We hypothesized that laparoscopy would result in more cellular apoptosis than open surgery, providing a clue to the reason for the diminished immune response following laparoscopy and the delayed and understated clinical presentation of laparoscopic bowel injury.

**MATERIALS AND METHODS**

Forty-four New Zealand white rabbits weighing 3 to 3.5 kg were acclimated to a climate- and light cycle-controlled environment for at least 7 days prior to surgery. The animals were allowed standard laboratory food, which was withheld only on the night prior to surgery. Ketamine at 0.5 mg/kg intramuscularly was given prior to general anesthesia.

The rabbits were randomized into four groups of 11 each. In three groups (N = 33), laparoscopy was performed, and bowel injury was created at 0, 1, or 5 hours after induction of pneumoperitoneum. The bowel injury in the fourth group was created during open laparotomy.

**Surgical technique**

In the three laparoscopy groups, pneumoperitoneum was induced in standard fashion using a Veress needle. Once an adequate pneumoperitoneum of 4 to 6 mm Hg was established, a 5-mm trocar was placed. A 10-mm laparoscope was then used to place a second 5-mm trocar under direct vision. Pneumoperitoneum was maintained for a period of 0, 1, or 5 hours. At the end of the specified time, 30-W monopolar electrosurgery was applied to the serosa of the bowel to create a superficial thermal injury (Fig. 1).

Following release of pneumoperitoneum, the rabbits were allowed to recover. Animals were euthanized at 0 days, 1 day, 1 week, or 2 weeks after surgery. At the time of sacrifice, 40 mL of peripheral blood was collected from each animal in heparinized tubes in order to conduct the apoptosis studies. The rabbits that were sacrificed at 0 days were euthanized under the same anesthetic as the surgical procedure.

A laparotomy with creation of a bowel injury using 30-W monopolar electrocautery was performed in 10 of the 11 rabbits in the open surgery group in a fashion analogous to the technique used in the laparoscopy groups. The injuries occurred at 0, 1, or 5 hours after laparotomy. The incisions were then closed and the animals allowed to recover. In the eleventh rabbit, no laparotomy or bowel injury was performed. Instead, a 2-inch incision was made deep into the abdominal musculature without entry into the abdominal cavity (control). This incision was closed and the animal allowed to recover. The animals were euthanized on a schedule similar to that used in the laparoscopy groups, and 40 mL of peripheral blood was drawn at that time for the apoptosis studies.

**Cell separation**

The blood collected was transferred to 15-cc sterile tubes containing separate layers of Histopaque 1119 and 1077 solu-

**FIG. 1.** Induction of injury to bowel serosa with 30-W monopolar electrocautery.
tions (Sigma) and spun at 800 × g for 30 minutes at 18°C. The mononucleocyte buffy-coat layer, containing both monocytes and lymphocytes, and the granulocyte buffy-coat layer, containing neutrophils, were aspirated and transferred to separate tubes. Subsequently, the cells were washed and spun for 5 minutes.

Apoptosis studies

By staining cells with H-33342 (Molecular Probes, Portland, OR) and propidium iodide (PI; Sigma-Aldrich), morphologic evaluation of monocyte, lymphocyte, and neutrophil apoptosis was performed. H-33342 stains the nuclei of live cells and identifies apoptotic cells by increased fluorescence and fragmented nuclei. Cells were prepared from the blood of animals in the control, laparoscopic, and open surgery groups. After 45 minutes of incubation, cells were treated with H-33342 (1 μg/mL) for 10 minutes and again incubated at 37°C. Subsequently, PI (final concentration 1 μg/mL) was added to each well. The percentage of live, apoptotic, and necrotic cells were recorded in eight random fields by two observers unaware of the experimental conditions (Fig. 2).

Statistical analysis

The unpaired t-test was used for comparison of values between groups. ANOVA was applied and a Neuman-Keuls multiple range test was used to calculate the P value and compare values in multiple groups. Statistical significance was defined as P < 0.05.

RESULTS

Neutrophil apoptosis

Neutrophil apoptosis was higher in the experimental groups than in the controls. In the 1-hour group, a significant increase in apoptosis was seen in the open laparotomy group compared with the laparoscopy group at 1 day and 1 week after surgery. Interestingly, the degree of apoptosis in the laparoscopic cohort was 50% that of open surgery at 1 day and 1 week. However, at 0 days and 2 weeks after surgery, the results in the two groups were similar (Fig. 3A).

In the 5-hour group, neutrophil apoptosis was significantly higher in the open surgery group than in the laparoscopy group at 1 week after surgery. No statistical difference was seen between the two groups at 0 and 1 day after surgery. None of the animals in the 5-hour group survived 2 weeks (Fig. 3B). At all measured time points, the laparoscopy group demonstrated more apoptosis than controls.

Lymphocyte apoptosis

In the 1-hour group, lymphocyte apoptosis was twofold to threefold higher in the open surgery group than in the laparos-
copy group at 1 day and 1 week after surgery. At 0 days, apoptosis was higher in the laparoscopy group than after open surgery. By the end of 2 weeks, no statistical difference was seen between the two experimental groups (Fig. 4A). In the 5-hour group, however, apoptosis was significantly higher in the laparoscopy group than in the open surgery group at 0, 1 day, and 1 week after surgery (Fig. 4B).

**Monocyte apoptosis**

In the 1-hour group, monocyte apoptosis was twofold to fivefold times higher in the open surgery group at 1 day, 1 week, and 2 weeks after surgery. At 0 days, apoptosis was higher in the laparoscopy group than in the open surgery group (Fig. 5A). In the 5-hour group, as in the lymphocyte study, apoptosis was significantly higher in the laparoscopy group at 0, 1 day, and 1 week after surgery (Fig. 5B).

**DISCUSSION**

Apoptosis is a form of physiologic cell death. The apoptotic process, as opposed to necrosis or toxic cell death, involves a series of coordinated morphologic changes in the affected cell making it more susceptible to ingestion by scavenger phagocytes. Characteristic features of apoptosis include membrane blebbing, chromatin condensation, DNA fragmentation, and formation of structures called apoptotic bodies. Apoptosis is observed in immature T cells on T-cell receptor stimulation, irradiated lymphocytes, cytotoxic T-lymphocyte target cells, metamorphosing tadpole tails, and regressing tumors. It is also seen in sepsis, malignant tumors, and surgical stress. As cells proceed to apoptosis, their functional activity declines.

Oka and associates studied the effects of surgical stress on lymphocyte apoptosis by taking blood samples from patients undergoing elective surgery. Their findings showed that cells committed to apoptosis appeared at 2 hours, peaked at 24 hours, and decreased by the 7th day after surgery.

Nishiguchi and associates compared the effects of surgical stress caused by laparoscopy-assisted and open colonic resection for colorectal carcinoma. Peripheral blood samples were obtained from each patient on preoperative day, 6 hours after the start of surgery, and 1, 2, 3, 5, and 7 days after surgery to determine lymphocyte apoptosis. No significant differences were seen between the open and laparoscopic groups in the apoptotic index at any of the time points. However, when these levels were compared with those in patients who underwent major open surgical procedures such as esophagectomy and gastrectomy, apoptosis was increased until 3 days after surgery in the latter group and was higher than in the laparoscopic or open surgery groups at 1, 2, 3, and 5 days after surgery.

Our study clearly shows that surgery, whether laparoscopic or conventional open, causes peripheral white blood cells to undergo apoptosis. However, in the 1-hour group, lymphocyte apoptosis was higher by two to three times in the open surgery group than in the laparoscopy group at 1 day and 1 week. This finding indicates that open surgery may induce substantial apoptosis of peripheral lymphocytes compared with laparoscopy. Some studies have shown that lymphocytes are susceptible to Fas-mediated apoptosis in the early postoperative period.

**FIG. 4.** Mean lymphocyte apoptosis in control (■), laparoscopy (□), and open surgery (■) groups injured at 1 hour (A) and 5 hours (B). *P < 0.05.

**FIG. 5.** Mean monocyte apoptosis in control (■), laparoscopy (□), open surgery (■) groups injured at 1 hour (A) and 5 hours (B). *P < 0.05.
Aside from the effects of tissue trauma, the presence of intraperitoneal infection and eventual sepsis may also play a role in lymphocyte apoptosis. All the animals subjected to electrocautery injury of the bowel had a perforation at the time of sacrifice, and spillage of bowel contents was evident in the animals that survived 1 week and longer. The effects of infection on apoptosis are vital in the clinical setting because premature, increased, or accelerated lymphocyte apoptosis may decrease the ability of the body to clear infection, resulting in less-effective mechanisms of host defense. Alternatively, apoptosis may be a marker of severe inflammation.

In the 5-hour group, lymphocyte apoptosis in the experimental groups was elevated compared with controls. However, it was significantly higher in the laparoscopy group than in the open surgery group. Interestingly, none of the animals that were subjected to a 5-hour open procedure survived 2 weeks, whereas the animals that sustained the same bowel injury during laparoscopy were able to survive to 2 weeks. Because apoptotic cells are rapidly phagocytosed in vivo, it is possible that the circulating apoptotic cells in the open surgery group were eliminated by phagocytosis more rapidly than those in the laparoscopy group. This may explain the higher level of apoptosis seen in the 5-hour laparoscopy group.

Among the leukocytes, neutrophils are the first immune cells to migrate toward sites of inflammation. Neutrophils have the shortest life span, dying via apoptosis within 72 hours. It has also been shown that neutrophils undergoing apoptosis are functionally ineffective and have poor microbicidal capability.

Neary and colleagues compared neutrophil apoptosis before and after open surgery and found a significant increase in systemic neutrophil apoptosis after surgery. Their results demonstrated that injury programs proinflammatory cells for early death in the immediate postoperative period. Similarly, Deloug and associates studied the effects of elective surgery under general anesthesia on circulating neutrophils. They observed that at 12 hours after surgery, neutrophil apoptosis was significantly higher than at baseline but returned to preoperative values by 24 hours after surgery. Those investigators concluded that open surgery triggers accelerated apoptosis in the immediate postoperative period. In another experimental study, Jacobi et al compared systemic inflammation after open surgery and laparoscopy in a murine model of peritonitis. They measured tumor necrosis factor (TNF-α) plasma concentrations instead of apoptosis and demonstrated that TNF-α increased 1 hour after fecal inoculation in both open surgery and laparoscopy groups. However, TNF-α showed significantly higher values in the open surgery group than in the laparoscopy group. In our study, neutrophil apoptosis was higher after bowel injury in open surgery than at 1 day and 1 week after laparoscopy. In contrast to the studies mentioned, we noted a persistent elevation of neutrophil apoptosis even after 2 weeks. Again, this may be an effect of the inflammation created by bowel injury.

Watson and coworkers documented the effects of exposure of the peritoneal cavity to air during open laparotomy and compared it with carbon dioxide insufflation in the murine model. That study demonstrated that air contamination of the peritoneal cavity induces lipopolysaccharide, an endogenous endotoxin, to translocate from the gastrointestinal tract into the peritoneal cavity, a phenomenon not seen during carbon dioxide laparoscopy. Bacterial translocation may induce a systemic inflammatory response and multiple organ dysfunction. In our open surgery model, it is possible that more cells were noted to be apoptotic as a result of longer exposure to air, inducing bacterial translocation and systemic inflammation.

The main function of monocytes in the immune system is to phagocytose infected or altered cells. They are also the main source of TNF and IL-1, which are proinflammatory cytokines responsible for enhanced postoperative systemic inflammation. Monocytes, therefore, play an important role in physiologic defense against bacterial infection. Gutt and associates studied the effects of different surgical techniques on the mononuclear phagocyte system (MPS) using a rat model. They examined MPS by performing an intravascular carbon clearance test during conventional fundoplication, laparoscopic fundoplication with pneumoperitoneum, and gasless laparoscopic fundoplication. Faster carbon clearance was associated with better preservation of phagocytic activity. Carbon clearance was fastest in the gasless laparoscopy group followed by conventional surgery and, lastly, by carbon dioxide pneumoperitoneum. The investigators concluded that pneumoperitoneum results in a significant reduction in the phagocytic activity of the MPS.

The results of our study show that at 5 hours, monocyte apoptosis is higher with laparoscopy than open surgery at 0, 1 day, and 1 week after surgery. On the other hand, monocyte apoptosis is higher with open surgery than with laparoscopy with only 1 hour of pneumoperitoneum. Aside from the effects of tissue trauma from creation of the bowel injury, it seems that a longer exposure to carbon dioxide pneumoperitoneum induces more monocytes to undergo apoptosis.

**CONCLUSION**

Open surgery results in a significant increase in programmed cell death in the immediate period following bowel injury. Laparoscopic surgery produces a delayed response that, after 2 weeks with bowel perforation, approaches that seen after open surgery. The difference in extent of cellular death may be attributable to blunting of the immune response by laparoscopy.

**REFERENCES**


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