Surg Endosc (1998) 12: 1035–1038

Surgical Endoscopy
Ultrasound and
Interventional Techniques

Increased tumor establishment and growth after open vs laparoscopic bowel resection in mice

J. D. F. Allendorf, M. Bessler, K. D. Horvath, M. R. Marvin, D. A. Laird, R. L. Whelan

Department of Surgery, Columbia University College of Physicians and Surgeons and the Columbia Presbyterian Medical Center, 161 Fort Washington Avenue, New York, NY 10032, USA

Received: 27 October 1997/Accepted: 19 January 1998

Abstract

Background: Surgery can suppress immune function and facilitate tumor growth. Several studies have demonstrated better preservation of immune function following laparoscopic procedures. Our laboratory has also shown that tumors are more easily established and grow larger after sham laparotomy than after pneumoperitoneum in mice. The purpose of this study was to determine if the previously reported differences in tumor establishment and growth would persist in the setting of an intraabdominal manipulation.

Methods: Syngeneic mice received intradermal injections of tumor cells and underwent either an open or laparoscopic cecal resection. In study 1, the incidence of tumor development was observed after a low dose inoculum; whereas in study 2, tumor mass was compared on postoperative day 12 after a high-dose inoculum.

Results: In study 1, tumors were established in 5% of control mice, 30% of laparoscopy mice, and 83% of open surgery mice ($p < 0.01$ for all comparisons). In study 2, open surgery group tumors were 1.5 times as large as laparoscopy group tumors ($p < 0.01$), which were 1.5 times as large as control group tumors ($p < 0.02$).

Conclusion: We conclude that tumors are more easily established and grow larger after open laparoscopic bowel resection in mice.

Key words: Laparoscopy — Surgery — Mouse — Tumor establishment — Tumor growth — Mouse mammary carcinoma

Although the clinical benefits of minimally invasive surgery in terms of postoperative pain, length of hospital stay, and time to return to work have been well established [4–7], there may be additional physiological benefits to laparoscopic techniques. Our laboratory has developed several small-animal models to study the immunological and oncological consequences of both laparoscopic and open surgery [3]. In rats, we compared immune function by serial delayed-type hypersensitivity (DTH) testing and demonstrated that cell-mediated immunity is better preserved after pneumoperitoneum than after sham laparotomy through postoperative day 5 [8]. Similar results were observed when rats underwent either an open or laparoscopic-assisted bowel resection [2]. Having documented postoperative differences in immune function and understanding that the immune system has been shown to play an integral role in defense against tumor establishment and proliferation for a number of malignancies, we hypothesized that there may be oncological benefits to minimally invasive surgery. Using a mouse model, we demonstrated that intradermal tumors are more easily established and grow larger after sham laparotomy than after pneumoperitoneum [1]. The goal of the present study is to determine if the differences in tumor establishment and growth observed after sham procedures would persist in the setting of an intraabdominal manipulation.

Materials and methods

Animals

Five- to six-week-old female C3H/He mice (Charles River Laboratories, Wilmington, MA, USA) were used for both experiments. These mice are immunocompetent and syngeneic to the mouse mammary carcinoma tumor line.

All studies were performed under protocols approved by the Columbia University Institutional Animal Care and Use Committee in accordance with FDA regulations. The animals were acclimated to a climate- and light-cycle–controlled environment for ⩾24 h prior to investigations. Mice were fed standard laboratory rodent chow and tap water ad libitum.

Tumor cell line

Both studies involved the use of mouse mammary carcinoma (MMC) cells derived from the MC2 cell line [9]; they were obtained from Dr. J. Vaage

Correspondence to: R. L. Whelan

of the Roswell Park Cancer Institute. Mouse mammary carcinoma is an immunogenic cell line that shows a plateau of maximal growth from 12 to 14 days after tumor cell inoculation. It is syngeneic to the C3H/He mouse strain. At a dose of 10,000 tumor cells (study 1), <10% of control mice are expected to develop a tumor nodule. At a dose of 1,000,000 tumor cells (study 2), >95% of control mice are expected to develop tumors. Eventually, 20% of these tumors are expected to spontaneously regress.

Tumor cell preparation and inoculation

On the day of operative intervention, tumor cells were prepared as a single-cell suspension for intradermal inoculation. MMC cells growing free-floating in RPMI 1640 medium supplemented with 10% fetal calf serum, 150 U/ml penicillin, and 150 mg/ml streptomycin were washed twice, counted, and resuspended in phosphate-buffered saline. In study 1, a suspension of 10^5 cells per ml was prepared, and mice were injected with 0.1 ml, for a total inoculum of 10^4 cells. In study 2, a suspension of 10^7 cells per ml was prepared, and mice were injected with 0.1 ml, for a total inoculum of 10^6 cells. Tumor cell viability was determined to be >95% by trypan blue exclusion.

On the day of intervention, mice were restrained, shaved, and injected in the dorsal skin with 0.1 ml of tumor cell suspension prior to beginning the surgical interventions.

Studies

Study 1: C3H/He threshold-dose tumor inoculum

A total of 115 C3H/He mice received an intradermal inoculation of 10^4 MMC cells on the day of operative intervention. All animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) in a total volume of 0.3 ml. After being anesthetized, the animals were randomly divided into one of three groups:

Group 1. Anesthesia control animals underwent no procedure and were returned to their cages.

Group 2. Laparoscopic cecectomy group mice underwent a laparoscopic-assisted cecal resection. The procedure was performed as previously described [5]. Briefly, the mouse was placed in Trendelenberg position and the abdomen was insufflated to a pressure of 3–5 mmHg with carbon dioxide gas through a 25-gauge cannula placed in the right upper quadrant. A 4-mm rigid laparoscope was inserted through a small incision created in the midline just caudal to the xiphoid, and a 2-mm operative port was created in the right lower quadrant. Under laparoscopic visualization, the cecum, which in mice is 1 to 2 cm in length, was grasped at its end and exteriorized. Extracorporeally, the cecum was ligated just distal to the ileocecal junction. The cecum was then resected and the stump was irrigated before being gently returned to the peritoneal cavity.

Group 3. Open cecectomy group animals underwent a cecal ligation and resection through a 4-cm midline incision. The operative time was standardized to 20 min for both procedures ($n = 40$ for anesthesia control and laparoscopic resection, $n = 32$ for open resection).

Mice were assessed weekly by blinded palpation of the dorsal skin for the presence or absence of a tumor nodule. If a tumor nodule was palpated, the mouse was killed, and the dorsal skin was reflected to confirm the presence of a tumor nodule by direct observation. Weekly observations were necessary so as not to miss tumors that established and regressed in the 1st postoperative month. On postoperative day 30, the remaining mice were killed, the dorsal skin overlying the tumor cell injection site was reflected, and the presence or absence of a tumor nodule was determined by direct observation. Several random specimens were analyzed histologically to confirm the presence of tumor. All data were collected in a blinded fashion.

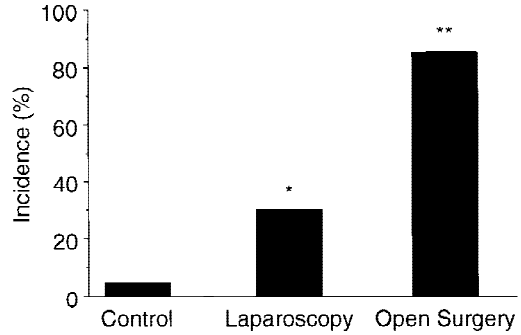

Fig. 1. Incidence of tumor establishment by postoperative day 30 after open versus laparoscopic cecectomy. *$p < 0.01$ versus control and open surgery, **$p < 0.01$ versus control and laparoscopy.

Study 2: C3H/He high-dose tumor inoculum

A total of 112 C3H/He mice received an intradermal inoculation of 10^6 MMC cells on the day of operative intervention. Animals were randomly assigned to one of three groups. As in study 1, anesthesia control mice underwent no procedure, laparoscopic resection group mice underwent a laparoscopic-assisted cecectomy, and open resection group mice underwent a cecectomy through a midline incision. Tumors were excised and weighed on postoperative day 12. This time point was chosen because it represents the beginning of the plateau phase in the normal growth curve of mouse mammary carcinoma. All data were collected in a blinded fashion ($n = 38$ for anesthesia control and open resection, $n = 35$ for laparoscopic resection).

Statistics

In study 1, differences among groups were analyzed for statistical significance by chi-square using Yate's correction for small numbers. In study 2, differences among groups were analyzed for statistical significance by ANOVA, followed by Student's *t*-test to determine *p* values.

Results

Study 1

By postoperative day 30, tumor nodules developed in 5% of control mice, 30% of laparoscopic resection group mice, and 83% of open resection group mice ($p < 0.01$ by chi-square for all comparisons), (Fig. 1).

Study 2

Tumor nodules developed in all mice. Tumor mass on postoperative day 12 showed a stepwise increase from the control group to the laparoscopic resection group to the open resection group. The open resection group tumors were 1.5-fold larger than laparoscopic resection group tumors and more than twice as large as control group tumors ($p < 0.01$ by Student's *t*-test for both comparisons). Laparoscopic resection group tumors were 1.5 times as large as control group tumors ($p < 0.02$ by Student's *t*-test) (Fig. 2). (Control

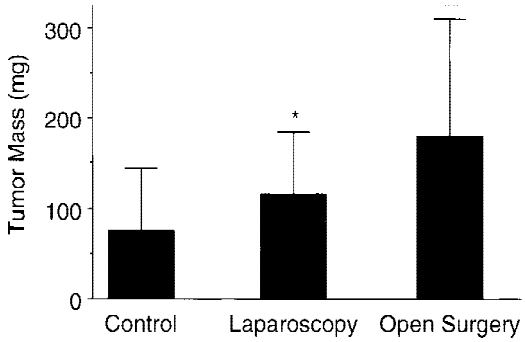

Fig. 2. Tumor mass (±SD) on postoperative day 12 after open versus laparoscopic cecectomy. *$p < 0.02$ versus control, **$p < 0.01$ versus control and laparoscopy.

$= 75 \pm 68$ mg, laparoscopic resection $= 115 \pm 68$ mg, open resection $= 180 \pm 132$ mg.)

Morbidity and mortality

Two mice in both the open and the laparoscopic resection groups were sacrificed intraoperatively for inadvertent injury to the bowel when gaining access to the peritoneum (2.8% vs 2.6% complication rate). There were no leaks from the site of the cecal ligation, and none of the mice died postoperatively.

Discussion

Previous work in our laboratory demonstrated that tumors are more easily established and grow larger after sham laparotomy than after pneumoperitoneum in mice [8]. These initial studies did not involve an intraabdominal procedure. In the current study, we overcame this hurdle by using a newly developed mouse model of laparoscopic bowel resection [5]. Using the same mouse strain and tumor line as in our previous studies, laparoscopic-assisted and open cecectomy were compared.

As shown in Fig. 3, the long cecum of the mouse permits resection of 1–2 cm of bowel without requiring a technically challenging anastomosis to reestablish bowel continuity. The model proved to be safe, reliable, and economical. There were no postoperative leaks and the intraoperative complication rate was similar to that of the open procedure (2.6% vs 2.8%). Using this model, we determined tumor establishment (study 1) and tumor growth (study 2) after open and laparoscopic bowel resection in mice.

In study 1, a threshold dose of tumor cells was injected into the dorsal skin immediately prior to surgical intervention. By postoperative day 30, tumors had developed in 83% of open resection group mice, 30% of laparoscopic resection group mice, and 5% of control group mice ($p < 0.01$ for all comparisons), (Fig. 1). These results are similar to those observed previously after sham interventions; significant differences between the laparoscopic and open groups persisted despite the addition of an intraabdominal procedure. Although not proven by this study, these results suggest that viable tumor cells remaining after resection would be better able to form a metastatic tumor nodule after open resection than after laparoscopic-assisted resection.

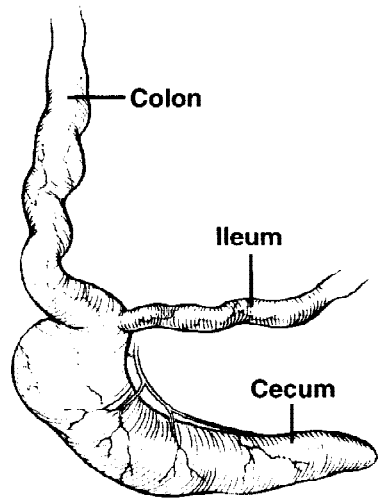

Fig. 3. Normal anatomy of the mouse colon, ileum, and cecum.

In study 2, a high dose of tumor cells was intradermally injected immediately prior to surgical intervention in order to study postoperative tumor growth. As expected, tumors developed in all animals and were excised and weighed on postoperative day 12. Open resection group tumors were 1.5 times as large as laparoscopic resection group tumors ($p < 0.01$), which were 1.5 times as large as control group tumors ($p < 0.02$) (Fig. 2). Despite the addition of an intraabdominal procedure, significant differences between groups persisted. These results suggest that tumor left behind at the time of surgery may grow less rapidly after laparoscopic resection than after open surgery. Thus, studies 1 and 2 demonstrate that the previously reported differences in tumor establishment and growth after sham procedures persisted in the setting of a bowel resection.

While several authors are actively investigating what factors may contribute to the development of tumor nodules at trocar sites, our investigation aimed to understand how systemic postoperative physiology affects tumor establishment and growth. We accomplished this by studying tumor behavior at a site distant from the surgical manipulation. Our model was designed to separate the local effects of carbon dioxide pneumoperitoneum and surgical manipulation from the systemic effects of postoperative physiology. The mechanism of port site recurrence needs to be studied with the understanding that techniques designed to minimize surgical trauma should limit, not enhance, tumor establishment and growth.

We conclude that tumors are more easily established and grow larger after open than after laparoscopic bowel resection in mice. Additional investigation is necessary to assess the behavior of other tumor lines and to identify the factors involved in the mechanism of differential tumor growth.

Acknowledgments. This investigation was made possible by generous support from the Association of Women Surgeons, the Ethicon division of Johnson and Johnson Corporation, and the United States Surgical Corporation.

References

1. Allendorf JDF, Bessler M, Kayton ML, Oesterling SD, Treat MR, Whelan RL (1995) Increased tumor establishment and growth after laparotomy versus laparoscopy in a murine model. Arch Surg 130: 649–653
2. Allendorf JDF, Bessler M, Whelan RL, Trokel M, Laird DA, Terry MB, Treat MR (1996) Better preservation of immune function after laparoscopic-assisted vs. open bowel resection in a murine model. Dis Colon Rectum 39 (Suppl):S67–72
3. Allendorf JDF, Bessler M, Whelan RL (1997) A murine model of laparoscopic-assisted intervention. Surg Endosc 11: 622–624
4. Barkun JS, Barkun AN, Sampalis JS (1992) Randomized controlled trial of laparoscopic versus mini cholecystectomy. Lancet 340: 1116–1119
5. Gadacz TR, Talamini MA (1991) Traditional versus laparoscopic cholecystectomy. Am J Surg 161: 336–338
6. Grace PA, Quereshi A, Coleman J (1991) Reduced postoperative hospitalization after laparoscopic cholecytectomy. Br J Surg 78: 160–162
7. Reddick EJ, Olsen DO (1989) Laparoscopic laser cholecytectomy. Surg Endosc 3: 131–133
8. Trokel MJ, Bessler M, Treat MR, Whelan RL, Nowygrod R (1994) Preservation of immune response after laparoscopy. Surg Endosc 8: 1385–1387
9. Vaage J, Pepin K (1985) Morphological observations during developing concomitant immunity against a C3H/He mammary tumor. Cancer Res 45: 659–666