Laparoscopic colonic resection consisted of the following procedures: 1) mobilization of the colon under CO_2 pneumoperitoneum, 2) mini-laparotomy with a skin incision about 5cm long, 3) division of mesentery, ligation of main vessels, and resection of tumor-bearing colon, 4) end-to-end anastomosis with interrupted layer-to-layer sutures, and 5) observation and irrigation of the peritoneal cavity under re-pneumoperitoneum using Sandwich-disc (12). Sandwich-disc is a device for instant closure of a minilaparotomy and rapid recreation of pneumoperitoneum, and enables easy sealing of the minilaparotomy wound during laparoscopy-assisted surgery.

Body temperature, serum C-reactive protein (CRP) and albumin levels, lymphocyte count, and weight loss during hospital stay were examined by the medical charts. Quality of life was estimated by the questionnaire which was mailed to and answered by the patients in November 1999. The questions consisted of 9 items concerning physical condition, mental condition, and performance status **(Table 1)**. Performance status was determined by the scale outlined by the Eastern Cooperative Oncology Group (13). The answers were analyzed in a quantitative fashion using a scoring system. The scores ranged from 1 indicating an excellent result with no symptoms, to 3 indicating an unsatisfactory result with severe symptoms. Average scores were calculated for each question, and results were expressed as mean ± standard deviation. The differences were statistically analyzed by the Student's *t*-test.

RESULTS

When the patients were compared between laparoscopic and open colonic resections, the mean age (64.0 years *vs.* 63.6 years), male-to-female ratio (1.36 *vs.* 1.05), and operation time (224 min *vs.* 236 min) were not different. However, the tumor size was smaller in the laparoscopic group than in the open group (2.8cm *vs.* 4.4cm, $p<0.05$).

Table 2 shows the clinical results after laparoscopic or open colonic resection. Laboratory data during the hospital stay showed that laparoscopic colectomy was different from open colectomy with regard to the body temperature (37.8°C *vs.* 38.0°C, $p<0.01$) and serum CRP level (6.34mg/dL *vs.* 11.15mg/dL, $p<0.01$) on postoperative day 1, serum albumin level (3.54g/dL *vs.* 3.36g/dL, $p<0.05$) and lymphocyte count (1354/mm^3 *vs.* 995/mm^3, $p<0.01$) on postoperative day 7, and weight loss on postoperative day 14 (2.08kg *vs.* 2.93kg, $p<0.01$; 3.95% *vs.* 5.45%, $p<0.01$).

Nineteen patients (73%) with laparoscopic colectomy and 68 (78%) with open colectomy replied to the questionnaire. **Table 3** shows scores for each question after laparoscopic or open colonic resection. Although all patients with laparoscopic colectomy were satisfied with their surgical results (question No. 6, mean score of 1.00), none of the 9 questions reached a statistically significant difference between the two groups. Both laparoscopic and open colonic resections were similarly accepted by the patients as a good operation that they would recommend to others (1.105 *vs.* 1.206), and total score was not significantly different between the two groups (10.95 *vs.* 11.81).

DISCUSSION

This study clarified that laparoscopic colectomy,

TABLE 1 Scoring System assessing Quality of Life after Colonic Resection

Questions	Answers	Score
1. How is your physical condition?	Good	1
	Fair	2
	Poor	3
2. Do you ever have wound pain?	Never	1
	Sometimes	2
	Often	3
3. How was your wound pain immediately after operation?	Not painful	1
	Painful but well-tolerated	2
	Painful and needed analgesics	3
4. Have you ever visited a doctor because of intestinal obstruction?	Never	1
	Yes, but recovered without surgery	2
	Yes, and was treated surgically	3
5. Do you ever have physical troubles after operation?	Never	1
	Sometimes	2
	Often	3
6. Are you satisfied with your surgical results?	Satisfied	1
	Unsatisfied, a little	2
	Unsatisfied, seriously	3
7. How is your wound condition?	Good	1
	Fair	2
	Poor	3
8. Can you recommend your operation to others as a good procedure?	Yes, surely	1
	Yes, probably	2
	No	3
9. How do you feel today? (Choose one which mostly represents your mood)	A or B	1
	C	2
	D or E	3

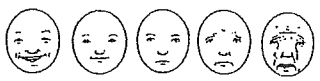

TABLE 2 Clinical Data after Colonic Resection

	Laparoscopic (n=26)	Open (n=87)	*P* value
Postoperative day 1			
Body temperature, °C			
Mean	37.78±0.38	38.02±0.41	<0.01
CRP, mg/dL			
Mean	6.34±2.75	11.15±5.43	<0.01
Postoperative day 7			
Albumin, g/dL			
Mean	3.54±0.32	3.36±0.38	<0.05
Lymphocyte, /mm^3			
Mean	1354±626	995±422	<0.01
Postoperative day 14			
Albumin, g/dL			
Mean	3.68±0.31	3.58±0.35	NS
Lymphocyte, /mm^3			
Mean	1282±427	1296±673	NS
Weight loss, kg			
Mean	2.08±1.18	2.93±1.21	<0.01
Weight loss, %			
Mean	3.95±2.00	5.47±1.98	<0.01

NS: not significant.

TABLE 3 Score for Each Quality-of-life Question after Colonic Resection

Question	Laparoscopic (n=19)	Open (n=68)	*P* value
1	1.474±0.513	1.426±0.498	0.7176
2	1.105±0.315	1.147±0.432	0.6975
3	1.526±0.612	1.603±0.715	0.6718
4	1.158±0.501	1.206±0.534	0.7267
5	1.263±0.452	1.515±0.680	0.1328
6	1.000±0.000	1.103±0.306	0.1481
7	1.158±0.375	1.221±0.418	0.5536
8	1.105±0.315	1.206±0.407	0.3225
9	1.158±0.375	1.338±0.536	0.1731
Total	10.947±1.682	11.809±2.241	0.1237

when compared with conventional open colectomy, was less invasive and more beneficial with regard to the surgical stress and nutritional damage after colonic resection for cancer. However, both operations were favorably accepted by the patients, and quality of life and performance status after operation were not different between the two procedures.

Many studies have shown a favorable outcome of laparoscopic surgery for colon cancer. Laparoscopic technique improves short-term results after colectomy including postoperative pain, bowel function, and activity of daily living (3,4,7,14). Recently, the findings were confirmed by prospective randomized trials comparing laparoscopic and conventional approaches (8,15). Stage *et al.* showed that patients with laparoscopic colonic resection were discharged earlier and suffered less pain (15). Milsom *et al.* indicated that laparoscopic techniques offered a faster recovery of pulmonary and gastrointestinal function (8). Thus, it has been indicated that laparoscopic colectomy for cure of colon cancer is less invasive compared with conventional open colectomy.

Other studies, however, have found no obvious benefit from laparoscopic resection of colon cancer (16,17). Bokey *et al.* described that among 66 patients in whom laparoscopic resection was attempted, 12 experienced cardiac complications, 9 had respiratory complications, and 3 died from myocardiac infarction (16). One prospective randomized trial showed that postoperative reduction in pulmonary function as shown by forced vital capacity and forced expiratory volume in one second was identical in the laparoscopic and open colonic resections (17).

Recently, Schwenk *et al.* demonstrated that pulmonary function was better preserved and recovered faster after laparoscopic operation, and suggested that the use of laparoscopic approach might decrease the incidence of pulmonary complications after colorectal surgery (18). Their prospective randomized trial also clarified that patients who had undergone laparoscopic colonic resection experienced less fatigue, and indicated that postoperative quality of life was improved in patients with laparoscopic procedure (9). Thus, the usefulness of laparoscopic surgery for colon cancer is still controversial. Most discussion has been focused on short-term results (19,20), and little is known about the long-term outcome concerning physical condition, mental condition, and performance status.

Since physical condition and performance status after colorectal surgery are strongly influenced by the location of tumor and type of operation (21,22), our study included only patients with colon cancer treated by colonic resection and hand-sewn anastomosis. Although all patients with laparoscopic colonic resection were satisfied with their surgical results, scores for each question assessing the postoperative physical and mental conditions were not different between the patients with laparoscopic colectomy and those with open colectomy. The results showed that both procedures were favorably accepted by the patients who had undergone resection of colon cancer, and were different from the study comparing the quality of life between the patients with laparoscopic gastrectomy and those with open gastrectomy (23).

With regard to the laboratory data during hospital stay, postoperative increase of body temperature and serum CRP level was less pronounced after laparoscopic colectomy than conventional open colectomy. The results showed that laparoscopic approach minimized surgical stress during and immediately after operation. Furthermore, postoperative decrease of serum albumin level, lymphocyte count, and body weight was less marked in patients with laparoscopic colectomy than those with open colectomy. Thus, surgical stress and nutritional damage after colonic resection for cancer might be decreased by the use of laparoscopic techniques (20,24). Since this study was non-randomized and based on a retrospective clinical analysis, the conclusions must be confirmed by a large series of patients enrolled in a prospective randomized trial.

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Surgical Technique Influences Bowel Function after Low Anterior Resection and Sigmoid Colectomy

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ABSTRACT

Background/Aims: Since June 1996, we have changed surgical strategies to preserve the pelvic autonomic nerve and abandon high ligation of the inferior mesenteric artery. The aim of this study was to clarify the influence of this surgical technique on subjective bowel function of patients with low anterior resection and sigmoid colectomy for cancer.

Methodology: Forty-eight patients who underwent low anterior resection or sigmoid colectomy for cancer during June 1996 and February 2000 replied to the questionnaire which consisted of eight categories of bowel symptoms. Subjective bowel function and operative data of these patients were compared with those obtained from 84 patients with low anterior resection or sigmoid colectomy during April 1984 and May 1996.

Results: When recent series were compared with previous series, the frequency of bowel movement at night (21% *vs.* 60%, $p<0.01$) and patient's own judgment as fair or poor (0% *vs.* 29%, $p<0.01$) was decreased in patients with low anterior resection; whereas the frequency of defecation >2 per day (5% *vs.* 34%, $p<0.01$), difficulty in emptying (32% *vs.* 71%, $p<0.01$), and incomplete evacuation (32% *vs.* 66%, $p<0.05$) was decreased in patients with sigmoid colectomy. Patient judged as poor bowel function was less frequent in the recent group after low anterior resection (25% *vs.* 71%, $p<0.01$) and sigmoid colectomy (18% *vs.* 42%, $p<0.05$) compared with the previous group. Operative data including volume of blood loss, frequency of transfusion, and length of resected specimen were also different between the two groups.

Conclusions: Surgical technique had a significant impact on bowel function following low anterior resection and sigmoid colectomy for cancer. When high ligation of the inferior mesenteric artery is abandoned and the pelvic autonomic nerve is preserved by careful technique, postoperative bowel dysfunction in patients with rectosigmoid colon cancer can be minimized.

KEY WORDS: Surgical technique; Surgical strategies; Bowel function; Low anterior resection; Sigmoid colectomy; Colorectal cancer

INTRODUCTION

Colorectal cancer is an increasing malignancy in Japan, and about 35,000 persons die of the disease annually. Radical surgery with extended lymph node dissection removes the primary tumor and regional lymph nodes (1), but increases the postoperative complications (2). Although sphincter-saving resection followed by colorectal anastomosis is preferred in patients with rectal cancer (3), some experience poor results of bowel function, including frequent bowel movements and nocturnal incontinence (4,5).

Manometric studies indicate that altered bowel habits after low anterior resection are derived from reduced rectal compliance (6), impaired anal sphincter (7,8), and damage to pelvic floor muscle (9). Despite that risk factors for postoperative bowel and sexual dysfunction have been largely analyzed, these studies show that poor bowel function is determined by the location of tumor and height of anastomosis and offer little information in the way of improving functional outcome of any given patient after presentation (10).

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Since June 1996, we have given our careful consideration to the bowel function following surgery of rectosigmoid colon cancer, and changed surgical strategies to preserve the pelvic autonomic nerve and to ligate and cut the inferior mesenteric artery just below the branch of the left colonic artery. Although several studies show that surgical strategies have an immediate impact on the outcome of patients (11,12), whether changes in surgical technique influence postoperative bowel function is unknown. The aim of this study was to assess the effect of alternative surgical technique on the subjective bowel function of patients with low anterior resection or sigmoid colectomy for cure of cancer.

METHODOLOGY

From June 1996 to February 2000, 52 patients underwent low anterior resection or sigmoid colectomy for cancer in the Department of Surgery I, Oita Medical University. In all patients, the pelvic autonomic nerve was preserved and the inferior mesenter-

ic artery was cut just below the branch of the left colonic artery, except for one patient in whom high ligation was carried out due to metastatic lymph nodes at the root of inferior mesenteric artery. Most patients with low anterior resection were treated with a circular stapler for colorectal anastomosis and no patient underwent perioperative radiation. All patients were alive, but four were accompanied by metastatic recurrence in the liver, one in the lung, and one in the locoregional area.

TABLE 1 Bowel Function after Low Anterior Resection and Sigmoid Colectomy

Categories of symptoms	Low anterior resection (n=24)	Sigmoid colectomy (n=22)	*P* value
(1) Defecation >2 per day			
No	15(63)	21(95)	
Sometimes or often	9(37)	1(5)	<0.01
(2) Difficulty in emptying			
No	8(33)	15(68)	
Mild or severe	16(67)	7(32)	<0.05
(3) Incomplete evacuation			
No	10(42)	15(68)	
Sometimes or often	14(58)	7(32)	0.071
(4) Diarrhea			
No	15(63)	14(64)	
Sometimes or often	9(37)	8(36)	NS
(5) Soiling			
No	23(96)	20(91)	
Sometimes or often	1(4)	2(9)	NS
(6) Bowel movement at night			
No	19(79)	20(91)	
Sometimes or often	5(21)	2(9)	NS
(7) Gas/stool discrimination			
Perfect	18(75)	18(82)	
Imperfect or lacking	6(25)	4(18)	NS
(8) Patient's own judgment			
Good	24(100)	21(95)	
Fair or poor	0(0)	1(5)	NS

( ) = percentages; NS = not significant.

TABLE 2 Bowel Function after Low Anterior Resection

Categories of symptoms	Before May 1996 (n=48)	After June 1996 (n=24)	*P* value
(1) Defecation >2 per day			
No	26(54)	15(63)	
Sometimes or often	22(46)	9(37)	NS
(2) Difficulty in emptying			
No	9(19)	8(33)	
Mild or severe	39(81)	16(67)	NS
(3) Incomplete evacuation			
No	12(25)	10(42)	
Sometimes or often	36(75)	14(58)	NS
(4) Diarrhea			
No	26(54)	15(63)	
Sometimes or often	22(46)	9(37)	NS
(5) Soiling			
No	35(73)	23(96)	
Sometimes or often	13(27)	1(4)	NS
(6) Bowel movement at night			
No	19(40)	19(79)	
Sometimes or often	29(60)	5(21)	<0.01
(7) Gas/stool discrimination			
Perfect	31(53)	18(75)	
Imperfect or lacking	17(47)	6(25)	NS
(8) Patient's own judgment			
Good	34(71)	24(100)	
Fair or poor	14(29)	0(0)	<0.01

( ) = percentages; NS = not significant.

A questionnaire was mailed to these 52 patients, and 48 (92%) replied to the questionnaire. The questions concerning the subjective bowel habits included the following eight categories of bowel symptoms: frequency of defecation per 24 hours, difficulty in emptying, sensation of incomplete evacuation, diarrhea, soiling, bowel movement at night, disability to discriminate between gas and stool, and patient's own judgment on functional results of the operation. The patients were divided into the following two groups: good bowel function showing less than half of symptoms (≤3 categories) and poor bowel function showing more than half of the symptoms (≥4 categories).

Operative findings including the volume of blood loss and need for blood transfusion were examined by operation records. Pathologic findings including the length of resected specimen and Dukes stage were obtained from histopathology reports. Outcomes and data from the recent 4 years' experiences were compared with those from the previous 12 years' series as historical controls (13). Differences were analyzed for statistical significance by the chi-square test for categoric variables and *t*-test for continuous variables.

RESULTS

There were 24 patients with low anterior resection and 22 with sigmoid colectomy. Bowel function of these patients is displayed in **Table 1**. After low anterior resection, patients were often complicated with defecation more than twice a day, difficulty in emptying, and incomplete evacuation.

Bowel function after low anterior resection of the recent series during June 1996 and February 2000 was compared with that of the previous series during April 1984 and May 1996 **(Table 2)**. All categories showed improvement of the symptoms, and the frequency of bowel movement at night (21% *vs.* 60%, $p<0.01$) and patient's own judgment as fair or poor (0% *vs.* 29%, $p<0.01$) was significantly decreased. Patient judged as having poor bowel function was different between the two series (25% *vs.* 71%, $p<0.01$).

Table 3 shows that all categories after sigmoid colectomy were improved in the recent group compared to the previous group, and the frequency of defecation more than twice a day (5% *vs.* 34%, $p<0.01$), difficulty in emptying (32% *vs.* 71%, $p<0.01$), and incomplete evacuation (32% *vs.* 66%, $p<0.05$) was significantly decreased. Patient judged as having poor bowel function was different between the two series (18% *vs.* 42%, $p<0.05$).

Although the mean age of patients and distribution of Dukes stage of tumors were not different between the recent group and previous group, the blood loss during low anterior resection (197mL *vs.* 638mL, $p<0.01$), blood loss during sigmoid colectomy (95mL *vs.* 300mL, $p<0.01$), frequency of blood trans-

fusion after low anterior resection (4% *vs.* 50%, $p<0.01$), length of resected specimen in low anterior resection (161cm *vs.* 266cm, $p<0.01$), and length of resected specimen in sigmoid colectomy (164cm *vs.* 260cm, $p<0.01$) were significantly different between the two groups.

DISCUSSION

Our study clarified that surgical technique had an immediate and significant impact on bowel function following low anterior resection and sigmoid colectomy for cancer. When high ligation of the inferior mesenteric artery was abandoned, the pelvic autonomic nerve was well preserved, and blood loss and transfusion was minimized by careful and gentle operative technique, postoperative bowel dysfunction was significantly decreased in patients with rectosigmoid colon cancer.

Several authors demonstrate that in patients with low anterior resection, the increased bowel movements, incontinence for loose stools, need to wear a pad, and need to return to the toilet after defecation are common, and the patient's judgment of the functional results is sometimes bad (14). Some authors emphasize that the height of anastomosis is a variable of major importance, and the lower rectal anastomosis results in worse bowel function after operation on the rectum (10). Others stress that division of the inferior mesenteric artery and mobilization and transection of the rectum alter autonomic innervation of the internal sphincter, which might affect postoperative bowel and sexual functions (8).

In the previous study, we demonstrated that the mean number of defecations per day and frequency of patients with night stools were more after low anterior resection than after sigmoid colectomy (13). When risk factors for postoperative bowel dysfunction were analyzed separately for low anterior resection and sigmoid colectomy, poor bowel function after low anterior resection was correlated with high ligation of inferior mesenteric artery, impairment of pelvic autonomic nerve, and aggressive surgery which needed blood transfusion; whereas poor bowel function after sigmoid colectomy was linked to an increased length of resected specimen (13).

In June 1996, therefore, we changed surgical strategies to preserve the pelvic autonomic nerve (15), to ligate and cut the inferior mesenteric artery just below the branch of the left colonic artery, and to reduce operative bleeding by gentle handling and careful technique. Surgical and pathological data of the present study showed that all but one patient (98%) underwent high ligation of the inferior mesenteric artery and preservation of the pelvic autonomic nerve. The volume of blood loss, frequency of blood transfusion, and length of resected specimen were much decreased in the recent series than in the previous series.

In the present study, the frequency of patient's own judgment as good bowel function was high both in patients with low anterior resection (100%) and in those with sigmoid colectomy (95%). These results

TABLE 3 Bowel Function after Sigmoid Colectomy

Categories of symptoms	Before May 1996 (n=38)	After June 1996 (n=22)	*P* value
(1) Defecation >2 per day			
No	25(66)	21(95)	
Sometimes or often	13(34)	1(5)	<0.01
(2) Difficulty in emptying			
No	11(29)	15(68)	
Mild or severe	27(71)	7(32)	<0.01
(3) Incomplete evacuation			
No	13(34)	15(68)	
Sometimes or often	25(66)	7(32)	<0.05
(4) Diarrhea			
No	21(55)	14(64)	
Sometimes or often	17(45)	8(36)	NS
(5) Soiling			
No	31(82)	20(91)	
Sometimes or often	7(18)	2(9)	NS
(6) Bowel movement at night			
No	27(71)	20(91)	
Sometimes or often	11(29)	2(9)	0.067
(7) Gas/stool discrimination			
Perfect	30(79)	18(22)	
Imperfect or lacking	8(21)	4(18)	NS
(8) Patient's own judgment			
Good	30(79)	21(95)	
Fair or poor	8(21)	1(5)	0.083

( ) = percentages; NS = not significant.

were associated with the decreased frequency of recent patients judged as poor bowel function after low anterior resection (25%) and sigmoid colectomy (18%). Because the age of patients and Dukes stage of tumors were not different between the recent series and previous series, a significant improvement of postoperative bowel function between the two series was considered to be derived not from patient factor or tumor factor but from surgical factor.

High ligation of the inferior mesenteric artery facilitates a complete dissection of lymph nodes along the inferior mesenteric artery and around its origin from the aorta (1), but often sacrifices thoracolumbar sympathetic nerves and superior hypogastric nerves (2). Although high ligation of the inferior mesenteric artery was advocated to ensure aggressive lymph node dissection which might decrease tumor recurrence (16,17), many studies revealed that no survival advantage was brought out by such procedures (18-20). Furthermore, alteration of bowel function after low anterior resection is affected by surgical technique (13), and fecal incontinence is associated with intraoperative bleeding (14). In the operative treatment of rectosigmoid colon cancer, therefore, high ligation of the inferior mesenteric artery should be abandoned and the pelvic autonomic nerve should be preserved with careful operative technique (15).

Recent studies show that clinical strategies have an important impact on the operative outcomes (11,12), including the recurrence of tumors (21), prognosis of patients (22), and cost-effectiveness (23). Our study added substantial evidence that surgical tech-

nique had a significant influence on bowel function after low anterior resection and sigmoid colectomy for cure of cancer. However, these results related to surgical technique and reported by individual surgeons must be confirmed and reproduced by population-based studies or randomized controlled trials.

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

T lymphocyte subsets and Th1/Th2 balance after laparoscopy-assisted distal gastrectomy

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Abstract
Background: Laparoscopic surgery provides for a less invasive procedure than open surgery in patients with gastric cancer, but the immune responses after laparoscopic surgery for early gastric cancer remain unknown.
Methods: Peripheral blood mononuclear cells from 20 patients with early gastric cancer who underwent laparoscopy-assisted distal gastrectomy (LADG) or open distal gastrectomy (ODG) were obtained; the cell surface molecules and intracellular cytokines (IFN-gamma and IL-4) were measured by flow cytometry.
Results: The populations of T lymphocytes after LADG, including CD3-, 4-, 8-, 57-, and HLA-DR-positive lymphocytes, showed patterns similar to those after ODG. The production of IFN-gamma as Th1 cell function decreased significantly on the third postoperative day after ODG but increased after LADG. The production of IL-4, representing Th2 cell function, increased postoperatively after ODG but not after LADG.
Conclusions: When compared with ODG, LADG contributes to the preservation of postsurgical Th1 cell-mediated immune function.

Key words: Gastric cancer — Laparoscopic surgery — Surgical stress — T lymphocyte subset — Th1/Th2 balance

Major surgery and trauma cause significant immunosuppression in patients [16, 19], and this immunosuppression is associated with high morbidity and mortality rates. Immunosuppression after major surgery leads to infection and tumor progression [8, 9]. There is a direct relation between the degree of surgical invasion and the degree of immunosuppression [2]. Therefore, less invasive surgeries are preferred.

Laparoscopic surgery, as a minimally invasive surgery, has been in use since the 1980s and is now performed worldwide for the treatment of gastrointestinal disease [11, 17, 18]. With the development of new devices and safe techniques, the application of laparoscopic surgery has been expanded to cancer. We performed the first laparoscopy-assisted distal gastrectomy (LADG) in 1991, and since that time we have performed the procedure successfully in about 120 patients [14]. With LADG, in comparison to conventional open distal gastrectomy (ODG), there are several advantages including earlier recovery, less pain, and less impaired pulmonary function [1, 15], but the effect of LADG on immunological responses remains unknown.

A relation between laparoscopic surgery and fewer immunological changes after surgery has been reported. The host defense is less depressed after laparoscopic cholecystectomy than after cholecystectomy under minilaparotomy, as assessed by natural killer cell cytotoxicity, neuroendocrine response, and metabolic substances [12, 13]. Also, the numbers of circulating T lymphocytes are shown to be greater after laparoscopic cholecystectomy than after minilaparotomy cholecystectomy [26].

To clarify differences between LADG and ODG from the standpoint of postsurgical immunological response, we compared the changing populations of T lymphocytes and the Th1/Th2 balance after LADG to those after ODG.

Materials and methods

Patients and surgery

The study included 20 patients with early gastric cancer admitted to the Department of Surgery I, Oita Medical University, between April 1999 and January 2002 [15]. After providing written informed consent, the patients were randomly assigned to either LADG group (n = 10) and an ODG group (n = 10) with Billroth-I reconstruction on the day before operation by use of numbered, sealed envelopes that were stratified by the surgeon.

All patients with early gastric cancer who were inadequate for endoscopic mucosal resection because of a risk of perigastric lymph-node metastasis participated to this study. The exclusion criteria were (1) age over 80 years, (2) operative cardiovascular risk greater than

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Table 1. Clinicopathological findings of patients undergoing LADG and ODG

Characteristics	LADG (n = 10)	ODG (n = 10)	p value
Age (year)	69.2 ± 10.3	63.2 ± 13.3	N.S.
Sex (male/female)	7/3	6/4	N.S.
Height (cm)	156.8 ± 8.1	159.1 ± 6.1	N.S.
Weight (kg)	58.2 ± 9.3	60.9 ± 9.2	N.S.
Body mass index (BMI)	28.3 ± 4.7	25.2 ± 6.1	N.S.
Concurrent illness (absent/present)	4/6	3/7	N.S.
Hypertension	4	5	
Ischemic heart disease	2	1	
Diabetes mellitus	0	2	
Operative findings			
Operation time (min)	225.5 ± 35.2	179:6 ± 37.6	<0.05
Blood loss (g)	134.0 ± 110.8	205.5 ± 75.7	N.S.
Complications (absent/present)	8/2	8/2	
Atelectasis/pneumonia	0	0	
Delayed gastric emptying	2	2	
Oncologic findings			
Size (cm)	2.0 ± 1.5	2.6 ± 1.6	N.S.
Gross type (elevated/depressed)	2/8	1/9	N.S.
Histologic type (well/poorly)	7/3	5/5	N.S.
Depth (mucosa/submucosa/muscularis)	8/1/1	4/5/1	N.S.
Lymph-node metastasis (absent/present)	9/1	10/0	N.S.
TNM classification (IA/IB/II)	9/0/1	9/1/0	N.S.
Outcome (alive/dead)	10/0	10/0	N.S.

Data are given as the mean ± SD or number
LADG, Laparoscopy-assisted distal gastrectomy; ODG, open distal gastrectomy; N.S., not significant

that of New York Heart Association class II, (3) operative pulmonary risk greater than that of Hugh-Jones class II, or (4) severe liver disease (Child class B or C) or renal dysfunction.

All patients were premedicated with 10 mg of oral diazepam the night before the surgery, and 0.5 mg of intramuscular atropine sulfate and 50 mg of intramuscular hydroxyzine hydrochloride 1 h before surgery. The protocol for anesthesia was the same in all patients: induction with propofol of identical doses (2.0 to 2.5 mg/kg) and vecuronium 0.1 mg/kg given intravenously, and maintenance with N_2O/O_2/sevoflurane (67%/33%/0.5 to 4.0%). In addition, epidural anesthesia was performed.

Both LADG and ODG were performed by the same experienced surgeon (SK) with the same surgical team [15]. LADG was carried out as previously described: (1) laparoscopic dissection of the greater and lesser omentum, (2) ligation and division of the right gastroepiploic and left gastric vessels under pneumoperitoneum, and (3) resection of the distal two-thirds of the stomach followed by handsewn anastomosis between the gastric remnant and duodenal stump through a 5-cm-long minilaparotomy incision [1, 15, 22]. ODG was performed in the usual manner through an upper midline laparotomy incision from the xiphoid process to the umbilicus. Postoperative analgesia in all patients was by epidural block, i.e., 0.25% bupivacaine hydrochloride (0.5 mL/h) and buprenorphine hydrochloride (0.02 mg/h) for 2 days.

Analysis of immunological response after surgery

Blood was taken at 7:00 a.m. on the preoperative day and at the same time on the first, third, and seventh postoperative days. Ten mL of blood was collected by peripheral venepuncture with a heparinized syringe and used for analysis of T lymphocyte subsets and the Th1/Th2 balance.

Changes in T lymphocytes after surgery were determined by flow cytometry [14]. Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation with Ficoll-Paque Plus (Amersham Pharmacia Biotech, Stockholm, Sweden). PBMCs were washed twice with phosphate-buffered saline (PBS) and once with PBS and 0.2% bovine serum albumin (BSA; Sigma Chemical, St. Louis, MO, USA) and 0.1% sodium azide (Wako Pure Chemical, Osaka, Japan). Monoclonal antibodies (Becton Dickinson, San Jose, CA, USA) were phycoerythrin (PE)-conjugated anti-CD3 and HLA-DR, and fluorescein isothiocyanate (FITC)-conjugated anti-CD4, -CD8, and -CD57. PBMCs were separated into tubes, 20 μL of monoclonal antibody was added for two-color staining, and cells were incubated for 30 min at 37°C in the dark. Cells were washed twice, and the surface molecules were analyzed on a FACS Calibur flow cytometer (fluorescence-activated cell sorter, Becton Dickinson, San Jose, CA, USA).

The Th1/Th2 balance was determined by measurement of intracellular IFN-gamma and IL-4 production as described previously [3, 20]. In brief, separated PBMCs were washed, counted, and resuspended at 2×10^6 per tube in RPMI 1640 medium containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and 10 μg/mL of brefeldin-A (Sigma-Aldrich Corp., St. Louis, MO, USA) and then stimulated with 100 μg/mL of phorbol 12-myristate-13-acetate (Sigma) and 1 μg/mL of ionomycin (Sigma). Four tubes were prepared; two were stimulated, and the other remained unstimulated. The cells were incubated at 37°C in 5% carbon dioxide for 4 h. After incubation, cells were washed and suspended in PBS with 1% BSA and 0.1% sodium azide, and 20 μL of peridinin chlorophyll protein-conjugated anti-CD3 monoclonal antibody or isotype control was added for staining of cell surface molecules. The cells were incubated at 4°C for 30 min. The cells were then fixed with 4% paraformaldehyde at 4°C for 20 min and washed twice. For intracellular cytokine staining, cells were suspended in PBS containing 1% BSA and 0.5% saponin, then incubated with 20 μL of FITC-conjugated anti-IFN-gamma/PE-conjugated anti-IL-4 monoclonal antibody at 4°C for 30 min in the dark. Cells were washed twice with PBS/BSA/saponin and then with PBS/BSA without saponin to allow for membrane closure. Samples were analyzed on a FACS Calibur, and results were analyzed with CellQuest software (both Becton Dickinson).

Statistical analysis

Within-group comparisons were carried out with the Wilcoxon matched-pairs signed rank test, and between-group analysis was performed on baseline-corrected data with the Mann–Whitney U-test. A two-tailed probability of less than 5% ($p < 0.05$) was considered significant.

Results

Age, sex, height, weight, body mass index, and concurrent illness did not differ between the groups (Table 1).

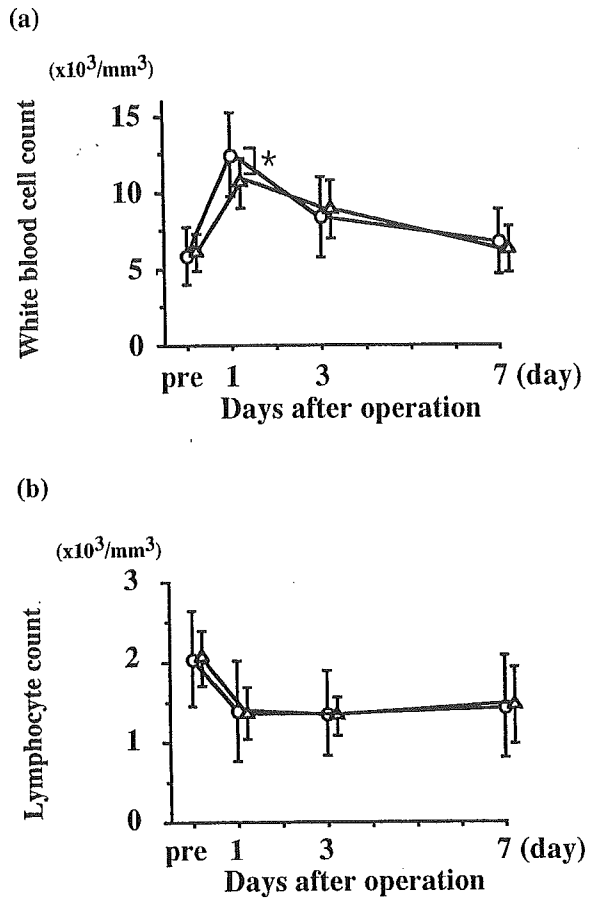

Fig. 1. Variations in the white blood cell (a) and lymphocyte (b) count of patients undergoing LADG (triangle) or ODG (circle). White blood cell count was significantly elevated on the first postoperative day in ODG compared to LADG (*$p < 0.05$). Values are expressed as mean ± SD.

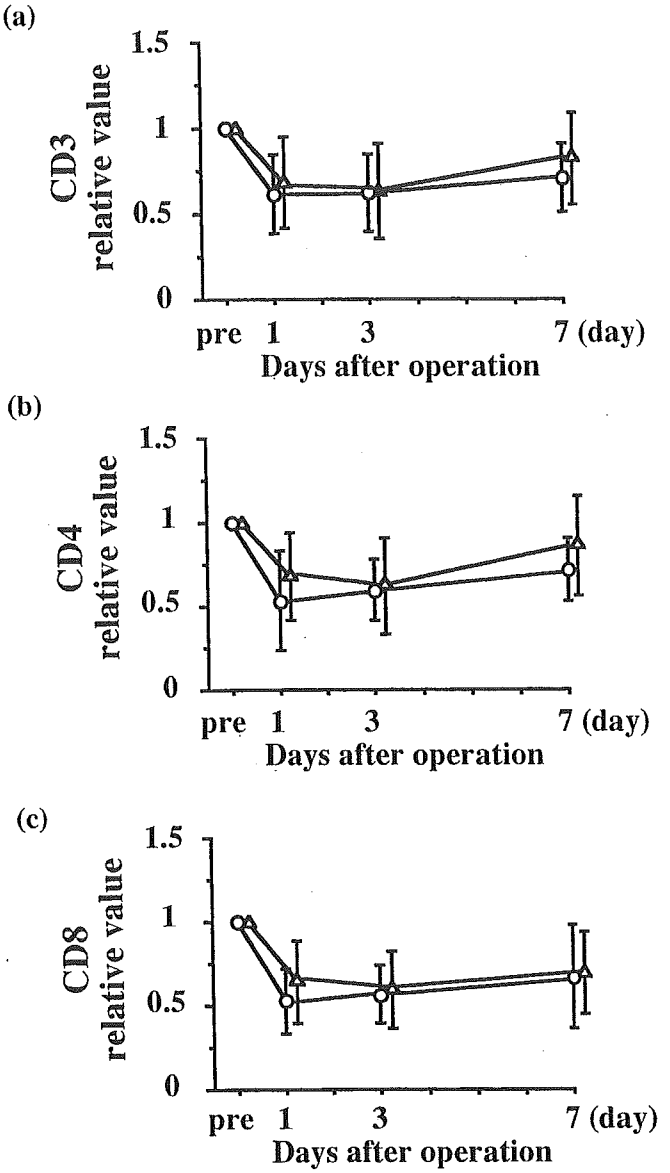

Fig. 2. Variations in the CD3 (a), CD4 (b), and CD8 (c) positive cells levels in patients undergoing LADG or ODG. Values are expressed as mean ± SD.

The operation time was greater for LADG than for ODG ($p < 0.05$), but the mean blood loss in either group was the same between the two procedures. There were no major complications after surgery. Pathological examination also showed the same tumor stage in both groups.

The number of white blood cells increased on the first day after surgery and then returned to preoperative levels by 7 days after surgery in both groups. The peak white blood cell count was significantly higher in the ODG group than in the LADG group ($p < 0.05$, Fig. 1a). The total number of lymphocytes decreased after surgery in both groups; and the difference between groups was not significant (Fig. 1b).

The populations of T lymphocytes after surgery are shown in comparison to the populations before surgery in Figs. 2 and 3. CD3, CD4, and CD8 expression decreased on the first postoperative day in both groups and then gradually returned to the preoperative levels by the seventh postoperative day. CD3, CD4, and CD8 expression did not differ statistically between the two groups (Fig. 2). CD57, a marker of activated NK cells, and HLA-DR, a marker of activated lymphocytes, showed changes similar to those of the other cell surface molecules (Fig. 3), and differences in expression of CD57 and HLA-DR did not differ between the two groups. Thus, the populations of T lymphocytes were altered similarly after LADG and ODG.

The Th1/Th2 balance was evaluated by immunostaining of intracellular cytokines IFN-gamma and IL-4 in circulating CD3-positive T lymphocytes. The IFN-gamma production was slightly increased postoperatively in the LADG group, but it was depressed in the ODG group (Fig. 4a). The IFN-gamma level on the third postoperative day after ODG was significantly lower than that after LADG ($p < 0.05$). IL-4 production showed almost no change in the LADG group; it increased postoperatively in the ODG group, but the difference was not significant (Fig. 4b). Thus, the normal Th1/Th2 balance was preserved after LADG, but Th1 cell function was significantly decreased after ODG.

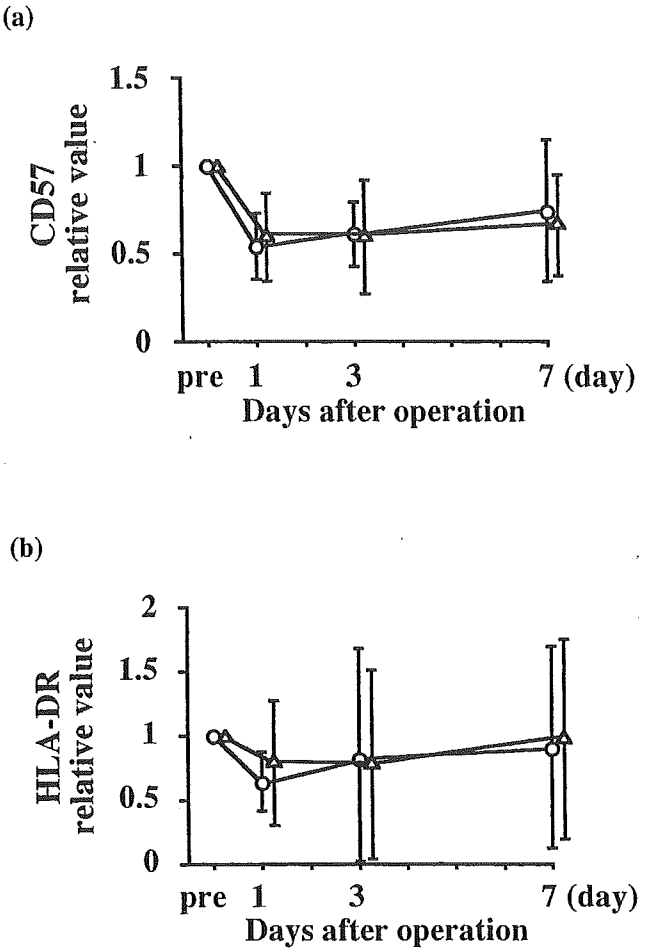

Fig. 3. Variations in the CD57 (a) and HLA-DR (b) positive cells levels in patients undergoing LADG or ODG. Values are expressed as mean ± SD.

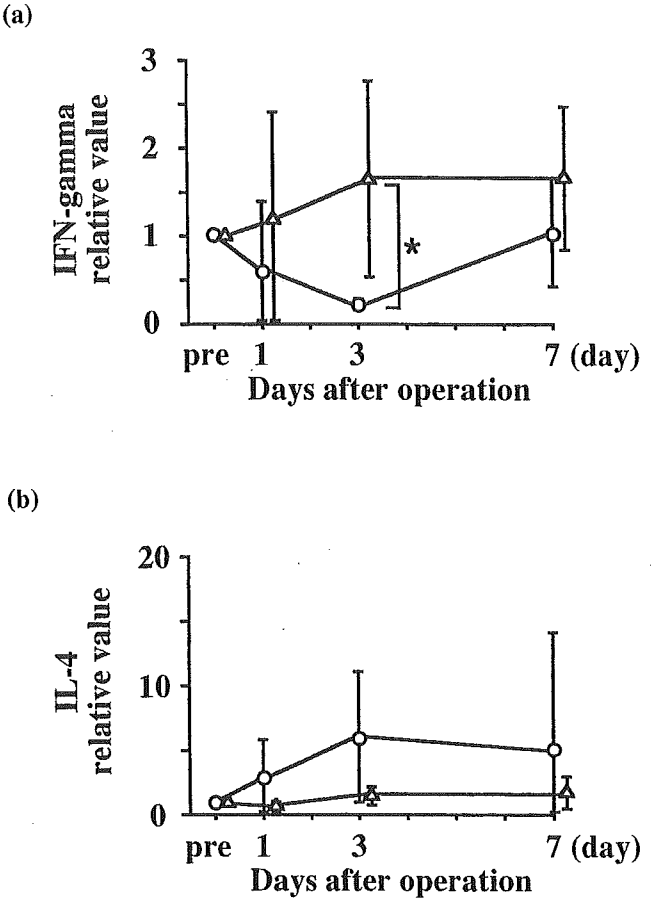

Fig. 4. Variations in the intracellular interferon-gamma (a) and interleukin-4 (b) positive cells level in patients undergoing LADG and ODG. IFN-gamma was significantly decreased on the third postoperative day in ODG compared to LADG (*$P < 0.05$). Values are expressed as mean ± SD.

Discussion

In our study comparing the effect of LADG vs ODG on immune response, changes in the populations of T lymphocytes, i.e., in CD3-, CD4-, CD8-, CD57-, and HLA-DR-positive lymphocytes, did not differ significantly between the two patient groups. Although Th1 cell function was significantly suppressed after ODG, and Th2 cell function was stimulated, Th1/Th2 balance was well preserved after LADG.

Tlymphocyte suppression is reported to be caused by surgical stress, infection, and cancer [5, 25]. Walker et al. showed by flow cytometric analysis that postoperative suppression of T lymphocyte subsets was less after laparoscopic cholecystectomy than after minilaparotomy cholecystectomy [26]. Gitzelmann et al. showed in animal studies that delayed-type hypersensitivity and the tumor rejection reaction representing cell-mediated immune function were better preserved by laparoscopy than by laparotomy [10]. In the present study, the changes in T lymphocytes after LADG were nearly the same as those after ODG. Ordemann et al. reported no significant difference in CD4- and CD8-positive T lymphocyte counts in relation to laparoscopic vs conventional colorectal resection [21]. Changes in T lymphocyte subsets after surgery may be associated with the degree of surgical stress, including the demands of minilaparotomy, operation time, and operative blood loss.

The Th1/Th2 balance is also affected by surgical stress and cancer, and the balance is important for anticancer immunity [7, 24]. Tabata et al. showed that Th2-dominant status develops in patients with digestive tract cancer [24]. Berguer et al. showed that surgical stress induces the Th1/Th2 balance toward a Th2-type immune response [3]. There have been several studies on the effect of laparoscopic surgery on the Th1/Th2 balance. Brune et al. showed that the production of IFN-gamma and IL-2 from CD3 and CD28 antibody-stimulated-T lymphocytes decreased postoperatively in association with open cholecystectomy but not laparoscopic cholecystectomy [4]. Decker et al. showed that the postoperative Th1/Th2 balance shifts toward Th2 in conventional cholecystectomy in comparison to that after laparoscopic cholecystectomy [7]. These studies were conducted by measuring plasma or supernatant concentrations of Th1 and Th2 cytokines produced by stimulated T lymphocytes. Flow cytometry has been used more recently to detect intracellular cytokines [20]. This method can be used to show the Th1/Th2 balance *in vivo* because cytokine production can be

measured without stimulation. Our study, measuring intracellular Th1 and Th2 cytokine production, showed that, with respect to gastrectomy, the Th1/Th2 balance is less impaired by laparoscopy-assisted procedure than by the conventional open procedure.

Suppression of immunity after surgery is associated with tumor growth, invasion, and metastasis. Da Costa et al. showed in animals that stimulation of tumor growth after open surgery is due to suppression of NK cell activity [6]. In a previous mouse study, we showed lung metastasis to be increased after open surgery in comparison to that after laparoscopic surgery because of the induction of acute-phase cytokines [23]. The present study showed preservation of the Th1/Th2 balance after LADG, as a cell-mediated immune function, whereas there was no difference in the morbidity and mortality between LADG and ODG groups in this study because of small sample size (data not shown). If further examinations for T lymphocyte subsets and Th1/Th2 balance after LADG and ODG are performed, we may be able to obtain some favorable immunological advantages for laparoscopic surgery.

In conclusion, laparoscopy-assisted distal gastrec tomy, when compared with conventional open distal gastrectomy, shows well-preserved cell-mediated immune function as indicated by Th1/Th2 balance. The data support the usefulness of laparoscopic gastric surgery. Further examination into the induction of cytokines, activation of second messengers in immune cells, and association between tumor progression and immunological response after LADG is needed to clarify the immunological response after LADG and the usefulness of LADG for cancer surgery.

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Digestive Endoscopy (2003) **15**, 280–283

ORIGINAL ARTICLE

LEARNING CURVE FOR LAPAROSCOPY-ASSISTED DISTAL GASTRECTOMY

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Background: Laparoscopy-assisted distal gastrectomy (LADG) has proved to be useful in the management of early gastric cancer. The aim of the present study was to examine the learning curve for LADG and clarify any technical problems.
Methods: The study included 75 consecutive patients who underwent LADG between 1994 and 2002. All operations were performed by a single surgeon and with a surgical team who were skilled in laparoscopic procedures, but new to LADG. Patients were divided into three groups according to the surgeon's level of experience at the time of surgery: Group I ($n = 25$, surgeries performed between November 1994 and April 1997), Group 2 ($n = 25$, surgeries performed between May 1997 and January 2000), and Group 3 ($n = 25$, surgeries performed between February 2000 and August 2002). We considered Groups 1, 2 and 3 to reflect the surgeon's beginning, intermediate and advanced levels of experience, respectively. Operation time, blood loss and incidence of complications were analyzed and compared between groups.
Results: Operation time and blood loss did not differ between Groups 1, 2 and 3 (operation time: 236 *vs* 258 *vs* 225 min; blood loss: 157 *vs* 198 *vs* 144 mg, respectively). Postoperative complications occurred in nine patients (12%); the incidence did not differ between groups. The most frequent complication was wound infection (4%), followed by anastomotic stenosis (3%). There were no intraoperative complications or conversions to open surgery.
Conclusions: There is no learning curve for LADG, when it is performed by a skilled surgeon and surgical team. Proficiency in basic laparoscopic techniques and open gastric surgery is easily adapted to safe completion of LADG.

Key words: complication, laparoscopic surgery, laparoscopy-assisted distal gastrectomy, learning curve, stomach.

INTRODUCTION

During the past 10 years, laparoscopic surgery, including mucosal resection, local resection and distal gastrectomy, has been introduced and widely accepted in Japan as a treatment for patients with early gastric cancer.[1–4] The number of laparoscopy-assisted distal gastrectomies (LADG) has doubled annually since 1994,[5] and several studies have shown that in comparison to conventional open gastrectomy, LADG involves less surgical trauma, less pain, faster recovery, shorter hospitalization, and a better postoperative quality of life.[6–10]

Laparoscopy-assisted distal gastrectomy consists of ligation and division of major vessels, full mobilization and resection of the stomach, and gastroduodenal anastomosis. Early criticism of laparoscopic gastrectomy focused on the complexity and technical difficulty of this procedure, but there has been no study of the learning curve of surgeons new to LADG.

To examine the learning curve for LADG and clarify the technical problems, we reviewed the first 75 LADG performed by one surgeon. The cases were divided into three groups according to the surgeon's level of experience at the time of surgery, and operation time, blood loss and intra- and postoperative complications were compared between groups.

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MATERIALS AND METHODS

Laparoscopy-assisted distal gastrectomy was introduced to the Department of Surgery I, Oita Medical University in 1994. Between 1994 and 2002, 107 LADG with perigastric lymph node dissection were performed in patients with early gastric cancer. Our study included the first 75 consecutive LADG performed by a single skilled surgeon (SK) with a surgical team. Resection of the stomach and perigastric lymph node dissection were performed by the single surgeon and reconstruction was mostly performed by the remaining members of the surgical team. Indications for LADG at our department are (i) tumor confined to the mucosa not indicated for endoscopic mucosal resection (EMR); and (ii) tumor invading the upper submucosal layer.

The cases were divided into groups according to the surgeon's level of experience with LADG at the time of surgery. Group 1 comprised the surgeon's first 25 LADG patients, who underwent surgery between November 1994 and April 1997. Group 2 comprised the surgeon's second set of 25 patients, who underwent surgery between May 1997 and January 2000. Group 3 comprised the surgeon's most recent set of 25 patients, who underwent surgery between February 2000 and August 2002. We considered Groups 1, 2 and 3 to reflect the surgeon's beginning, intermediate and advanced levels of experience, respectively. Data comprising patient

Table 2. Clinical results of laparoscopy-assisted distal gastrectomy in gastric cancer patients

	Group 1 ($n = 25$)	Group 2 ($n = 25$)	Group 3 ($n = 25$)
Operative time (min)			
Mean ± SD	236 ± 45	258 ± 36	225 ± 39*
Range	165–353	175–325	140–300
Blood loss (mg)			
Mean ± SD	157 ± 124	198 ± 124	144 ± 166
Range	10–500	35–450	10–620
Complications			
Absent/Present	24/1	21/4	21/4
Wound infection		1	2
Pancreatic fistula		1	
Anastomotic stenosis		1	1
Pneumonia	1	1	
Cholecystitis			1

* $P < 0.01$ (*vs* Group 2).

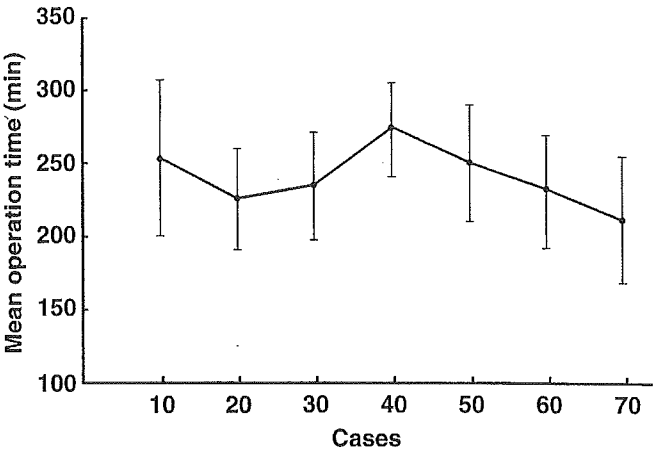

Fig. 1. Operation time of chronological groups of 10 patients each. Data are expressed as mean ± SD.

stenosis (3%) and pneumonia (3%). There was no statistically significant difference in the incidence of postoperative complications between the three groups (Table 2). There were no intraoperative complications, and no patients required conversion to conventional open surgery.

All patients, except one who died of cerebral bleeding, remained alive and disease free during the follow-up period of 5–96 months (median, 50 months).

DISCUSSION

Surgeons in Japan have recently altered treatment strategies for early gastric cancer to reflect new developments in diagnostic instrumentation and treatment technologies.[12,13] As noted earlier, the proportion of patients with gastric cancer treated laparoscopically has increased.[5] Laparoscopic gastric surgery is increasingly selected because of the favorable effect on postoperative course and the cost benefit.[6–9,14]

Laparoscopic cholecystectomy is used frequently by most general surgeons, and a study in the USA showed that surgeons who have performed more than 20 laparoscopic cholecystectomies have reduced complication rates.[15] Laparoscopic colectomy and laparoscopic gastrectomy are more complex procedures than laparoscopic cholecystectomy. Previous studies showed a more prolonged learning curve to master the technique of laparoscopic colectomy than that required for laparoscpic cholecystectomy.[16,17] In our series of laparoscopic gastrectomies, operation time, blood loss and incidence of postoperative complications did not differ between Groups 1, 2 and 3. There were no intraoperative complications, nor patients requiring conversion to conventional open surgery. Moreover, in previous studies, when performed by a skilled and experienced surgeon, the operation time for LADG was not significantly longer than that for conventional open distal gastrectomy.[8,18] The surgeon in the present study had performed hundreds of laparoscopic cholecystectomies before performing the first LADG and was an expert in gastric cancer surgery at that time. The members of the surgical team were also competent in laparoscopic cholecystectomy and conventional open gastric surgery. Our findings indicated that knowledge of basic laparoscopic techniques, acquired by experience with laparoscopic cholecystectomy, and proficiency in open gastric surgery prepared the surgeon to perform laparoscopic gastrectomy safely and easily.

Common complications of LADG are anastomotic stenosis and anastomotic leakage.[6,7,12] The sixth nationwide survey of endoscopic surgery conducted by the Japan Society of Endoscopic Surgery[5] showed that 253 postoperative complications occurred in 2600 patients who underwent LADG (10%); the most frequent complication was anastomotic stenosis (4%), followed by wound infection (2%) and anastomotic leakage (2%). In our study, there were nine complications in the 75 patients, including two that were anastomotic stenoses. These complications resulted from injury and excessive devascularization of the duodenal wall during isolation of the duodenum, and some technical difficulty in performing gastroduodenal anastomosis through a 5-cm-long minilaparotomy.[18] To avoid these complications, we recommend the following techniques. During mobilization of the duodenal bulbus, avoid injuring the duodenum with the laparoscopic coagulating shears, and minimize devascularization. To reduce difficulties in reconstruction by the Billroth I method with hand-sewn anastomosis, extend the minilaparotomy from 5 to 7 cm, shift the minilaparotomy to the right side from the midline, or use autosuture.

We conclude from our study that for a skilled surgeon and surgical team, there is no learning curve for laparoscopic gastric surgery with respect to operation time, blood loss and incidence of complications. Proficiency in basic laparoscopic techniques and open gastric surgery translate easily to safe completion of laparoscopic gastric surgery. To avoid postoperative complications, surgeons must be careful while performing gastroduodenal anastomosis through the minilaparotomy during LADG.

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age, sex, height, weight, history of previous abdominal surgery, operation time, blood loss, and intra- and postoperative complications were obtained from the medical and surgical records. Tumor characteristics including location, size, gross type, histological type, depth of wall invasion and presence or absence of lymph node metastasis, were determined from the pathology reports. Anastomotic stenosis was defined as an event that required additional treatment such as endoscopic dilatation. The presence of pancreatic fistula was confirmed by a high concentration of amylase in the discharge.

Laparoscopy-assisted distal gastrectomy, as described previously in detail,[1,11] consisted of the following procedures. Under pneumoperitoneum, laparoscopic dissection of the lesser and greater omentum and full mobilization of the stomach (ligation and division of the right gastroepiploic vessels, right gastric vessels, and left gastric vessels); and resection of the distal two-thirds of the stomach and reconstruction by the Billroth I method with hand-sewn anastomosis performed through a 5-cm-long minilaparotomy incision.

Categoric variables were compared between groups, by chi-squared test or Fisher's exact test. Continuous variables are presented as mean values with standard deviations and between-group deferences were analyzed by unpaired t-test. A P value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of patients in Group 3 was statistically greater than that of patients in Group 1, but other patient characteristics including sex, height, weight and body mass index were similar among all three groups (Table 1). Appendectomy was the most common previous surgery in all groups, and no patients had undergone laparotomy of the upper abdomen. Prior surgeries were statistically more frequent in Group 3 than in Group 1. Pathological characteristics of the resected tumor including location, size, gross type, depth of wall invasion and lymph node metastasis were similar among the three groups.

Operation time, blood loss and complications are shown for all three groups in Table 2. There was no association between the surgeon's level of experience and the operation time; the mean operation time in Group 2 was greater than that in either of the other two groups. This finding led us to further subdivide the 75 cases into seven chronological groups of 10 patients each for a more detailed view of the surgeon's learning curve; however, we observed no significant difference in mean operation time between these groups (Fig. 1). The mean blood loss in each of the three original groups was 200 mg or less, with no significant difference between groups (Table 2). Postoperative complications occurred in nine patients (12%). The most frequent complication was wound infection (4%), followed by anastomotic

Table 1. Patients and tumors in early gastric cancer

	Group 1 ($n=25$)	Group 2 ($n=25$)	Group 3 ($n=25$)
Patient			
Age, years (mean ± SD)	64.9 ± 9.5	66.6 ± 10.6	71.6 ± 9.0*
Sex			
Male/Female	15/10	17/8	16/9
Height (cm; mean ± SD)	157.6 ± 8.9	158.6 ± 8.4	156.5 ± 8.4
Weight (kg; mean ± SD)	53.5 ± 8.9	57.3 ± 8.5	52.5 ± 8.8
Body mass index (mean ± SD)	21.5 ± 2.8	22.7 ± 2.2	21.3 ± 2.5
Previous abdominal surgery			
Absent/Present	19/6	16/9	13/12*
Appendectomy	5	6	8
Hysterectomy	1	1	1
Oophorectomy			1
Open cholecystectomy			1
Laparoscopic cholecystectomy		1	2
LWR of the stomach		1	
Tumor			
Location			
Lower/Middle	11/14	10/15	11/14
Size (cm; mean ± SD)	2.2 ± 0.3	2.3 ± 0.2	2.1 ± 0.3
Gross type			
Elevated/Depressed	7/18	8/17	4/21
Histological type			
Well-/Poorly differentiated	22/3	20/5	19/6
Depth of invasion			
M/SM/MP	19/5/1	15/8/2	15/8/2
Lymph node metastasis			
Absent/Present	25/0	23/2	21/4

* $P < 0.05$ (*vs* Group 1).
LWR, laparoscopic wedge resection; M, mucosa; MP, muscularis propria; SM, submucosa.

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SURGERY TODAY

Less Impaired Cell-Mediated Immune Response in the Murine Peritoneal Cavity After CO_2 Pneumoperitoneum

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Abstract

Purpose. We compared changes in the populations of peritoneal T lymphocytes and natural killer (NK) cells after CO_2 pneumoperitoneum and laparotomy to clarify whether pneumoperitoneum affects cell-mediated immune responses in the peritoneal cavity.

Methods. We analyzed and compared populations of T lymphocytes and NK cells among peritoneal exudative cells (PECs) collected from 185 female mice subjected to pneumoperitoneum, laparotomy, or anesthesia only. PECs were collected postoperatively, and the populations of T lymphocytes and NK cell subsets were analyzed by flow cytometry. The NK cell cytotoxicity (NKCC) of PECs and splenocytes was measured.

Results. The populations of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes in the PECs continued to increase up until postoperative day (POD) 7 after laparotomy. The CD4/8 ratio on POD 3 was significantly lower after laparotomy than after pneumoperitoneum. The percentages of NK cells in the pneumoperitoneum group were significantly lower than those in the laparotomy group. On POD 1, the NKCC of splenocytes was less impaired in the pneumoperitoneum group than in the laparotomy group (10.3% vs 5.0%, $P < 0.05$).

Conclusion. Laparoscopic surgery is preferable to open surgery because it results in less impairment of systemic and intraperitoneal cell-mediated immune responses.

Key words Pneumoperitoneum · Peritoneal cavity · Immune response

Introduction

Laparoscopic surgery is preferred to open surgery for gastrointestinal diseases because it is much less invasive.[1,2] With the development of new devices and techniques, the application of laparoscopic surgery has extended from benign to malignant diseases,[2,3] although its suitability for advanced cancer remains controversial because it is still suspected that laparoscopic procedures can promote the progression of cancer through port-site metastasis, peritoneal dissemination, or liver metastasis.[4–6]

The immunosuppression caused by surgical trauma, neuroendocrine responses after surgery, and growth factors and cytokines secreted during wound healing are thought to be risk factors for tumor recurrence after surgery.[7] Several studies have been conducted to compare systemic immune responses after laparoscopic surgery with those after conventional open surgery. In both the clinical setting and in animal experiments, the systemic cell-mediated immune response, including T-lymphocyte function and natural killer (NK) cell activity, is less impaired after laparoscopic surgery than after conventional open surgery.[8,9] However, few studies have examined immune response in the peritoneal cavity.[10,11]

Our previous studies showed that pneumoperitoneum with carbon dioxide (CO_2) caused morphological changes in peritoneal mesothelial cells and the secretion of hyaluronic acid in the peritoneal cavity.[12,13] Thus, it is possible that CO_2 pneumoperitoneum influences cell-mediated immune responses and that the populations and function of T lymphocytes and NK cells in the peritoneal cavity are associated with anticancer immunity.

The purpose of this study was to document changes in the populations of peritoneal T lymphocytes and NK cells over time after CO_2 pneumoperitoneum, compared with changes after laparotomy, and to compare

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the NK cell cytotoxicity (NKCC) of peritoneal exudative cells (PECs) and splenocytes after these procedures.

Materials and Methods

We used 185 female Balb/c mice, 6–8 weeks of age and weighing 20–25 g. All mice were acclimatized to a climate- and light cycle-controlled environment for at least 5 days before the start of any treatment. Mice were given a standard laboratory diet and tap water ad libitum. All studies were performed under the guidelines for animal experimentation of Oita Medical University.

The influences of surgical procedures on the populations of intraperitoneal T lymphocytes and NK cells subsets after CO_2 pneumoperitoneum were examined first. Mice were divided into a pneumoperitoneum group (n = 50), a laparotomy group (n = 50), and a control group (n = 50). All the mice were anesthetized by diethyl ether, and their abdomens were shaved and disinfected with 70% alcohol. Using adhesive tape, they were fixed to an operating table, in the supine position. A convenient murine pneumoperitoneal model was used for the CO_2 pneumoperitoneal group.[14] After placing 21- and 22-gauge intravenous catheters intraperitoneally in the left and right lower quadrants, the peritoneum was insufflated with CO_2 gas and maintained at 4–6 mmHg for 30 min, then deflated. In the laparotomy group, a 3-cm midline incision was made from the xiphoid to the pubis, and closed 30 min later with 3-0 polyglycolic acid sutures. Mice in the control group were only anesthetized and not subjected to any operative procedure.

Ten mice from each group were killed on days 0 (baseline), 1, 3, 7, and 10 after the respective procedures, and PECs were collected by washing the intra-abdominal cavity with 10 ml of phosphate-buffered saline (PBS) solution through an intraperitoneal 18-gauge catheter. The collected PECs were washed twice with PBS, and the total cell count was calculated. The subpopulations of T lymphocytes and NK cells in the PECs were also counted by single-color immunofluorescence staining of cell surface molecules and by cytometric analysis.[10,11,15] Staining was with fluorescein isothiocyanate (FITC)-conjugated antimouse monoclonal antibodies recognizing CD3, CD4, CD8, NK1.1, and $IgG_{2a\ kappa}$ (control) (PharMingen, San Diego, CA, USA).

PECs suspended in PBS were divided between five polystyrene tubes and FITC-conjugated antimouse monoclonal antibodies were added to each tube at 1 μg per 1×10^6 cells. Control antibody was added to each tube at 20 μg per 1×10^6 cells. Tubes were then stored at 4°C in the dark for 30 min. After the cells were washed twice with PBS, they were analyzed on a FACS Calibur (Becton Dickinson, San Jose, CA, USA) flow cytometer. Instrument calibration was performed daily with Calibrite Beads according to the recommendations of the manufacturer, and results were analyzed with CellQuest software (both Becton Dickinson).

For the evaluation of NKCC in PECs and splenocytes, a chromium release assay (^{51}Cr radionuclide, Daiichi Chemical, Tokyo, Japan) was carried out. Thirty-five mice were divided into a CO_2 pneumoperitoneum group (n = 10), a laparotomy group (n = 10), and a control group (n = 15), which were all subjected to the procedures described above. Five mice from each group were killed on postoperative days (PODs) 1 and 3, and five mice from the control group were killed just after being anesthetized. PECs and spleens were harvested 1 and 3 days after the respective procedure. For the purification of splenocytes, spleens were teased on a stainless mesh screen.

PECs and splenocytes were washed three times in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin and streptomycin. Cells were assessed by trypan blue exclusion to assure greater than 95% viability. PECs or splenocytes were resuspended in supplemented RPMI to a concentration of 5.0×10^5 viable cells/100 μl for use as effector cells. Yac-1 cells, which are T-cell lymphoma cells, served as target cells. Viable Yac-1 cells, 1.0×10^6, were suspended in 100 μl of supplemented RPMI, labeled with 3.7 MBq ^{51}Cr, and incubated at 37°C for 60 min. After incubation, labeled Yac-1 cells were washed three times in supplemented RPMI and resuspended at a concentration of 5.0×10^3 cells/100 μl.

NKCC was determined as previously described.[16,17] Briefly, 100 μl of effector cell suspension at various dilutions (100:1, 50:1, 25:1, 12.5:1, 6.25:1) was incubated in round-bottomed microtiter plates with 5.0×10^3 Yac-1 cells in 100 μl supplemented RPMI for 4 h in a 37°C humidified 5% CO_2 atmosphere. Spontaneous release was determined by the incubation of Yac-1 cells alone, and maximum release was induced by lysing the Yac-1 cells with sodium dodecyl sulfate. A 100-μl aliquot was removed from each well, and disintegrations were counted in an auto-gamma scintillation spectrometer (ARC-2000, Aloka, Osaka, Japan). Specific cytotoxicity was calculated according to the following formula:

$$\% \text{ of NKCC} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximum release} - \text{Spontaneous release}} \times 100$$

Statistical analysis was performed with Student's t-test. A P value of less than 0.05 was considered significant.

Results

The number of PECs harvested was similar in the three groups at each time point (Table 1). PEC lymphocytes were also calculated by the flow cytometer and shown as the percentage of lymphocytes to PECs. There were no statistical differences among the three groups (Fig. 1).

$CD3^+$, $DC4^+$, and $CD8^+$ cells are expressed as the percentage of T lymphocytes among the total PEC lymphocytes. In the pneumoperitoneum group, the percentages of $CD3^+$, $DC4^+$, and $CD8^+$ cells increased from the baseline value up until postoperative day (POD) 3, then returned to the baseline value by POD 10 (Fig. 2, data of $CD4^+$ and $CD8^+$ cells, not shown). In the laparotomy group, the percentages of $CD3^+$, $DC4^+$, and $CD8^+$ cells increased up until POD 7, then returned to the baseline value. The peak percentage of $CD3^+$ cells on POD 7 was significantly higher in the laparotomy group than in the pneumoperitoneum group (25.5% ± 14.3% *vs* 12.2% ± 4.6%). The changes in $CD4^+$ and $CD8^+$ cells were similar to those in the $CD3^+$ cells, and there were significant differences between the two groups on POD 7 (21.3% ± 10.4% *vs* 10.7% ± 6.6%, 5.9% ± 2.0% *vs* 2.6% ± 1.4%). The CD4/8 ratio on POD 3 was significantly lower in the laparotomy group than in the pneumoperitoneum group (Fig. 3).

The percentage of NK cells in PECs increased during the early period after pneumoperitoneum and laparotomy, and returned to preoperative levels by POD 10 (Fig. 4). The percentages of NK cells in the laparotomy group on PODs 1, 3, and 7 were significantly higher than those in the pneumoperitoneum group on the same days (16.1% ± 10.7% *vs* 7.6% ± 3.3%, 11.5% ± 2.9% *vs* 5.6% ± 3.2%, 6.3% ± 2.7% *vs* 2.6% ± 1.2%).

The NKCC of splenocytes on POD 1 was less impaired after pneumoperitoneum than after laparotomy (10.3% ± 1.5% *vs* 5.0% ± 1.1%, $P < 0.05$) (Fig. 5). However, the difference in the NKCC of the PECs between the two groups was not significant.

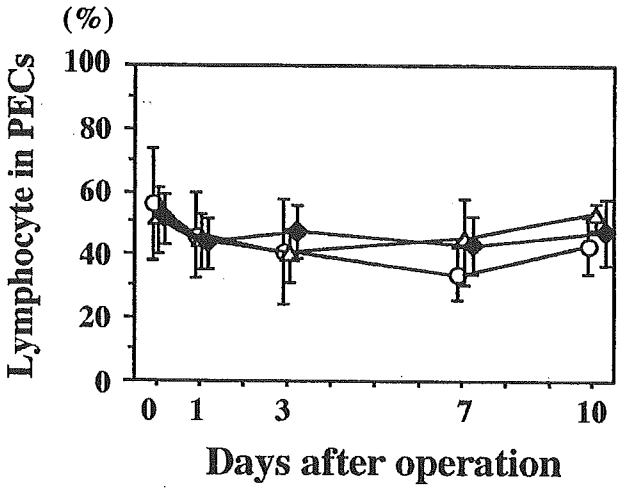

Fig. 1. Variations of lymphocytes in peritoneal exudative cells (*PECs*) in pneumoperitoneum (*open triangles*), laparotomy (*open circles*), and control groups (*closed diamonds*). There were no significant differences among the groups. Values are expressed as mean ± SD

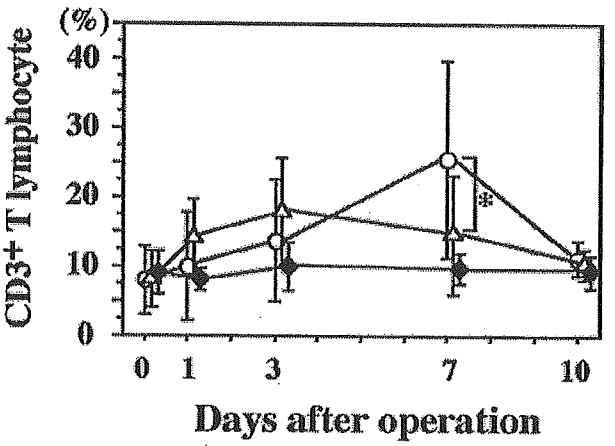

Fig. 2. Variations of $CD3^+$ lymphocytes in peritoneal exudative cells in the pneumoperitoneum (*open triangles*), laparotomy (*open circles*), and control groups (*closed diamonds*). Values are expressed as mean ± SD. $*P < 0.05$

Table 1. Number of peritoneal exudative cells harvested

Group	Days after surgery					
($\times 10^6$ cells/mouse)	0	1	3	7	10	*P* value
Pneumoperitoneum	4.0 ± 2.1	5.9 ± 2.2	5.9 ± 1.6	7.0 ± 2.7	6.2 ± 0.6	NS
Laparotomy	3.6 ± 3.1	6.0 ± 2.2	5.6 ± 1.4	6.5 ± 2.2	5.0 ± 1.7	NS
Control	4.8 ± 1.0	7.4 ± 1.8	6.7 ± 1.7	6.5 ± 1.7	6.0 ± 1.4	NS

Data are expressed as mean ± SD
NS, not significant

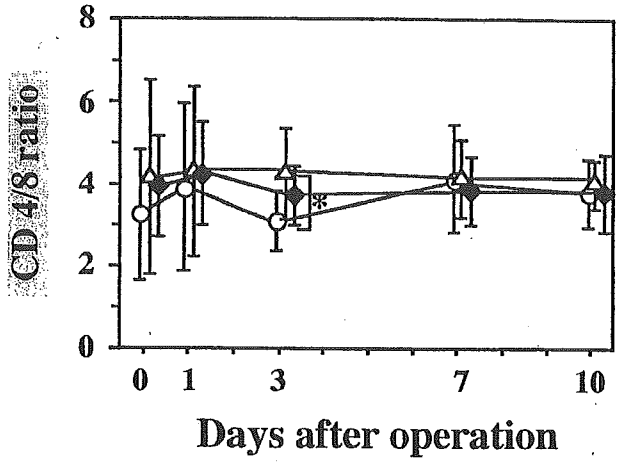

Fig. 3. Variations of CD4/8 ratio in the pneumoperitoneum (*open triangles*), laparotomy (*open circles*), and control groups (*closed diamonds*). Values are expressed as mean ± SD. $*P < 0.05$

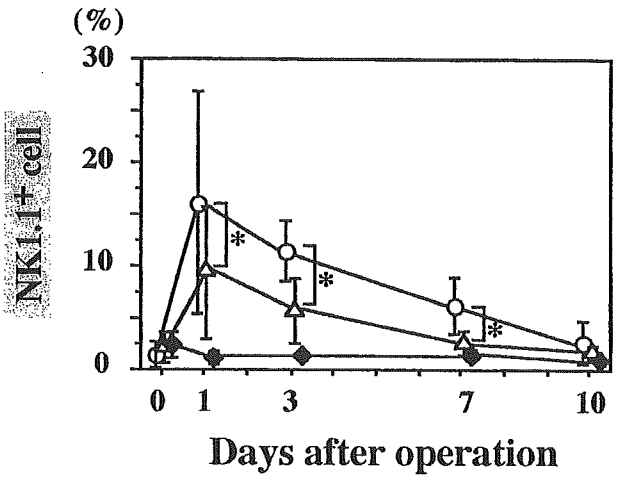

Fig. 4. Variations of NK1.1$^+$ cells in the pneumoperitoneum (*open triangles*), laparotomy (*open circles*), and control groups (*closed diamonds*). Values are expressed as mean ± SD. $*P < 0.05$

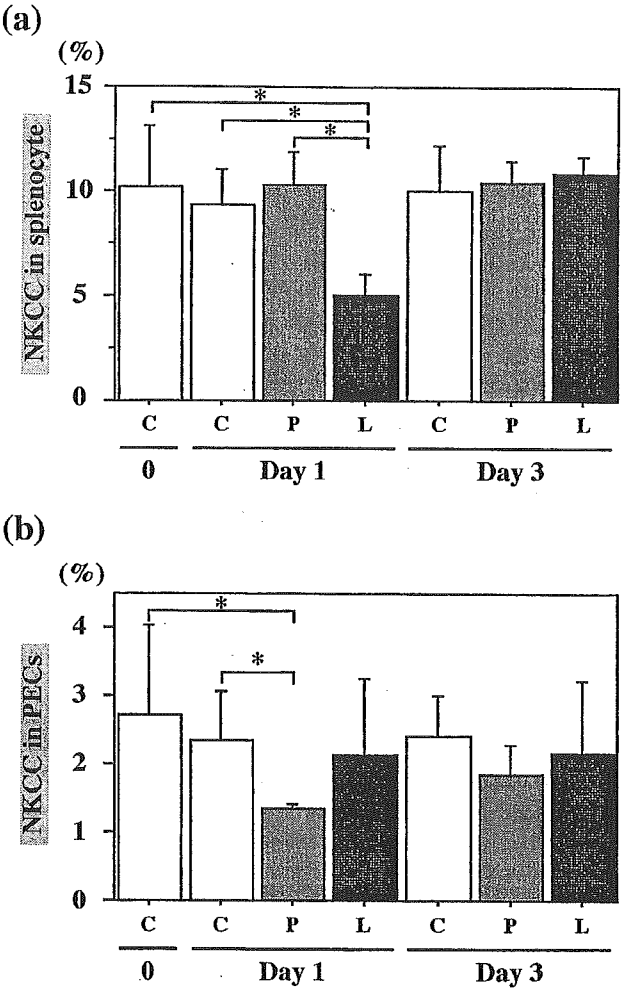

Fig. 5. Variations of natural killer cell cytotoxicity in splenocytes (**a**) and peritoneal exudative cells (**b**) in the laparotomy, pneumoperitoneum, and control groups. The effector and target ratios were 100:1 and 50:1 for the splenocytes and peritoneal exudative cells, respectively. *C*, control; *P*, pneumoperitoneum; *L*, laparotomy. Values are expressed as mean ± SD. $*P < 0.05$

Discussion

This animal study showed the effects of CO_2 pneumoperitoneum on cell-mediated immune response in the peritoneal cavity. Although the changing pattern of T-lymphocyte populations in the peritoneal cavity after CO_2 pneumoperitoneum was similar to that after laparotomy, the day-3 CD4/8 ratio decreased to a lesser degree after pneumoperitoneum than after laparotomy. The population of NK cells in the peritoneal cavity increased further after laparotomy than after pneumoperitoneum, but the peritoneal NKCC after pneumoperitoneum did not differ significantly from that after laparotomy. Thus, cell-mediated immune response in the murine peritoneal cavity was less impaired after CO_2 pneumoperitoneum than after laparotomy.

Several reports have been published on the systemic cell-mediated immune response after laparoscopic surgery compared with that after open surgery. Using a rat model, Trokel et al. showed that delayed-type hypersensitivity (DTH) response was better preserved after laparoscopy than after laparotomy.[9] Gitzelmann et al. and Gleason et al. also showed that cell-mediated immune function was better preserved after CO_2 pneumo-