ORIGINAL ARTICLE

Immunological Effects of Laparoscopic vs Open Colorectal Surgery

A Prospective Clinical Study

Matthias W. Wichmann, MD; Thomas P. Hüttl, MD; Hauke Winter, MD; Fritz Spelsberg, MD; Martin K. Angele, MD; Markus M. Heiss, MD; Karl-Walter Jauch, MD

Hypothesis: Laparoscopy has become a popular approach for the surgical treatment of benign and even malignant colorectal diseases. Several authors have reported better preserved immunity in patients undergoing laparoscopic compared with conventional colorectal surgery. The present study addresses the hypothesis that specific and nonspecific immunity are differently affected by laparoscopic and conventional colorectal surgery.

Design: Nonrandomized control trial.

Setting: University hospital.

Patients: Seventy prospectively enrolled patients with colorectal diseases undergoing laparoscopic (n=35) or open (n=35) surgery.

Main Outcome Measures: Blood samples were taken prior to surgery as well as on days 1, 3, and 5 after surgery. Cell numbers of lymphocyte subpopulations as well as natural killer cells were determined by flow cytometry, and interleukin 6 and C-reactive protein serum levels were measured.

Results: Significant differences between study and control patients ($P<.05$) were detected regarding circulating interleukin 6 and C-reactive protein levels with a reduced proinflammatory response to surgery in patients after laparoscopic surgery. Furthermore, postoperative natural killer cell counts were significantly higher in patients after laparoscopic surgery. The levels of B lymphocytes and T lymphocytes and helper T–cell counts and cytotoxic (suppressor) T–cell counts did not show significant differences after open or laparoscopic surgery.

Conclusions: Our findings indicate a less pronounced proinflammatory response to surgical trauma in patients after minimally invasive surgery. The nonspecific immune response appears to be less affected by laparoscopic surgery when compared with open surgery while the specific cell-mediated immunity is equally affected. These findings are important because a divergent effect on specific and nonspecific immunity of laparoscopic surgery for colorectal disease has not been reported before.

Arch Surg. 2005;140:692-697

Author Affiliations:
Department of Surgery, Ludwig-Maximilians University, Klinikum Grosshadern, Munich, Germany.

THE DEVELOPMENT OF MINIMALLY invasive surgery has allowed major changes in the surgical treatment of various benign and malignant diseases, especially because it limits surgical trauma.[1] During recent years, the laparoscopic approach has developed as an interesting therapeutic alternative for the resection of various colorectal diseases. This procedure has been shown to be feasible in most patients with benign disease and can be performed without an increase of perioperative morbidity and mortality rates.[2-7] Because the surgical trauma is limited, the laparoscopic approach usually allows for a rapid return to preoperative activity levels with significantly shorter hospitalization. Recent reports also indicate reduced rates of postoperative ileus, wound infection, and cardiorespiratory complications after laparoscopic surgery when compared with the open approach.[1,8,9]

Recently, the Clinical Outcomes of Surgical Therapy Study Group[10] has reported that laparoscopic-assisted colectomy and open colectomy for colon cancer provide comparable long-term results in a multi-institutional setting. These findings of a large multi-institutional study support the data published by Lacy et al,[11] who were able to show that laparoscopic surgery was superior to open surgery in colon cancer therapy in a single-center study when comparing morbidity, hospital stay, tumor recurrence, and cancer-related survival.

Experimental and clinical data, therefore, suggest that laparoscopic surgery is also suitable for the treatment of malignant disease. It appears that laparoscopic

resection of colorectal cancer is associated with clinically relevant benefits during the first weeks after surgery and that it can be performed with the same intention of radical treatment as conventional resection.[10-12] A recent study, however, reported that only minimal, short-term quality-of-life benefits could be observed with laparoscopic-assisted colectomy when compared with open colectomy for colon cancer.[13] Nonetheless, a finding that the laparoscopic approach to colorectal cancer results in less immunosuppression may have implications for the long-term prognosis of patients with cancer.[7]

Despite the promising clinical results, only limited information is available regarding the perioperative immunological effects of laparoscopic surgery when compared with conventional open large-bowel and rectal surgery. This issue is of major clinical interest because the reduced surgical trauma should result in reduced postoperative immune dysfunction in patients undergoing laparoscopic surgery, thus contributing to clinical and oncologic advantages for these patients.

Until now, it has been reported that the degree of postoperative inflammation is reduced after laparoscopic surgery.[14,15] Other groups also observed significantly better preservation of lymphocyte subpopulations, neutrophil function, and cell-mediated immunity after laparoscopic vs open colorectal surgery.[16-19] Furthermore, it has been observed that cell-mediated immunity, as assessed by delayed-type hypersensitivity testing in humans, is better preserved after laparoscopic vs open colorectal resection.[20] This lesser degree of operative stress was also confirmed by experimental animal studies by Kuntz et al.[21]

This prospective clinical study was performed to evaluate perioperative immune parameters in 70 patients with various colorectal diseases. We herein analyze the effects of laparoscopic and open surgery on proinflammatory cytokine levels (interleukin 6 [IL-6]) and C-reactive protein (CRP) levels. Furthermore, we measured lymphocyte subpopulations, leukocyte and granulocyte counts, and circulating natural killer (NK) cells before surgery and on days 1, 3, and 5 after surgery. This study, therefore, allows assessment of the effects of laparoscopic and conventional colorectal surgery on specific and nonspecific immune responses after major abdominal surgery.

METHODS

Patients with known immunological dysfunction (advanced liver disease, HIV [human immunodeficiency virus] infection, hepatitis C virus infection), drug addiction, and cardiac or pulmonary insufficiency were excluded from the study. Patients recruited for laparoscopic resection were not considered for analysis when converted to the open procedure (n=2 during the study period).

During the study period, we asked all patients who had surgery on one minimally invasive surgical ward (that of M.M.H.) to participate in our clinical case-control study. Control patients were recruited on another surgical ward (that of T.P.H.). Patient selection was based on their admission to different surgical wards in the same institution. All patients recruited for our clinical case-control study were informed that additional blood was taken during the perioperative period for immunological evaluation, and written consent was obtained.

Table. Monoclonal Antibodies Used for Flow-Cytometric Determination of Lymphocyte Subpopulations*

Reagent for Fluorescein or Phycoerythrin	CD for Fluorescein or Phycoerythrin	Monoclonal Antibodies
Simultest LeucoGATE	CD45/CD14	Immunological 3-part differential (lymphocytes, monocytes, neutrophils)
Isotype control	IgG1/IgG2a	Irrelevant antibodies to quantify nonspecific staining
Leucine-4/Leucine-3a	CD3/CD4	Helper/inducer T lymphocytes
Leucine-4/Leucine-2a	CD3/CD8	Cytotoxic/suppressor T lymphocytes
Leucine-4/Leucine 11c+19	CD3/CD16/56$^+$	Natural killer cells (CD3$^-$, CD16/56$^+$)
Leucine-4/Leucine-12	CD3/CD19	T lymphocytes, B lymphocytes

*All monoclonal antibodies were purchased from Becton Dickinson, San Jose, Calif.

Minimally invasive colorectal surgery was performed as a laparoscopic-assisted procedure with removal of the resected specimen via a horizontal minilaparotomy (5 cm) just above the mons pubis. Laparoscopic surgery was done using a 4-trocar technique with 1 trocar (10 mm) inserted via a paraumbilical incision (camera port). Two additional (5- or 10-mm) trocars were inserted in the right and left lower abdomen, and 1 trocar (12 mm) was inserted in the midline just above the mons pubis (the site of the minilaparotomy). After removal of the resected specimen and preparation of the stapler anastomosis, we closed the minilaparotomy and reintroduced pneumoperitoneum.

Conventional colorectal surgery was performed via a vertical midline incision ranging from 5 to 10 cm above the umbilicus to the mons pubis. After we removed the resected specimen, we performed a stapler anastomosis.

Blood samples were taken on the day before surgery as well as on days 1, 3, and 5 after surgery.

Peripheral venous blood samples were collected in EDTA collection tubes (Kabe, Nümbrecht-Elsenroth, Germany). The monoclonal antibodies used for immunophenotyping were purchased from Becton Dickinson (San Jose, Calif). The samples were prepared by labeling 50 µL of whole blood with 10 µL of monoclonal antibody for 10 minutes in the dark using the antibody combinations indicated in the **Table**. The monoclonal antibodies were conjugated to the fluorochrome fluorescein or phycoerythrin. The blood samples subsequently underwent a hypotonic lysis of red blood cells for 10 minutes (FACS Lysing Solution; Becton Dickinson) and were washed with phosphate buffered saline. The samples were made into pellets (300 g, room temperature, 5 minutes) and then resuspended in 150 µL of phosphate buffered saline solution. The fluorescence was measured using a FACScan (Becton Dickinson) within 60 minutes after processing the samples.

Fluorescence-activated cell sorter analysis was performed on a FACScan flow cytometer after calibration with CaliBRITE beads (Becton Dickinson) using the AutoCOMP software package (Becton Dickinson). A minimum of 10 000 cells were measured for each determination. For 2-parameter evaluation dot plots and quadrant statistics, we used the SimulSET software package (Becton Dickinson). The lymphocyte populations were automatically gated, including at least 98% of all lymphocytes measured within each sample.

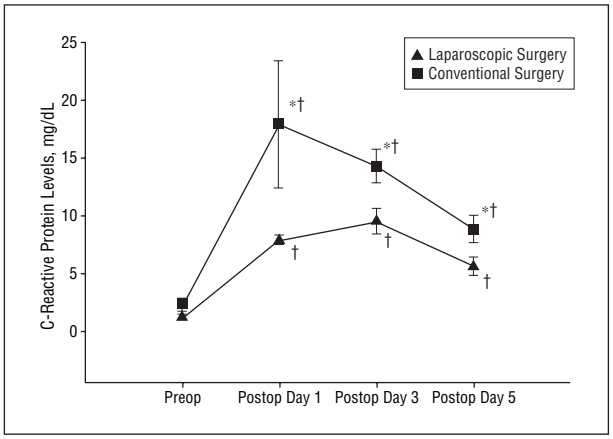

Figure 1. Circulating C-reactive protein levels as determined by immunoturbidimetry (Olympus, Hamburg, Germany) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for laparoscopic vs open surgery; dagger, $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

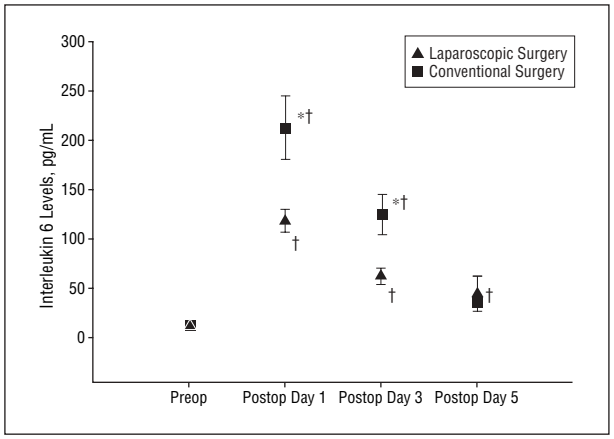

Figure 2. Circulating interleukin 6 levels as determined by enzyme-linked immunosorbent assays (Biosource, Nivelles, Belgium) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for laparoscopic vs open surgery; dagger, $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

Peripheral venous blood samples were collected in serum collection tubes (Kabe) and were subsequently centrifuged at 300 g for 15 minutes at 4°C. Serum aliquots were subsequently stored at –80°C until assayed for IL-6.

Circulating serum IL-6 levels were determined using enzyme-linked immunosorbent assays (Biosource, Nivelles, Belgium) as described by the manufacturer. The concentration of IL-6 present in the samples was determined at 450 nm on a Bio-Tek plate reader (EL-311; Bio-Tek Instruments Inc, Winooski, Vt). C-reactive protein was measured with the immunoturbidimetric method (Olympus, Hamburg, Germany).[22]

Data were analyzed using 1-way analysis of variance, analysis of variance on ranks, the Newman-Keuls test, and the Dunn test. Differences were considered statistically significant at $P<.05$. Data are presented as mean±SEM.

RESULTS

PATIENTS

Among the study patients (laparoscopic colorectal surgery), the mean±SEM patient age was 64.2±1.6 years, and most study patients were men (71%). The majority of study patients underwent surgery for colorectal cancer (76%). The mean±SEM length of surgery was 188±28 minutes. During the early postoperative period (until discharge from the hospital), complications (anastomotic leakage, surgical site infection, hemorrhage) occurred in 23% of the study patients. One patient died from myocardial infarction during the postoperative period (mortality rate, 5%).

Among the control patients (open colorectal surgery), the mean±SEM patient age was 61.5±1.6 years, and most control patients were men (57%). Again, most control patients underwent surgery for colorectal cancer (77%). The mean±SEM length of surgery was significantly shorter when compared with patients undergoing laparoscopic surgery, 104±13 minutes ($P<.05$). During the early postoperative period, complications were observed in 20% of the control patients.

PROINFLAMMATORY MEDIATORS

After both laparoscopic and open colorectal surgery, we observed a significant increase of circulating CRP levels (**Figure 1**). This increase was significantly higher in patients after conventional surgery when compared with patients after minimally invasive surgery.

After conventional and laparoscopic surgery, we observed a significant increase in serum IL-6 levels (**Figure 2**), which were significantly higher in patients after open surgery during the early postoperative period (days 1 and 3) and were comparable in both patient groups on day 5 after surgery.

MARKERS OF CELL-MEDIATED IMMUNE RESPONSE

After laparoscopic as well as conventional surgery, we saw a rapid but insignificant drop of circulating B lymphocytes ($CD19^+$), which was significantly different from preoperative values only in patients after laparoscopic surgery on postoperative day 5 (**Figure 3**).

We observed a significant depression of circulating T-lymphocyte ($CD3^+$) cells, which lasted until day 5 after surgery and did not differ between both patient groups (**Figure 4**).

A significant depression of circulating helper T cells ($CD3^+$ and $CD4^+$) was observed (**Figure 5**). No differences were detected when comparing study and control patients.

We observed a lasting significant depression of circulating cytotoxic (suppressor) T cells ($CD3^+$ and $CD8^+$) (**Figure 6**). The depression of cell counts was comparable in study and control patients.

We observed an initial significant depression of NK-cell ($CD16/56^+$ and $CD3^-$) counts in both patient groups (**Figure 7**). In patients who had minimally invasive surgery, this depression was reversed on day 5, and circulating NK cells were significantly higher on days 1 and 5 after surgery, when compared with patients after open colorectal surgery.

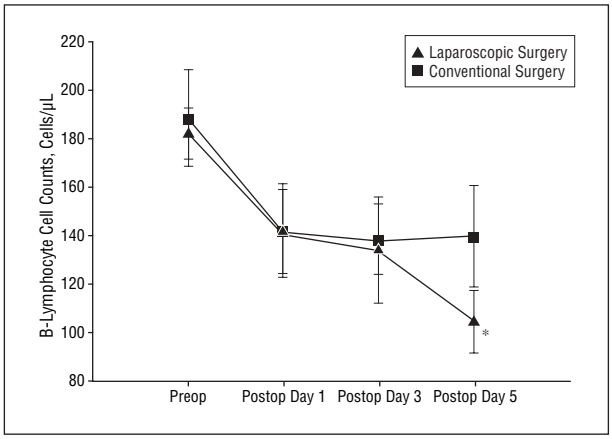

Figure 3. B-lymphocyte cell counts as determined by flow cytometry ($CD19^+$) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

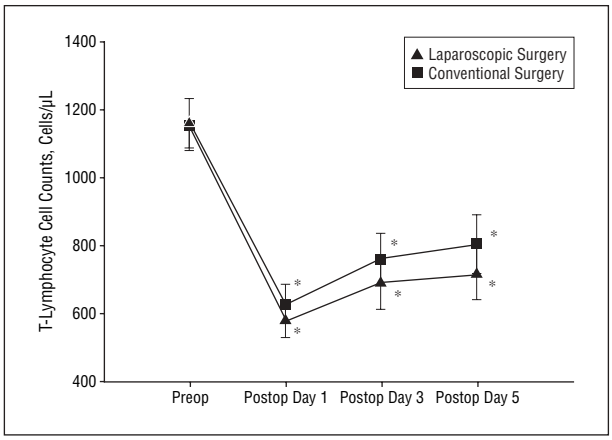

Figure 4. T-lymphocyte cell counts as determined by flow cytometry ($CD3^+$) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

COMMENT

Laparoscopic surgery is as safe as open colorectal surgery, and laparoscopic colorectal cancer surgery is feasible but should be performed within clinical studies and in surgical centers that have sufficient experience with laparoscopic colorectal surgery for benign diseases.[6,7,23] In addition to the ongoing discussion about the oncologic safety of this approach, it is still not completely clear whether the laparoscopic approach offers significant immunological advantages over the conventional open approach.[23,24] Whelan et al[20] recently reported a significantly better preservation of delayed-type hypersensitivity responses after laparoscopic vs conventional surgery. These findings indicate better preserved cell-mediated immune responses in patients after laparoscopic colorectal surgery.[20] The postoperative immune dysfunction is important for patients undergoing surgery for benign as well as malignant disease because it influences the rate of infectious complications as well as the growth of disseminated tumor cells.[25,26] Especially in patients with cancer, better preserved postoperative immunity could result in better long-term oncologic results.

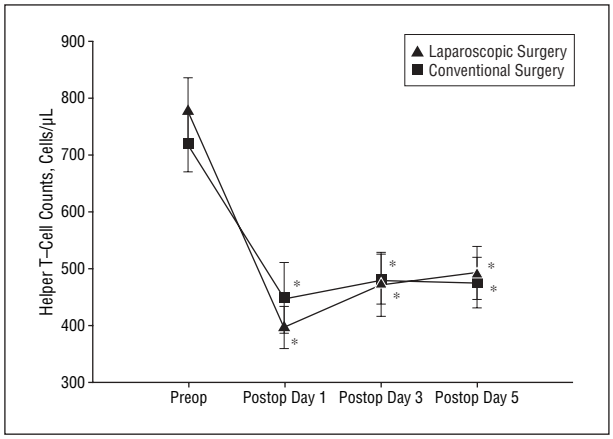

Figure 5. Helper T–cell counts as determined by flow cytometry ($CD3^+$ and $CD4^+$) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

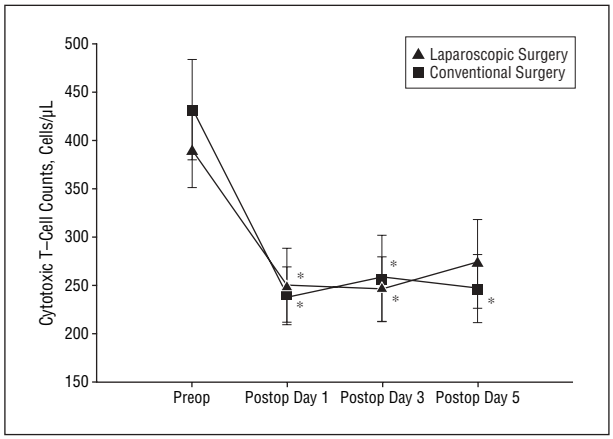

Figure 6. Cytotoxic (suppressor) T–cell counts as determined by flow cytometry ($CD3^+$ and $CD8^+$) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

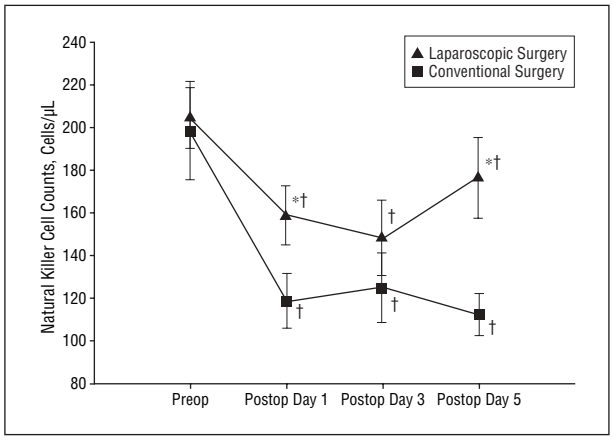

Figure 7. Natural killer cell counts as determined by flow cytometry ($CD3^-$ and $CD16/56^+$) in patients with colorectal disease treated with open and laparoscopic surgery. Asterisk indicates $P<.05$ for laparoscopic vs open surgery; dagger, $P<.05$ for preoperative (Preop) vs postoperative (Postop) values.

Recently, the Clinical Outcomes of Surgical Therapy Study Group[10] demonstrated in a multicenter setting with

872 patients that laparoscopic-assisted colectomy and open colectomy for colon cancer provide comparable long-term results. These authors provided good evidence to suggest that the laparoscopic approach is an acceptable alternative to open surgery for colon cancer. The findings reported by this study group support the results of Lacy et al,[11] who even reported better oncologic and clinical results of laparoscopic surgery when compared with open surgery.

We conducted this prospective clinical study to address the issue of potential differences in postoperative immunological alterations after conventional and laparoscopic colorectal surgery. In addition, this work focused on the potential differences in trauma-induced alterations of specific and nonspecific immunity.

As done in other clinical studies, we assessed immune function by measuring circulating immunocompetent cells (ie, helper T cells, cytotoxic T cells, and NK cells) as well as circulating IL-6 and CRP.[27-30] It has been reported that the functional capacity of NK cells correlates well with absolute cell numbers.[31] Furthermore, absolute numbers of circulating immunocompetent cells have been considered good indicators of the individual patient's immune function.[27-30]

Our findings show that minimally invasive surgery results in a less pronounced proinflammatory response to surgical trauma. In addition, we observed that the effector cells of the nonspecific immune response (NK cells) are less affected by laparoscopic surgery. The effector cells of the specific immune response (helper T cells and cytotoxic T cells), however, are equally depressed after conventional open as well as laparoscopic surgery for colorectal diseases.

In postoperative immune function, it has been reported that the systemic immune response was better preserved after laparoscopic surgery than after open surgery.[19,20,32] Nonetheless, Gupta and Watson[32] also showed that laparoscopic surgery was associated with a more severe suppression of intraperitoneal cell-mediated immunity, which may be relevant in the treatment of malignant disease. Braga et al[24] recently observed significantly fewer infectious complications and faster recovery after laparoscopic colorectal surgery when compared with open surgery. Moreover, it was reported that the suppressive effect of open surgery on the whole T-cell population was more evident 15 days after operation whereas no suppressive effect was found in the laparoscopic group.[24] These authors hypothesized that laparoscopy induced a less pronounced local inflammation than open surgery, which would allow for a faster return of T_H1 cells into circulation.[24] This hypothesis is in agreement with the lesser degree of proinflammation reported here as well as by other groups.[14,15] Nonetheless, our observation of significantly depressed helper T–cell counts after both laparoscopic and conventional colorectal surgery does not support this hypothesis of differently affected T_H1 cells and is at odds with the findings reported by Braga et al[24] and others who described better preserved cell-specific immunity after laparoscopic surgery.[19,20]

Our findings of better preserved nonspecific immune functions in patients after laparoscopic colorectal surgery help explain the reported lower rate of infectious complications in these patients.[24] Furthermore, it is interesting to note that the NK cell counts were less affected after laparoscopic colorectal surgery, but a more severe significant depression was observed after conventional surgery. This finding is relevant to the discussion of the potential oncologic advantages of laparoscopy because NK cells are important in controlling the growth of metastatic tumor cells, which are disseminated during surgical manipulation.[31,33] Moreover, it has been reported that NK cell cytotoxicity increases with the number of NK cells, and low preoperative levels of NK-cell cytotoxicity have been shown to correlate with an increased risk of colorectal cancer recurrence.[31] Cristaldi et al[34] also observed a less pronounced reduction of NK cells after laparoscopic surgery.

The findings of significant differences between patients after laparoscopic and conventional colorectal surgery regarding the release of CRP and IL-6 suggest a more pronounced proinflammatory response in patients undergoing conventional surgery. This observation confirms findings reported by Leung et al,[35] who detected significantly smaller peaks of circulating IL-1β, IL-6, and CRP levels in a group of 34 patients undergoing laparoscopic or conventional resection of rectosigmoid carcinoma. It is not clear whether these differences have immunological relevance because a certain degree of proinflammation is required for the initiation of host defense mechanisms as well as for the activation of repair processes after tissue trauma.[36,37] It is nonetheless well known that an overwhelming inflammatory response to surgical trauma may ultimately lead to organ dysfunction.[38,39]

After laparoscopic as well as conventional colorectal surgery, we observed a significant depression of circulating B and T lymphocytes as well as helper T cells and cytotoxic T cells. This observation is in agreement with the findings of other groups who have previously shown that a significant depression of these cell counts is associated with immune dysfunction and may even promote tumor growth.[28,40-42] The findings presented here indicate a comparable significant depression of circulating mediators of the specific immune system (ie, helper T–cell and cytotoxic T–cell counts). This observation is in agreement with the findings of Tang et al,[30] who also observed comparable changes of immune responses in patients undergoing laparoscopic or conventional colorectal cancer surgery. Other investigators, however, reported significantly better preservation of lymphocyte subpopulations, neutrophil function, and cell-mediated immunity after laparoscopic vs open colorectal surgery.[16-18] Obviously, additional prospective studies are required to elucidate the important question of whether laparoscopy offers significant advantages regarding specific immune function in patients with colorectal diseases. If we consider together all of the studies that have investigated systemic immunological function after laparoscopic and open surgery in both experimental models and in clinical settings, we see their outcomes have consistently demonstrated that laparoscopic approaches are associated with less overall disturbances of the systemic immune function (Gupta and Watson[32] provide a review). Nonetheless, our findings do not confirm better preserved specific immune response in patients undergoing laparoscopic colorectal surgery, as previously reported by Whelan et al.[20]

It is not known whether the observed immunological effects of laparoscopic colorectal surgery are of direct oncologic relevance and contribute to the observed comparable or even better oncologic results of laparoscopic surgery for malignant colorectal disease, which have been reported in the literature.[10,11]

The long-term effects of immunosuppression in response to treatment and development of metastases still remain obscure. However, we can assume that our observation of better preserved nonspecific immunity in patients after laparoscopic colorectal surgery has beneficial effects on perioperative infectious complication rates—which is true for patients with benign as well as malignant colorectal disease. In addition, our findings are of clinical interest because a divergent effect on specific and nonspecific immunity of laparoscopic surgery for colorectal disease has not been previously reported.

Accepted for Publication: November 30, 2004.
Correspondence: Matthias W. Wichmann, MD, Department of Surgery, Ludwig-Maximilians University, Klinikum Grosshadern, Marchioninistrasse 15, 81377 Munich, Germany (Matthias.Wichmann@med.uni-muenchen.de).

REFERENCES

1. COLOR Study Group. COLOR: a randomized clinical trial comparing laparoscopic and open resection for colon cancer. *Dig Surg*. 2000;17:617-622.
2. Seow-Choen F, Eu KW, Ho YH, Leong AF. A preliminary comparison of a consecutive series of open versus laparoscopic abdomino-perineal resection for rectal adenocarcinoma. *Int J Colorectal Dis*. 1997;12:88-90.
3. Wexner SD, Reissman P, Pfeifer J, Bernstein M, Geron N. Laparoscopic colorectal surgery. *Surg Endosc*. 1996;10:133-136.
4. Köckerling F, Rose J, Schneider C, et al. Laparoscopic colorectal anastomosis: risk of postoperative leakage. Results of a multicenter study. Laparoscopic Colorectal Surgery Study Group (LCSSG). *Surg Endosc*. 1999;13:639-644.
5. Köckerling F, Schneider C, Reymond MA, et al. Early results of a prospective multicenter study on 500 consecutive cases of laparoscopic colorectal surgery. Laparoscopic Colorectal Surgery Study Group (LCSSG). *Surg Endosc*. 1998;12:37-41.
6. Fazio VW, López-Kostner F. Role of laparoscopic surgery for treatment of early colorectal carcinoma. *World J Surg*. 2000;24:1056-1060.
7. Maxwell-Armstrong CA, Robinson MH, Scholefield JH. Laparoscopic colorectal cancer surgery. *Am J Surg*. 2000;179:500-507.
8. Stewart BT, Stitz RW, Lumley JW. Laparoscopically assisted colorectal surgery in the elderly. *Br J Surg*. 1999;86:938-941.
9. Basse L, Madsen JL, Billesbølle P, Bardram L, Kehlet H. Gastrointestinal transit after laparoscopic vs open colonic resection. *Surg Endosc*. 2003;17:1919-1922.
10. Clinical Outcomes of Surgical Therapy Study Group. A comparison of laparoscopically assisted and open colectomy for colon cancer. *N Engl J Med*. 2004;350:2050-2059.
11. Lacy AM, Garcia-Valdecasas JC, Delgado S, Castells A, Taurá P, Piqué JM. Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet*. 2002;359:2224-2229.
12. Müller JM, Schwenk W, Jacobi CA, Böhm B. Endoscopic surgery: fit for malignancy? *World J Surg*. 1999;23:808-815.
13. Weeks JC, Nelson H, Gelber S, Sargent D, Schroeder G. Short-term quality-of-life outcomes following laparoscopic-assisted colectomy vs open colectomy for colon cancer: a randomized trial. *JAMA*. 2002;287:321-328.
14. Feussner H, Siewert JR. Reduktion des Zugangstraumas: gesicherte Vorteile [Reduction of access trauma: safe advantages]. *Chirurg*. 2001;72:236-244.
15. Schwenk W, Jacobi C, Mansmann U, Bohm B, Muller JM. Inflammatory response after laparoscopic and conventional colorectal resections. *Langenbecks Arch Surg*. 2000;385:2-9.
16. Kehlet H, Nielsen HJ. Impact of laparoscopic surgery on stress responses, immunofunction, and risk of infectious complications. *New Horiz*. 1998;6:S80-S88.
17. Bessler M, Whelan RL, Halverson A, Treat MR, Nowygrod R. Is immune function better preserved after laparoscopic versus open colon resection? *Surg Endosc*. 1994;8:881-883.
18. Carey PD, Wakefield CH, Thayeb A, Monson JRT, Darzi A, Guillou PJ. Effects of minimally invasive surgery on hypochlorous acid production by neutrophils. *Br J Surg*. 1994;81:557-560.
19. Bolla G, Tuzzato G. Immunologic postoperative competence after laparotomy vs laparotomy. *Surg Endosc*. 2003;17:1247-1250.
20. Whelan RL, Franklin M, Holubar SD, et al. Postoperative cell mediated immune response is better preserved after laparoscopic vs open colorectal resection in humans. *Surg Endosc*. 2003;17:972-978.
21. Kuntz C, Wunsch A, Bay F, Windeler J, Glaser F, Herfarth C. Prospective randomized study of stress and immune responses in laparoscopic vs conventional colonic resections. *Surg Endosc*. 1998;12:963-967.
22. Hoechtlen-Vollmar W, Menzel G, Bartl R, Lamerz R, Wick M, Seidel D. Amplification of cyclin D1 gene in multiple myeloma: clinical and prognostic relevance. *Br J Haematol*. 2000;109:30-38.
23. Wichmann MW, Meyer G, Angele MK, Schildberg FW, Rau HG. Recent advances in minimally invasive colorectal cancer surgery. *Onkologie*. 2002;25:318-323.
24. Braga M, Vignali A, Gianotti L, et al. Laparoscopic versus open colorectal surgery: a randomized trial on short-term outcome. *Ann Surg*. 2002;236:759-767.
25. Salo M. Effects of anaesthesia and surgery on the immune response. *Acta Anaesthesiol Scand*. 1992;36:201-220.
26. Shigemitsu Y, Saito T, Kinoshita T, Kobayashi M. Influence of surgical stress on bactericidal activity of neutrophils and complications of infection in patients with esophageal cancer. *J Surg Oncol*. 1992;50:90-97.
27. Ordemann J, Jacobi CA, Schwenk W, Stösslein R, Müller JM. Cellular and humoral inflammatory response after laparoscopic and conventional colorectal resections: results of a prospective randomized trial. *Surg Endosc*. 2001;15:600-608.
28. Lennard TWJ, Shenton BK, Borzotta A, et al. The influence of surgical operations on components of the human immune system. *Br J Surg*. 1985;72:771-776.
29. Tartter PI. Preoperative lymphocyte subsets and infectious complications after colorectal cancer surgery. *Surgery*. 1988;103:226-230.
30. Tang CL, Eu KW, Tai BC, Soh JGS, Machin D, Seow-Choen F. Randomized clinical trial of the effect of open versus laparoscopically assisted colectomy on systemic immunity in patients with colorectal cancer. *Br J Surg*. 2001;88:801-807.
31. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. *Arch Surg*. 1987;122:1264-1268.
32. Gupta A, Watson DI. Effect of laparoscopy on immune function. *Br J Surg*. 2001;88:1296-1306.
33. Tartter PI, Martinelli G, Steinberg B, Barron D. Changes in peripheral T-cell subsets and natural-killer cytotoxicity in relation to colorectal cancer surgery. *Cancer Detect Prev*. 1986;9:359-364.
34. Cristaldi M, Rovati M, Elli M, et al. Lymphocytic subpopulation changes after open and laparoscopic cholecystectomy: a prospective and comparative study on 38 patients. *Surg Laparosc Endosc*. 1997;7:255-261.
35. Leung KL, Lai PB, Ho RL, et al. Systemic cytokine response after laparoscopic-assisted resection of rectosigmoid carcinoma: a prospective randomized trial. *Ann Surg*. 2000;231:506-511.
36. Jeschke MG, Wolf SE, DebRoy MA, Herndon DN. The combination of growth hormone with hepatocyte growth factors alters the acute phase response. *Shock*. 1999;12:181-187.
37. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448-454.
38. Faist E, Wichmann MW. Immunologie bei Schwerverletzten [Immunology in the severely injured]. *Chirurg*. 1997;68:1066-1070.
39. Baue AE. MOF/MODS, SIRS: an update. *Shock*. 1996;6:S1-S5.
40. Faist E, Mewes A, Strasser T, et al. Alteration of monocyte function following major injury. *Arch Surg*. 1988;123:287-292.
41. Eggermont AM, Steller EP, Sugarbaker PH. Laparotomy enhances intraperitoneal tumor growth and abrogates the antitumor effects of interleukin-2 and lymphokine-activated killer cells. *Surgery*. 1987;102:71-78.
42. Weese JL, Ottery FD, Emonto SE. Do operations facilitate tumor growth? an experimental model in rats. *Surgery*. 1986;100:273-277.