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Comparison of interleukin-6, interleukin-8, and granulocyte colony–stimulating factor production by the peritoneum in laparoscopic and open surgery

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Abstract
Background: Human mesothelial cells secrete a variety of cytokines. The levels of postoperative serum inflammatory cytokines are thought to reflect the magnitude of surgical stress.
Methods: Pieces of peritoneum were obtained immediately upon and 1 h after entry into the abdominal cavity in nine patients undergoing laparoscopic surgery and 11 patients undergoing open surgery. The samples were cultured and interleukin (IL)-6, IL-8, and granylocyte colony–stimulating factor (G-CSF) levels in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA). Expression of IL-6, IL-8, and G-CSF mRNAs was examined by RT-PCR.
Results: At 1 h after laparotomy, the amounts of IL-6 and G-CSF produced by the peritoneum were significantly greater than those obtained immediately after the procedure, but this difference was not observed with laparoscopic surgery. Reverse transcription–polymerase chain reaction (RT-PCR), which showed an increase in the expression of cytokine mRNAs at 1 h after laparotomy, was compatible with these results.
Conclusion: The lower levels of cytokine production by the peritoneum suggest that laparoscopic surgery is associated with lower degree of surgical stress.

Key words: Peritoneum — Laparoscopy — Laparotomy — Interleukin (IL)-6 — IL-8 — Granulocyte colony–stimulating factor

Laparoscopic surgery (LS) is now widely accepted by surgeons as an alternative to conventional open surgery (OS). LS has many advantages, including quicker recovery, less wound pain, better cosmesis, and a shorter hospital stay [3, 8]. It is thought that these advantages may be due to a decrease in surgical stress; the procedure is less traumatic overall.

Activation of the cytokine network is an important part of the acute response to stimuli such as surgical trauma. It has been shown that the serum levels of interleukin (IL)-6, IL-8, and granulocyte colony–stimulating factor (G-CSF) increase after surgery and correlate with the magnitude of surgical stress [9, 13, 18]. It has also been reported that postoperative serum levels of inflammatory cytokines are lower with LS than with OS [10, 11]. In addition, high levels of IL-6 and IL-8 have been detected in exudate from the peritoneal fluid after abdominal surgery [13, 14], suggesting that cytokines produced by the abdominal cavity may be a main source of serum cytokines. It was reported recently that mesothelial cells established from human peritoneum secrete a variety of cytokines [7, 20]. Therefore, it is quite possible that peritoneal mesothelial cells participate in the inflammatory response during abdominal surgery and that the difference in the degree of surgical stress between LS and OS may be due to inflammatory cytokine production by peritoneal cells. However, there have been no reports of the influence of surgical insult on cytokine production by peritoneal cell.

To determine the difference in the magnitude of surgical insult between LS and OS, we compared IL-6, IL-8, and G-CSF production by peritoneal tissue obtained immediately upon and at 1 h after entry into the abdominal cavity in patients undergoing LS and OS.

Patients and methods

From May 1998 to June 2000, 20 patients (15 men and five women, ages 29–75 years) who underwent elective surgery under general anesthesia in the Department of Surgery II, Yamaguchi University School of Medicine, were included in this study. General anesthesia was maintained by ventilation with 50% nitrous oxide in oxygen and isoflurane as needed. None of the patients had chemotherapy, immunotherapy, or radiotherapy prior to surgery, and none showed any evidence of an acute phase reaction such as leukocytosis or low- or

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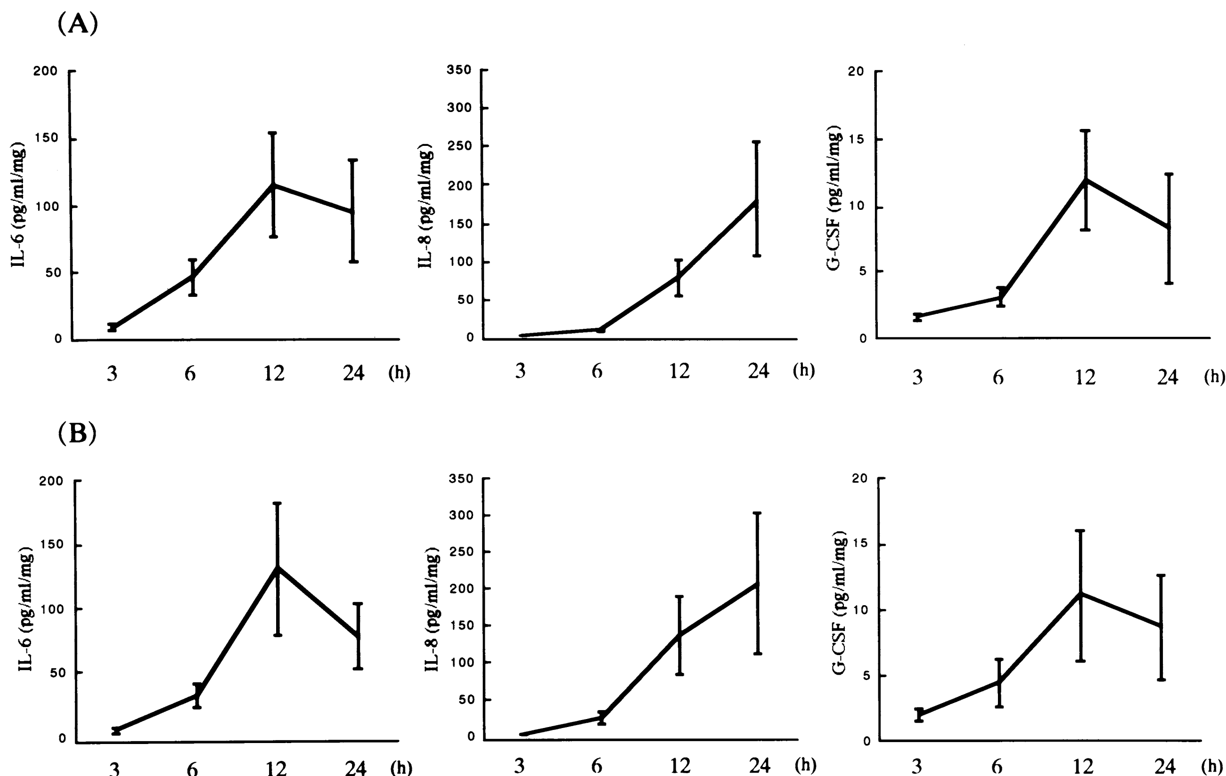

Fig. 1. Time course of IL-6, IL-8 and G-CSF production by cultured peritoneal tissue. **A** Laparoscopic surgery group, **B** Open surgery group.

Table 1. Patient characteristics

	LS Group (n = 9)	OS Group (n = 11)
Age (years)	55.7 ± 4.6*	65.0 ± 3.5*
Sex ratio (male/female)	6 / 3	9 / 2
Surgical procedures		
	Colectomy : 4	Distal gastrectomy : 7
	Cholecystectomy : 3	Proximal gastrectomy : 3
	Nissen's procedure : 1	Rectal amputation : 1
	Partial resection of stomach : 1	

LS, Laparoscopic surgery; OS, open surgery
* mean ± SE

high-grade fever. The patients were divided into the following two groups: a laparoscopic surgery group (LC group; $n = 9$) and an open surgery group (OS group; $n = 11$). All laparoscopic surgeries were performed by pneumoperitoneum with carbon dioxide (CO_2) at a pressure of 8–10 mmHg. Table 1 shows the patient characteristics.

Informed consent was obtained from all patients. The study protocol was approved by the Institutional Review Board for Experiments on Human Subjects at the Yamaguchi University School of Medicine.

Preparation of the peritoneum

Peritoneal tissue samples were obtained immediately upon (0 h) and at 1 h after entry into the abdominal cavity in both groups. Samples were taken from the right upper abdominal wall, a site far from the surgical field. The samples were resected with surgical shears after expansion by injection of saline into the subperitoneal space. The samples were divided into four pieces and weighed twice. They were then immediately transferred to 2 ml of DMEM (GIBCO BRL, Grand Island, NY, USA) containing 10% fetal calf serum (Sigma Chemical Co., St. Louis, MO, USA) and antibiotics (penicillin, 100 units/ml; streptomycin, 100 µg/ml). Specimens were incubated for 3, 6, 12, and 24 h at 37°C in 5% CO_2 atmosphere. The culture supernatants were stored at −80°C until cytokine assay. Peritoneal samples obtained at 0 h and 1 h from two patients were frozen immediately at −80°C for later mRNA analysis.

IL-6, IL-8, and G-CSF assays

Levels of IL-6, IL-8, and G-CSF in culture supernatants were measured with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions (human IL-6 Immunoassay Kit; Biosource, Camarillo, CA, USA; human IL-8 Immunoassay Kit; Otsuka Pharmaceutical Co., Tokushima, Japan; Human G-CSF Immunoassay Kit; Otsuka). Detection limits were 2 pg/ml for the IL-6 assay and 20 pg/ml for the IL-8 and G-CSF assays. Lower levels were considered undetectable. Levels of IL-6, IL-8, and G-CSF produced per 1 mg of peritoneum were determined.

RNA Extraction

Total RNA was isolated with Trizol reagent (GIBCO BRL) according to the manufacturer's protocol. Sufficient amounts of RNA for reverse transcription–polymerase chain reaction (RT-PCR) were obtained

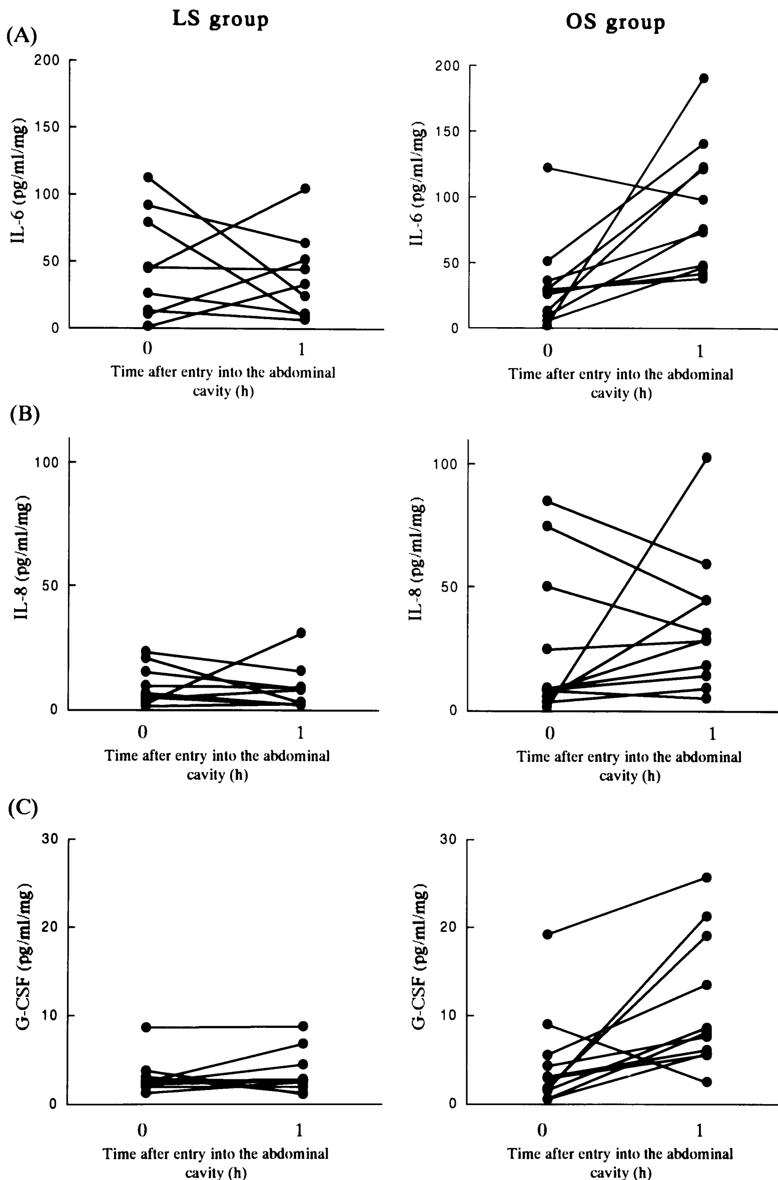

Fig. 2. Peritoneal IL-6 (**A**), IL-8 (**B**), and G-CSF (**C**) production by cultured peritoneum at 6 h incubation for the LS group and the OS group. LS, laparoscopic surgery; OS, open surgery.

with three peritoneal samples (one at 0 h and two at 1 h) from two OS patients. However, in the LS group, the sample volumes were not adequate to permit extraction of RNA.

Conventional RT-PCR for analysis of the expression of IL-6, IL-8, and G-CSF mRNAs

RT-PCR amplification was performed with an RNA PCR Kit (AMV) Ver. 2.1 (TaKaRa, Tokyo, Japan) and a modified version of our previously described procedure [6]. Briefly, 1 μl of total RNA (1 μg) was added to 19 μl of RT mix, which comprised 2 μl 10× RNA PCR buffer, 4 μl $MgCl_2$, 1 μl random 9-mers, 2 μl dNTP mixture, 0.5 μl RNase inhibitor, 8.5 μl distilled water, and 1 μl reverse transcriptase. After mixing, the samples were first incubated at 30°C for 10 min, and then at 55°C for 30 min. The reverse transcriptase was inactivated by incubation at 98°C for 5 min. A 10-μl aliquot of the 20 μl of resulting cDNA was used for PCR amplification with primers for IL-6 (BioSource International), IL-8 (Stratagene, La Jolla, CA, USA), G-CSF (Stratagene), and β-actin (internal control). The cDNA was added to 40 μl of PCR mixture, which was comprised of 4 μl of ×10 RNA PCR buffer (100 mM Tris-HCL, pH8.3, 500 mM KCL, 15 mM $MgCl_2$, 0.01% gelatin; Perkin-Elmer/Cetus, San Diego, CA, USA), 3 μl of 10 mM $MgCl_2$, 31.7 μl of distilled water, 0.5 μl of 10 μM 5′-primer, 0.5 μl of 10 μM 3′-primer, and 0.3 μl (1.5 units) of Taq polymerase (Perkin-Elmer/Cetus).

Approximately 50 μl of the reaction mixture was overlaid with 20 μl of mineral oil, and cycling was performed with a thermal cycler (PC-700; ASTEC, Fukuoka, Japan) according to the following procedure: denaturation at 94°C for 1 min, annealing at 55°C for 45 sec, and extension at 72°C for 2-min PCR amplifications of IL-6, IL-8, G-CSF, and β-actin were performed for 32, 34, 32, and 30 cycles, respectively. The expected sizes of the amplification products were 510 base pairs (bp) for IL-6, 200 bp for IL-8, 471 bp for G-CSF, and 1224 bp for β-actin. The PCR products were analyzed by 1% agarose gel electrophoresis.

Statistical analysis

We used two-way analysis of variance (ANOVA) for repeated measures for comparison of the kinetics of each cytokine in the culture supernatants of peritoneal samples. Comparisons of cytokine production between samples obtained at 0 h and 1 h and between the LS and OS groups were performed by paired and unpaired *t*-test, respectively. A *p* value of < 0.05 was considered statistically significant. Data are expressed as mean ± standard error (SE).

Results

Time course of IL-6, IL-8, and G-CSF production from the peritoneum

Figure 1 shows the time course of production of IL-6, IL-8, and G-CSF in supernatants of cultures of 1 mg of peritoneal tissue obtained immediately (0 h) after entry into the abdominal cavity in both the LS and OS groups. There were no significant differences between the groups; IL-8 levels increased in a time-dependent manner, but IL-6 and G-CSF levels peaked at 12 h of incubation. The standard errors for the levels of each cytokine at 12 h and 24 h of incubation were much greater than those at 3 h and 6 h of incubation. On the basis of these results, we selected 6 h as the appropriate incubation time for statistical analysis.

IL-6, IL-8, and G-CSF production by peritoneal tissues obtained by laparoscopic or open surgery

Figure 2 shows the levels of IL-6, IL-8, and G-CSF at 6 h of incubation in culture supernatants of peritoneal tissue obtained at 0 h and 1 h after entry into the abdominal cavity. IL-6 levels at 0 h vs 1 h were 47.0 ± 13.1 vs 39.1 ± 10.6 pg/ml/mg in the LS group and 32.3 ± 10.0 vs 90.8 ± 14.8 pg/ml/mg in the OS group. IL-8 levels at 0 h vs 1 h were 9.7 ± 2.7 vs 9.0 ± 3.1 pg/ml/mg in the LS group and 25.2 ± 9.2 vs 35.1 ± 8.4 pg/ml/mg in the OS group. G-CSF levels at 0 h vs 1 h were 3.1 ± 0.7 vs 3.5 ± 0.9 pg/ml/mg in the LS group and 4.6 ± 1.7 vs 11.2 ± 2.8 pg/ml/mg in the OS group.

In the LS group, cytokine levels did not differ between 0 h and 1 h. In contrast, in the OS group, IL-6 and G-CSF levels at 1 h were significantly higher than those at 0 h ($p = 0.008$ and $p = 0.011$, respectively). Although IL-8 levels did not increase significantly, its levels significantly increased at 1 h ($p < 0.02$) if three cases having high IL-8 levels at 0 h are excluded. Furthermore, IL-6, IL-8, and G-CSF levels at 1 h in the OS group were significantly higher than those in the LS group ($p = 0.014$, $p = 0.006$, and $p = 0.006$, respectively).

IL-6, IL-8, and G-CSF mRNA expression in peritoneal tissue

Two samples of peritoneum obtained at 0 h after laparotomy yielded no distinct RT-PCR products specific for IL-6, IL-8, and G-CSF; whereas four samples obtained at 1 h after laparotomy yielded IL-6, IL-8, and G-CSF RT-PCR products, with the exception of two samples that did not give G-CSF products (Fig. 3).

Discussion

In the present study, the levels of IL-6, IL-8, and G-CSF produced by the peritoneum after LS were significantly lower than those produced after OS. The increased expression of cytokine mRNAs by the peritoneum at 1 h after laparotomy supported these findings.

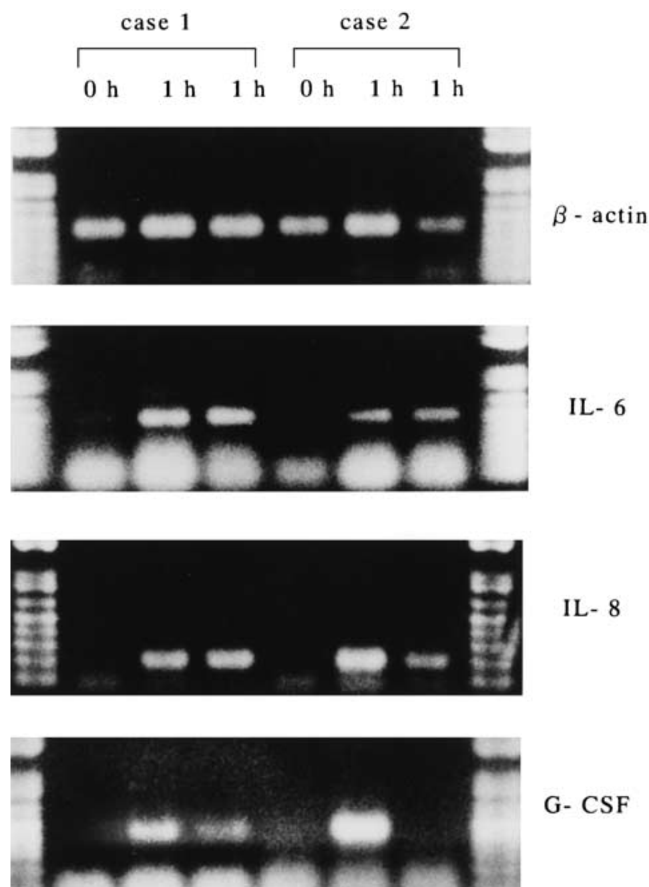

Fig. 3. Expression of IL-6, IL-8, and G-CSF mRNAs by peritoneal samples obtained immediately (0 h) and at 1 h after laparotomy.

During open surgery, stimuli such as exposure to the atmosphere, surgical manipulation, and direct instrumentation contribute to induce an inflammatory reaction in the peritoneum. In contrast, during laparoscopic surgery, the pneumoperitoneum and laparoscopic devices maintain an isolated warm and humid environment within the abdominal cavity, which protects the peritoneum and viscera from desiccation and extraneous injury [4]. The main difference between the two procedures appears to be the degree of stimulation of the peritoneum. In fact, it has been reported that changes in peritoneal response, including metabolism, immunity, and inflammation, may be associated with surgical stress [1, 4].

The peritoneum is a smooth, translucent serous membrane that lines the abdominal cavity as the parietal peritoneum and reflects onto the contained organs as the visceral peritoneum [5]. Recent studies have demonstrated that mesothelial cells established from human peritoneum secrete a variety of cytokines, including IL-1, IL-6, IL-8, granulocyte macrophage-CSF, macrophage-CSF, and G-CSF [7, 20]. Peritoneal macrophages and fibroblasts are also involved in the regulation of inflammation [12], forming a peritoneal cytokine network. Tsukada et al. [14] previously showed postlaparotomy levels of IL-6 and IL-8 in peritoneal fluid to be correlated significantly with operation time and amount of blood loss. Recently, Berge

et al. [2] measured cytokine levels in peritoneal fluid after abdominal surgery and concluded that TNF-α levels increase preceding intrabdominal complications. Therefore, we hypothesized that increased production of cytokines by the peritoneum reflects the degree of operative stress.

It has been reported that the postoperative levels of inflammatory cytokines in serum are lower with LS than with OS [10, 11]. The observation of lower levels of cytokines in serum after LS suggest that it is a reduced-stress procedure in comparison to OS. However, the primary source of the elevated serum cytokines after OS has not been clear. Ueo et al. [15] reported that production of IL-6 at the surgical wound site may be partially responsible for an increase in the serum IL-6 level during surgery. We previously compared serum IL-6 levels between open cholecystectomy (OC) and laparoscopic cholecystectomy (LC) [10]. Although the skin incisions were identical in size, the serum IL-6 levels observed after OC were significantly higher than those seen with LC. Therefore, the skin may not be a major source of IL-6 after surgery. Our data indicate that the peritoneum could be a significant source of serum IL-6 and G-CSF.

Some investigators have reported peritoneal metabolic or immune responses to CO_2 insufflation. Watson et al. [16] reported that there were significant increases in peritoneal tissue macrophage release of superoxide and tumour necrosis factor after laparoscopy with air and laparotomy compared with control procedures and CO_2 laparoscopy. West et al. [17] showed that cytokine expression was diminished in murine peritoneal macrophages exposed to CO_2. Specifically, they proposed that the diminished cytokine response of the peritoneal cells was the result of cellular impairment due to acidosis related to the CO_2 insufflation. Yoshida et al. [19] reported that the systemic cytokine response was significantly lower for laparoscopic cholecystectomy with pneumoperitoneum than for cholecystectomy by abdominal wall-lifting. These findings suggest that CO_2 may influence cytokine production by the peritoneal cells. However, the influence of CO_2 on the mesothelial cells of the peritoneum are still unclear. Further investigation is needed to determine whether CO_2 affects the cytokine production of mesothelial cells.

We observed that laparotomy provokes the production of inflammatory cytokines by the peritoneum. However, the comparatively low production of cytokines by the peritoneum when LS is performed indicates that surgical stress is comparatively low. Thus, our data provide evidence of an advantage for LS.

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