

Original Article

## Trans-Serosal Leakage of Proinflammatory Mediators during Abdominal Aortic Aneurysm Repair: Role of Phospholipase A<sub>2</sub> in Activating Leukocytes

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Gut barrier failure and the resultant translocation of luminal bacteria and bacterial products into the systemic circulation have been proposed as pathogenic mechanisms of multiorgan dysfunction syndrome (MODS) in open repair of abdominal aortic aneurysm (AAA). Our study aimed to demonstrate the direct release of gut-derived inflammatory mediators via the trans-serosal route in humans. Fifteen patients who underwent elective infrarenal open repair of AAA were randomized into two groups. In Group I patients (n = 10), the small intestine was exteriorized into a bowel bag. In Group II patients (n = 5), the small intestine was packed within the peritoneal cavity using large gauzes. We collected the bowel bag fluid in Group I and the ascites fluid, squeezed out from the gauzes at the end of surgery, in Group II. Leukocytes were collected from patients' blood samples. Incubation with the bowel bag fluid and ascites fluid caused a significant increase in both granulocyte pseudopod formation and CD11b expression compared to that with control fluid ( $p < 0.01$ ). The addition of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) inhibitor quinacrine abolished leukocyte activation by the bowel bag fluid. Based on these results, we consider that trans-serosal leakage of gut-derived mediators occurred during the open repair of AAA; further, sPLA<sub>2</sub> may be the most potent mediator in the activation of leukocytes among such gut-derived mediators in AAA surgery.

**Key words:** gut barrier function, abdominal aortic aneurysm, permeability, peritoneum, phospholipase A<sub>2</sub>

### INTRODUCTION

In critical illness, the gut is considered a critical organ in producing inflammatory mediators and transferring them systemically following the loss of its mucosal barrier function.<sup>1,2</sup> The perturbation of the intestinal mucosal barrier during open repair of abdominal aortic aneurysm

(AAA) has been reported in several studies.<sup>3,4</sup> The dysfunction of the barrier is associated with the entrance of gut-derived inflammatory mediators into systemic circulation. Such mediators activate neutrophils, which results in neutrophil sequestration to the site of inflammation and may provoke multiple organ dysfunction syndrome (MODS).<sup>5</sup> In ruptured AAA, systemic inflammation and subsequent MODS often occur.<sup>6,7</sup> Among the routes of transfer of the mediators from the gut to systemic circulation, two possible routes have been described in literature. One is via the gut mesenteric lymphatic system, and the other is through the portal venous circulation.<sup>2,8,9</sup> Previously, by using a rat model, we have demonstrated that gut-derived inflammatory mediators can be absorbed directly into the systemic circulation via the transperitoneal route.<sup>10</sup> Therefore, the identification of trans-serosal leakage of gut-derived mediators in humans

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### Patients' demographics

| Parameters                 | Group I    | Group II    | <i>p</i> Value  |
|----------------------------|------------|-------------|-----------------|
| Age (yrs)                  | 67.5 ± 8.9 | 73.4 ± 7.7  | N.S.            |
| AAA size (mm)              | 56.3 ± 8.1 | 58.0 ± 7.6  | N.S.            |
| Sex (M, F)                 | 8M, 2F     | 4M, 1F      | N.S.            |
| Operation time (min)       | 260 ± 36   | 320 ± 60    | <i>p</i> < 0.05 |
| Aortic clamping time (min) | 53.3 ± 7.2 | 82.0 ± 29.5 | <i>p</i> < 0.05 |
| Blood loss (mL)            | 587 ± 350  | 2047 ± 2080 | <i>p</i> < 0.05 |
| peritoneal fluid (mL)      | 114 ± 72   | 208 ± 121   | N.S.            |

N.S. : not significant

may suggest that the trans-serosal route could be another route for the transfer of the mediators from gut to systemic circulation in various clinical situations. To confirm this hypothesis, we collected the fluid that had leaked from the small intestine in the bowel bag or the ascites fluid that was absorbed by sponge gauzes during AAA repair; we then assessed the levels of proinflammatory mediators in these fluid samples.

As possible candidates of gut-derived pro-inflammatory mediators, we chose the following: endotoxin, IL-1, IL-6, IL-8, and phospholipase A<sub>2</sub> (PLA<sub>2</sub>). This was because previous studies have reported the importance of these mediators in gut-derived remote organ failure and MODS.<sup>2-4, 9-12</sup> We also tested the ability of the fluid to activate leukocytes and assessed which of the abovementioned molecules acted as the most potent mediator in activating leukocytes.

## PATIENTS AND METHODS

### Collection of Bowel Bag Fluid and Ascites Fluid during Surgery

The ethical committee of the Hamamatsu University School of Medicine approved this study. Written informed consent was obtained from each patient before participation in this study. We investigated 15 patients (12 males and 3 females) who underwent elective open repair of AAA with aorto-iliac bifurcated grafts between May 2007 and September 2009 (Table 1).

At the start of the surgery, the abdominal peritoneal cavity was washed with 50 mL of saline. The peritoneal lavage fluid was collected from the peritoneal space 5 min later and stored as the control fluid. Then, in order to exclude the contribution of the inherent peritoneal macrophage in the production of inflammatory mediators, patients were randomized into two groups based on the type of bowel

manipulation being performed. In Group I patients (n = 10), the small intestine was exteriorized into a plastic bowel bag during the surgery, and retracted to the right side over the abdominal wound to expose the infrarenal aorta. The bag was tightened just enough to retain but not strangulate the small intestine. Therefore, the source of the inflammatory mediators accumulated in the bag was mainly from trans-serosal gut leakage. In Group II patients (n = 5), the small intestine was packed within the peritoneal cavity using large sponge gauzes, in this case, the mediators in the ascites fluid absorbed in the sponge were derived from both gut trans-serosal leakage and inherent peritoneal macrophages. However, the levels of the gut trans-serosal leakage of the mediators might have differed between the two groups because of the different methods of bowel manipulation. Open repair of AAA was performed according to standard protocol. In all cases, both the infrarenal aorta and bilateral iliac arteries were clamped during reconstruction with a bifurcated synthetic graft (Hemashield Gold™, Boston Scientific, Natick, MA). The inferior mesenteric artery (IMA) was sacrificed in all cases because the blood flow to the sigmoid colon is supplied through collateral circulation, which was assessed by IMA stump pressure measurement and Doppler ultrasound technique. In Group I patients, the small intestine was returned from the bowel bag to the peritoneal cavity at the end of the surgery; thereafter, we collected the fluid accumulated in the bowel bag. In Group II, the ascites fluid was squeezed out from the sponge gauzes, collected, and its volume was measured. After collection, the fluid was centrifuged (1500 xg, 15 min), and its supernatant was stored at -70°C, until further investigation for determining its ability to activate naïve donor leukocytes and assay the levels of TNF-α, IL-1β, IL-6, IL-8, secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>), and endotoxin. The surgical duration, aortic clamp time,

and amount of blood lost were recorded retrospectively.

### **Blood Sampling to Test Leukocytes Activation**

Two months after surgery, 15 mL of venous blood was collected from each patient in the outpatient clinic. By adding the stored supernatant obtained from the bowel bag, ascites, or control fluid during the AAA surgery to a fraction of granulocytes, we tested the ability of the supernatant to activate leukocytes.

### **Observation of Pseudopod Formation in Naïve Leukocytes**

The leukocytes from the blood sample two months after surgery were processed as follows. After 40-min sedimentation of 20 mL of venous blood, granulocyte-rich plasma was collected, layered onto 3.5 mL of Histopaque (Sigma-Aldrich, Inc., St Louis, MO, USA), and centrifuged at 600  $\times g$  for 20 min. The leukocytes and erythrocytes (1 mL) were then suspended in 1 mL of Krebs-Henseleit solution layered onto 2.5 mL of 55% and 74% isotonic Percoll solution (Sigma-Aldrich, Inc., St Louis, MO, USA). After centrifugation at 600  $\times g$  for 15 min, the purified granulocyte layer was removed and resuspended in 1 mL of 10 mM phosphate-buffered saline (PBS). These control cells are referred to as naïve granulocytes. The ability of the bowel bag fluid or ascites fluid to activate leukocytes was determined by assessing pseudopod formation in naïve granulocytes. Suspended granulocytes (200  $\mu\text{L}$ ; 10,000 per  $\text{mm}^3$  in PBS) were mixed with 200  $\mu\text{L}$  of supernatant from the bowel bag fluid or ascites fluid from Group I and Group II patients, respectively. As a control, 200  $\mu\text{L}$  of suspended granulocytes were mixed with 200  $\mu\text{L}$  of the supernatant from the peritoneal lavage fluid obtained at the start of the surgery. The mixture was incubated for 10 min at room temperature.

Then, glutaraldehyde in PBS (3%, 100  $\mu\text{L}$ ) was added to arrest pseudopod formations. The fraction of activated leukocytes, i.e., cells with pseudopodia length  $> 1 \mu\text{m}$ , containing more than 200 cells, were counted under a light microscope after 10 min of incubation with the supernatant samples and used as an indicator of the level of cell activation.<sup>10</sup> The reproducibility of this test was within 5%, as determined by repeated counts of the same sample by the same or by different operators. In previous experiments, we observed that pseudopod formation after incubation with test plasma is accompanied by other forms of activation such as oxygen free radical formation or cell adhesion molecule up-regulation.<sup>13, 14</sup>

### **Analysis of Neutrophil CD11b Expression Using Flow Cytometry**

The purified granulocytes mixed with the supernatant obtained from the bowel bag, ascites, or peritoneal lavage fluid during AAA surgery were incubated with PE Mouse Anti-Human CD11b/Mac-1 (BD Biosciences, San Jose, CA, USA) for 30 min. After incubation, lysis of erythrocytes was performed with  $\text{NH}_4\text{Cl}$ .

All samples were analyzed using the EPICS XL flow cytometer (Beckman-Coulter, Inc., Fullerton, CA, USA) within 2 h of venipuncture. A total of 5,000 cells were acquired per measurement. A two-parameter light scatter dot plot was created and a software gate was set around the neutrophil granulocytes using EXPO 2 software (Beckman-Coulter, Inc., Fullerton, CA, USA). Measurement of neutrophil CD11b expression was carried out as follows: the granulocytes population was gated based on its light-scattering properties and this gate was then plotted as a frequency histogram of red fluorescence. Results are expressed as an arithmetic mean fluorescence intensity (MFI), which is a measure of the level of neutrophil CD11b expression (more than 97% of the granulocyte gates were positive for the CD11b receptor).

### **Measurement of Levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, Secretory Phospholipase A2 (sPLA2) in the Bowel Bag Fluid and Ascites Fluid**

We measured the levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and sPLA<sub>2</sub> in the bowel bag fluid and control group with commercial kits as follows: TNF- $\alpha$  and IL-1 with Human TNF- $\alpha$  Immunoassay kit and Human Interleukin-1 $\beta$  (hIL-1 $\beta$ ) ELISA kit, respectively (BioSource International, Inc., Camarillo, CA, USA); IL-6 and IL-8 with Quantikine<sup>®</sup> Human IL-6 Immunoassay and Human CXCL8/IL-8 Immunoassay, respectively (R&D Systems, Inc., Minneapolis, MN, USA) and sPLA<sub>2</sub> with sPLA<sub>2</sub> ELISA kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions.

### **Endotoxin Measurement of the Bowel Bag Fluid and Ascites Fluid**

The endotoxin concentration in the bowel bag fluid and ascites fluid was measured using Limulus amoebocyte lysate (LAL). The procedure was based on the Endotoxin Test by Wako (Wako Pure Chemical Industries, Osaka, Japan). Briefly, for sample pretreatment, 0.9 mL of Sample Pretreatment Solution (Wako Pure Chemical Industries, Osaka, Japan) and 0.1 mL of sample peritoneal fluid were mixed and agitated. The mixture was incu-

bated for 10 min at 70°C, cooled on ice and then used for the assay.

### Endotoxin Adsorption by Polymyxin B-immobilized Fiber Column (PMX)

To assess the effect of the endotoxin in the bowel bag fluid or ascites fluid on leukocyte activation, a polymyxin B-immobilized fiber column (PMX; Toray Industries Inc., Tokyo, Japan) was used to absorb the endotoxin in the fluid. Then, we assessed leukocyte activation based on pseudopod formation.

### Microbial Assessment of Bowel Bag Fluid and Ascites

Both the bowel bag fluid in Group I and ascites fluid in Group II was cultured immediately after surgery for aerobic and anaerobic organisms. For aerobic culture, blood agar, desoxycholate-hydrogen sulfite-lactose (DHL) agar and BTB agar were used. For anaerobic culture, brucella HK agar and, paramomycin-vancomycin brucella HK agar was prepared in an anaerobic jar prior to inoculation. The aerobic agar plates were incubated for 48 h at 35°C, and anaerobic plates for at least 48 h at 37°C in anaerobic jars. Microbial specimens were subjected to light microscopic examination.

### Statistical Analysis

All data were expressed as mean  $\pm$  SD. The differences between the two groups were assessed using paired Student's *t* test. The differences in the means among the three groups, and the effects of quinacrine on leukocyte activation were determined by one-way analysis of variance followed by Sheffe's test. All statistical analyses were performed with StatView 5.0 (SAS Institute Inc., Cary, NC, USA). A *p* value less than 0.05 was considered statistically significant.

## RESULTS

A total of 15 patients were recruited in this study. The demographic and surgical details of the patients are shown in Table. We performed open repair of AAA, using a bifurcated graft (Hemashield Gold™, Boston Scientific Co., Natick, USA) according to standard protocol. None of the patients underwent reconstruction of the IMA. Autotransfusion using a washing-salvaging autotransfusion device (Cell Saver) was performed in 11 patients. The operation time and aortic clamping time both were longer in Group II than in Group I. The amount of blood loss was also significantly greater in

Group II than in Group I. This was coincidental and occurred because several cases in Group II had a severely angulated aortic neck and multiple lumbar arteries which necessitated a longer duration for the completion of the proximal aortic procedures. All the patients were transferred from the intensive care unit the day after the surgery. Postoperative courses were uneventful in all patients. When we collected blood samples at our outpatient clinic two months after the surgery, all the patients had fully recovered and had returned to the previous routine activities of daily life.

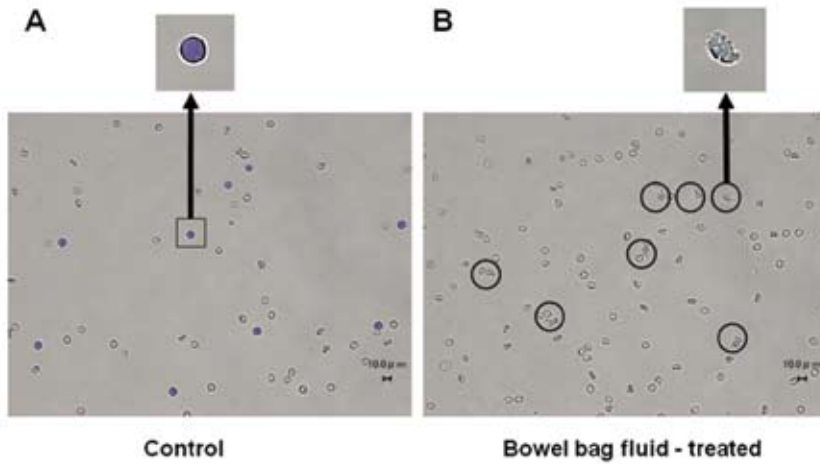
### Leukocyte Activation

Incubation of the leukocyte samples with bowel bag fluid (Group I) caused a significant increase in granulocyte pseudopod formation compared to that with ascites fluid (Group II) or abdominal lavage fluid (control group) ( $56.5 \pm 13.5$ , Group I;  $10.0 \pm 2.0$ , Group II;  $1.6 \pm 0.9$  %, control, respectively, *p* < 0.01 vs control) (Figs. 1 and 2A).

Similarly, the granulocyte expression of CD11b was greater in the bowel bag fluid-treated group (Group I) than that in the ascites fluid-treated group (Group II), and in the control group (MFI:  $104.9 \pm 28.6$ , Group I;  $66.5 \pm 23.0$ , Group II;  $31.2 \pm 8.5$ , control, respectively; *p* < 0.01 vs. control) (Fig. 2B). The effect of bowel bag fluid on leukocyte activation, based on both pseudopod formation and CD11b expression, was increased in a dose-dependent manner. (Figs. 3A, B)

### Analysis of Inflammatory Mediators in Bowel Bag Fluid

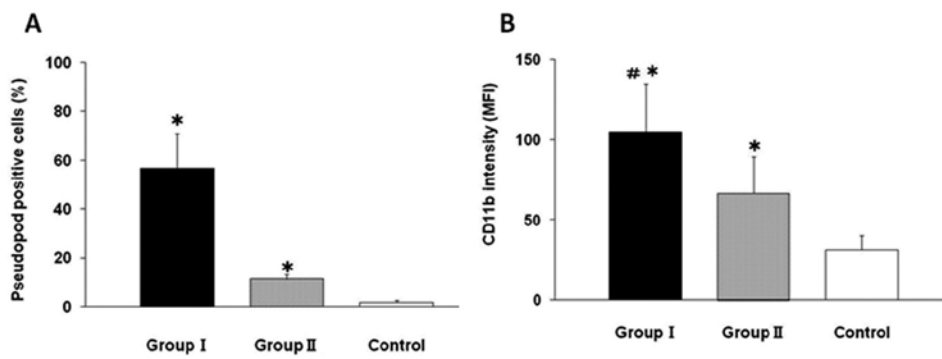
Bacterial cultures of bowel bag fluid did not show any positive bacterial growth in any of the samples. Among the inflammatory cytokines, IL-6 and IL-8 were elevated in Group I and Group II, compared to that in the control group (IL-6:  $4393.7 \pm 4642.9$  pg/mL, bowel bag fluid;  $2697.2 \pm 2226.7$ , ascites fluid;  $77.7 \pm 47.5$  pg/mL, control group, respectively, *p* < 0.05 vs control) (IL-8:  $3141.5 \pm 2613.1$ , bowel bag fluid;  $2963.0 \pm 1959.2$ , ascites fluid;  $0.79 \pm 2.1$  pg/mL, controlgroup, respectively; *p* < 0.05 vs control), while neither TNF- $\alpha$  nor IL-1 $\beta$  was detected in any of the groups. The levels of endotoxin in the bowel bag fluid were slightly elevated compared to those in the control group ( $16.6 \pm 12.0$ , bowel bag fluid;  $9.3 \pm 4.0$ , ascites fluid;  $0.52 \pm 0.20$  control group, respectively, *p* < 0.05 vs control). The levels of sPLA<sub>2</sub> in the bowel bag were significantly elevated compared to those in Group II and control group ( $890.3 \pm 104.7$  pg/mL, bowel bag fluid;  $397.7 \pm 72.8$  pg/mL ascites fluid;  $17.3 \pm 5.5$  pg/mL, control group, respectively; *p* < 0.05 vs. control) (Fig. 4).



**Fig. 1** Micrograph of granulocytes collected from a patient's blood sample for pseudopod formation test.

**A:** Incubation with control fluid (abdominal lavage fluid at the beginning of laparotomy). Granulocytes without pseudopod formation (framed rectangle) are observed.

**B:** Incubation with bowel bag fluid. Granulocytes with pseudopod formation are observed (circled).



**Fig. 2** Leukocyte activation.

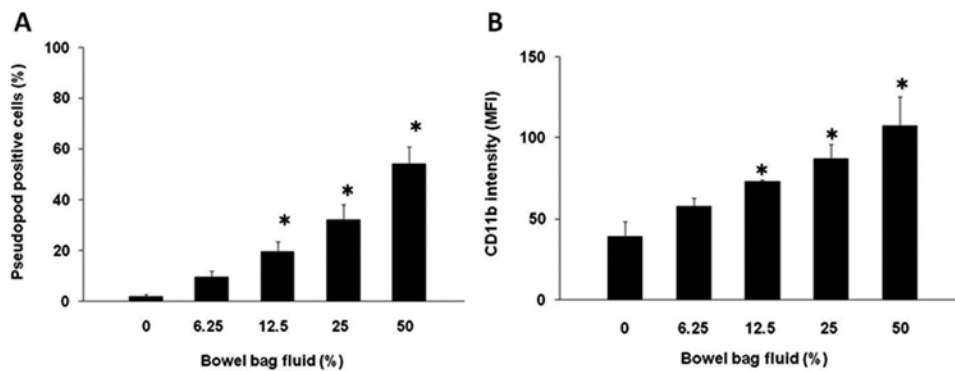
**A:** Pseudopod formation test after incubation with bowel bag fluid (Group I), ascites fluid (Group II), or control fluid.

**B:** Neutrophil CD11b expression with flow cytometry after incubation with bowel bag fluid (Group I), ascites fluid (Group II), or control fluid

MFI: Mean Fluorescence Intensity.

\* indicates  $p < 0.01$  vs. control group

# indicates  $p < 0.01$  vs. Group II

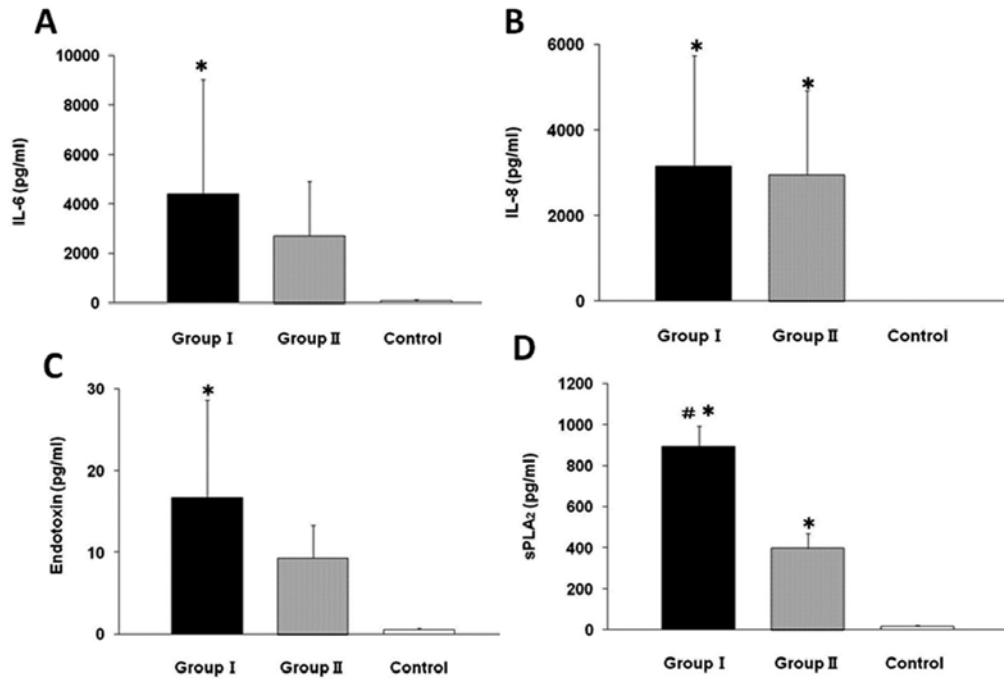


**Fig. 3** Dose response curve of leukocyte activation following addition of bowel bag fluid

**A:** Pseudopod formation test after incubation with 0–50% bowel bag fluid.

**B:** Neutrophil CD11b expression with flow cytometry after incubation with 0–50% bowel bag fluid.

\* Indicates  $p < 0.01$  vs. 0% (100% saline solution)

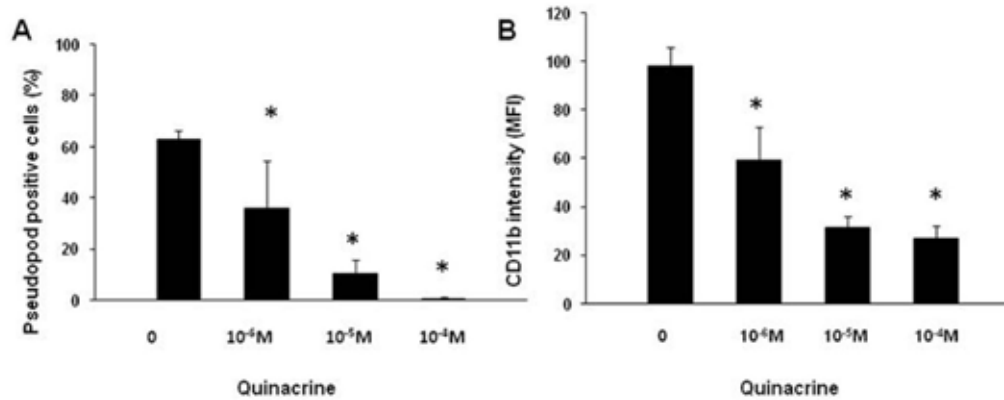


**Fig. 4** The levels of IL-6, IL-8, endotoxin, and secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) in bowel bag fluid. The bowel bag fluid (Group I), ascites fluid (Group II) and control fluid were collected, and their supernatants were stored until the measurement of various mediators.

**A:** IL-6, **B:** IL-8, **C:** endotoxin, **D:** sPLA<sub>2</sub>

\* indicates  $p < 0.01$  vs. control group

# indicates  $p < 0.01$  vs. Group II



**Fig. 5** Patients' leukocytes were incubated with bowel bag fluid with the addition of quinacrine at a dose range of 0 to 10<sup>-4</sup> M.

**A:** Pseudopod formation test

**B:** Neutrophil CD11b expression with flow cytometry

MFI: Mean Fluorescence Intensity

\* indicates  $p < 0.05$  vs. 0 M quinacrine group (without quinacrine).

### Identification of the Mediator Responsible for Leukocyte Activation

To demonstrate the effect of sPLA<sub>2</sub> on leukocyte activation, we tested quinacrine, a non selective PLA<sub>2</sub> inhibitor, to determine whether the agent could inhibit neutrophil pseudopod formation and CD11b expression. As **Fig. 5** shows, quinacrine inhibited both leukocyte pseudopod formation and neutrophil CD11b expression in a dose-dependent manner.

### DISCUSSION

An association between gut barrier failure and resultant intestinal cytokine response has been previously proposed. However, it has been difficult to directly demonstrate that the gut releases cytokines after gut ischemia and/or ischemia-reperfusion.<sup>15)</sup> In this study, we demonstrated the trans-serosal leakage of gut-derived pro-inflammatory mediators (IL-6, IL-8, and sPLA<sub>2</sub>) and endotoxin into bowel bag fluid. As control fluid, we used saline solution that had been exposed to the peritoneum for only 5 min, which was not discharged from the peritoneum. We used the fluid to eliminate the interference of powder present on the surgical gloves or avoid simple laparotomy. The potency of leukocyte activation was higher in the Group I fluid than in the Group II fluid; this finding suggested that the increased potency of leukocyte activation could be attributable to the fluid that had leaked trans-serosally from the gut and had accumulated in the bowel bag rather than to that produced by peritoneal inherent macrophages. However, a limitation of this study is the lack of direct evidence of trans-serosal leakage of the mediators from the gut. To confirm this leakage, the gut tissue should have been harvested to determine the upregulation of mRNA levels of IL-6, IL-8, and sPLA<sub>2</sub>. Further, we should have directly measured the levels of these molecules in the homogenized gut wall tissue and then compared the levels between Group I and Group II. However, gut tissue sampling during surgery is difficult in a clinical setting and is regarded as unethical. Therefore, the assessment of the levels of these mediators in the accumulated fluid in the bowel bag or gauzes was the only ethically approved method. On the basis of the indirect evidence, we speculate that the method of bowel manipulation method appeared to be important and did influence the trans-serosal leakage of sPLA<sub>2</sub>, which leads to leukocyte activation.

A previous study has also reported increased intestinal permeability in patients following elective and emer-

gency infrarenal aortic aneurysm surgeries.<sup>16)</sup> The mechanisms underlying this increased permeability are not entirely understood.

Among the inflammatory mediators that increased significantly in the bowel bag fluid, neither IL-6 nor IL-8 caused pseudopod formation; this was ascertained when the incubation of the patient's leukocytes with ten-fold concentrations of each cytokine did not induce any changes in pseudopod formation (data not shown). We then removed endotoxins from the bowel bag fluid by using a polymyxin B-immobilized fiber column before adding the fluid to the patient's leukocytes: however, no inhibition of pseudopod formation was observed (data not shown).

In this study, we believe that the trans-serosal leakage of proinflammatory mediators, especially the release of sPLA<sub>2</sub>, was caused by bowel manipulation. This phenomenon is probably not specific to surgery for AAA repair but commonly occurs during surgeries involving the use of a bowel bag.

Lau et al. demonstrated that altered permeability and subsequent portal endotoxemia were observed not in patients with retroperitoneal aneurysm repair but in patients who underwent transperitoneal aneurysm repair,<sup>3)</sup> suggesting that the mere manipulation of the intestine and its placement in a bowel bag is likely to have a deleterious effect on intestinal mucosa. Although the bowel bag drawstring was loosely cinched during surgery to avoid compression of the mesenteric root, exteriorization of the small intestine in a bowel bag placed the mesentery under traction and, the bowel was noted to be edematous at the end of the surgery: this might have perturbed intestinal perfusion and oxygen metabolism. The resultant ischemia may reduce cellular ATP levels. We and others have previously demonstrated that even a modest degree of cellular ATP depletion, if maintained for a prolonged period of time, is associated with an increased permeability in intestinal epithelial monolayers and mucosal hyperpermeability.<sup>17,18)</sup> A previous study has reported that pseudopod formation is induced by N-formylmethionyl-leucyl-phenyl-alanine (fMLP) and reflects actin polymerization in granulocytes.<sup>13)</sup> Cells that exhibit pseudopod formation have a higher propensity to be trapped in the microcirculation.<sup>19,20)</sup> The upregulated expression of CD11b in neutrophils promotes neutrophil aggregation in the systemic and pulmonary microvasculature causing neutrophil respiratory burst.<sup>21)</sup> Therefore, both pseudopod formation and CD11b expression have been used to assess leukocyte activation, although it is difficult to verify both

these changes in the same samples due to the technical limitations of the assay method.

Based on this study, we can safely say that the bowel bag fluid should be discarded and not allowed to spill back into the abdominal cavity because of its potential for activating leukocytes. For open repair of AAA, the method of the small intestine being packed within the peritoneal cavity using large sponge gauzes may be superior to that of using a bowel bag. However, this issue needs further study for greater clarity.

With regard to the routes of transfer for the mediators from the gut to systemic circulation, two possible routes have been described previously. One involves the gut mesenteric lymphatic system, and the other is the portal venous circulation.<sup>2, 8, 9)</sup> Previously, using a rat model, we have demonstrated that inflammatory mediators, which are produced by the ischemic gut during splanchnic aortic occlusion, can be absorbed directly into the systemic circulation via the transperitoneal route and can play a part in the development of MODS.<sup>10)</sup> In animal studies, peritoneal fluid drains to the celiac, and periportal lymph nodes, or to the diaphragmatic lymphatics entering the thoracic duct.<sup>22, 23)</sup> Others have shown that the removal of trans-serosally leaked gut-derived mediators using a bowel bag ameliorated lung injury after intestinal ischemia-reperfusion in rats.<sup>24)</sup> Peritoneal lavage has also been effective in inhibiting lipopolysaccharide-induced serum elevation of inflammatory cytokines in mice.<sup>25)</sup> These studies support our notion that gut mucosal barrier failure may be associated with trans-serosal leakage of inflammatory mediators and could contribute to systemic leukocyte activation. Our study clearly demonstrated evidence of trans-serosal leakage of chemical mediators from the gut in humans. Therefore, the peritoneal cavity could be a third route for gut-derived mediators to enter the systemic circulation, apart from mesenteric lymphatics and the portal circulation.

In this study, the patients' postoperative courses were uneventful. All patients resumed a normal diet within 4 days after surgery, suggesting that the damage to the intestinal barrier function due to bowel bag manipulation was transient and caused only subclinical effects on the patients' postoperative course. We speculate that gut mucosal barrier failure could be reversible once the perfusion is normalized and the energy metabolism is re-established as previous basic experiments have demonstrated.<sup>17)</sup> However, if the gut undergoes prolonged malperfusion as in ruptured cases, barrier failure may be sustained and cause systemic inflammation via gut-derived proinflammatory mediators. Among the various media-

tors we detected in the bowel bag fluid, PLA<sub>2</sub> showed the most potent effect on leukocyte activation.

PLA<sub>2</sub> comprises a diverse family of enzymes that release fatty acid lysophospholipids from the second carbon group of glycerol.<sup>26)</sup> PLA<sub>2</sub> is one of the key enzymes in the inflammatory process that regulates the synthesis of arachidonic acid and lysophospholipids.<sup>27)</sup> Previous studies have demonstrated the presence of the M-type sPLA<sub>2</sub> receptor on neutrophils,<sup>28)</sup> which has been implicated in diverse inflammatory states including pancreatitis and MODS following traumatic injury. Koike et al. demonstrated that the inhibition of group IIA PLA<sub>2</sub> ameliorated lung injury after gut ischemia-reperfusion.<sup>11)</sup> Moreover, Gonzalez et al. reported that the systemic administration of PLA<sub>2</sub> inhibitor ameliorated post-hemorrhagic shock.<sup>12)</sup> PLA<sub>2</sub> has been found in the Paneth cells of the small intestine.<sup>30)</sup> Further, the use of more selective inhibitors of sPLA<sub>2</sub> other than quinacrine might help in determining the specific type of PLA<sub>2</sub> involved in leukocyte activation among the PLA<sub>2</sub> groups.

Considering these results together, gut-derived PLA<sub>2</sub> may be a key mediator in postoperative systemic inflammation. Because no study has measured the plasma levels of PLA<sub>2</sub> after ruptured AAA, further investigations are necessary to confirm the association of PLA<sub>2</sub> with postoperative morbidities such as respiratory, renal and liver dysfunction and gastrointestinal complications. The importance of the trans-serosal route of inflammatory mediators in systemic inflammation as compared to the portal or lymphatic route remains to be elucidated.

## CONCLUSION

The gut placed in a bowel bag trans-serosally leaked various pro-inflammatory mediators during the open repair of AAA. On assaying the bowel bag fluid, we found that among the secreted mediators, PLA<sub>2</sub> possessed potent leukocyte activation ability, PLA<sub>2</sub> may thus be a critical mediator among gut-derived proinflammatory mediators in AAA surgery.

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