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Lung Injury After Intestinal Ischemia–Reperfusion May Be Avoided by the Reduced Absorption of Locally Produced Cytokines

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Abstract

Purpose. It has been suggested that inflammatory mediators such as cytokines released during intestinal ischemia and reperfusion increase permeability in the lungs. Cytokines exist at concentrations several hundred times higher at the site of inflammation than in the blood. When absorbed, the locally produced cytokines may affect multiple remote organs. We thus investigated whether the isolation of the intestine in a bag during ischemia and reperfusion can reduce subsequent lung injury.

Methods. Rats were divided into three groups: group 1, simple laparotomy (sham); group 2, intestinal ischemia and reperfusion (I/R); and group 3, intestinal ischemia and reperfusion with an intestinal bag (IB). Lung permeability was assessed using the Evans Blue leakage method. Cytokines (interleukin-1β, tumor necrosis factor α, interleukin-8) in the plasma and ascites were measured by enzyme-linked immunosorbent assay.

Results. The increase in lung permeability of I/R significantly decreased in IB (1.73 ± 0.48 vs 1.05 ± 0.22, $P < 0.01$). The plasma cytokine concentrations were also lower in IB than in I/R. In addition, the cytokine levels in the intestinal bag fluid were extremely high.

Conclusion. The isolation of the intestine during ischemia and reperfusion was found to reduce the degree of subsequent lung injury, possibly due to the reduced absorption of locally produced cytokines via the parietal peritoneum.

Key words Intestine · Ischemia · Reperfusion · Lung injury · Cytokine

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Introduction

Intestinal ischemia and reperfusion lead to an acute local inflammatory response, resulting in increased microvascular permeability.[1] In addition to local injury, reperfusion of the intestine leads to remote organ dysfunction.[2,3] Recently, various studies have been published on lung injury associated with intestinal ischemia and reperfusion. As a result, inflammatory mediators such as cytokines have been suggested to cause neutrophil sequestration and increase permeability in the lungs.

Interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-8 (IL-8) are typical cytokines involved in acute inflammation. Simpson et al. showed that interleukin-1 receptor antagonist (IL-1ra) and tumor necrosis factor binding protein (TNFbp) reduce pulmonary permeability and liver damage after intestinal ischemia and reperfusion.[4] Caty et al. found that anti-TNF antibody attenuated the increase in pulmonary microvascular permeability.[5] In addition, Sorkine et al. reported that soluble tumor necrosis factor receptors reduce bowel ischemia-induced lung permeability and neutrophil sequestration.[6] These results suggest that IL-1 and TNF play major roles in the lung injury associated with intestinal ischemia and reperfusion.

Interleukin-8 is thought to be an important cytokine in various forms of acute inflammatory diseases. Sekido et al. reported pulmonary neutrophil sequestration, lung injury, and increased IL-8 concentration in bronchial lavage fluid during pulmonary ischemia and reperfusion.[7] They also showed that lung injury decreases after the administration of anti-IL-8 antibody.[7]

The cytokine response is augmented via a positive-feedback mechanism. Cytokines stimulate neutrophils and promote neutrophil adhesion to the endothelium with adhesion molecules such as P-selectin[8] or MAC-1.[9] The adherent and stimulated neutrophils then release

various chemical mediators[10–13] which cause pulmonary microvascular injury.

Locally produced cytokines exist at concentrations several hundred times higher at the site of inflammation than in the blood.[14] They activate neutrophils which result in neutrophil sequestration to the site of inflammation, thus playing an important role in the local defense against pathogens.[15] In addition, they may also provoke multiple organ dysfunction. We reasoned that by preventing the absorption of locally produced cytokines we may be able to reduce the systemic effects of cytokines exerted on the lung. We therefore placed the intestine of the group 3 rats in a rubber bag during intestinal ischemia and reperfusion, thus separating the intestine from the parietal peritoneum. We then studied whether this procedure can prevent subsequent lung injury.

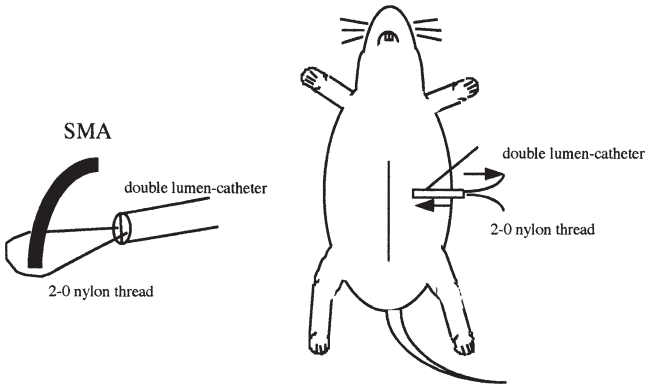

Fig. 1. Method of intestinal ischemia–reperfusion. A midline laparotomy was performed. Intestinal ischemia was achieved by the complete occlusion of the superior mesenteric artery (*SMA*) with the use of a double-lumen catheter. A 2-0 nylon thread was passed through the lumens of the catheter making a loop at the end. The nylon thread was pulled tight to occlude the SMA, and reperfusion was initiated by removing the catheter and thread

Materials and Methods

Animal Preparation

Animals were kept in a specialized facility, and the experimental protocol had been approved by the internal review board of the medical school of the university. Adult male Wistar rats (weighing 250–350 g) were used. The animals were fasted and allowed to drink only water for 24 h before the experiment. They were given pentobarbital sodium (25 mg/kg body weight) intraperitoneally, and the tail vein was cannulated. Anesthesia was maintained with pentobarbital sodium (12.5 mg/kg per hour). Lactate Ringer solution was infused (10 ml/kg per hour) from the tail vein.

Intestinal Ischemia and Reperfusion

A midline laparotomy was performed. Intestinal ischemia was achieved by a complete occlusion of the superior mesenteric artery (SMA) with the use of a double-lumen catheter. A 2-0 nylon thread was passed through the lumens of the catheter making a loop at the end. The nylon thread was pulled tight to occlude the SMA, and reperfusion was initiated by removing the catheter and thread (Fig. 1).

Experimental Design

The rats were divided into three groups as follows.

Group 1: a simple laparotomy (sham)
Group 2: intestinal ischemia for 90 min and reperfusion for 180 min (I/R)
Group 3: intestinal ischemia for 90 min and reperfusion for 180 min using an intestinal bag (IB); the intestine was placed in a condom during ischemia and reperfusion

Lung Permeability

The extravasation of Evans Blue was used as a marker for changes in capillary permeability and tissue edema.[17–20] Evans Blue strongly binds to serum albumin, and its distribution has been used as a marker for carrier albumin.[17,21,22] This technique compares favorably with the methodology using radiolabeled albumin and it is also more sensitive to microvascular dysfunction than the lung weight is.[21]

Evans Blue (25 mg/kg, i.v.; Sigma, St. Louis, MO, USA) was administered into the tail vein before intestinal ischemia. After intestinal reperfusion, a tracheotomy was performed, and the rats were ventilated with room air. After injecting heparin (500 U), a median sternotomy was performed, a 2-ml blood sample was taken from the superior vena cava (SVC), and the pulmonary artery was cannulated. The left auricle and SVC were cut off, and the lungs were infused with 20 ml saline at a pressure of 20 cmH_2O with the heart still beating to wash residual blood and Evans Blue from the pulmonary vascular bed (Fig. 2). The lungs and heart were then removed en bloc, and the left lung was used for the Evans Blue assay while the right one was used to calculate of the wet/dry ratio.

Details of the Evans Blue assay have been described previously.[17,20–22] Briefly, the left lung was weighed and homogenized in 2 ml of formamide (10%). It was then incubated at 37°C for 24 h, and the supernatant was separated by centrifugation at 5000 × *g* for 60 min. The

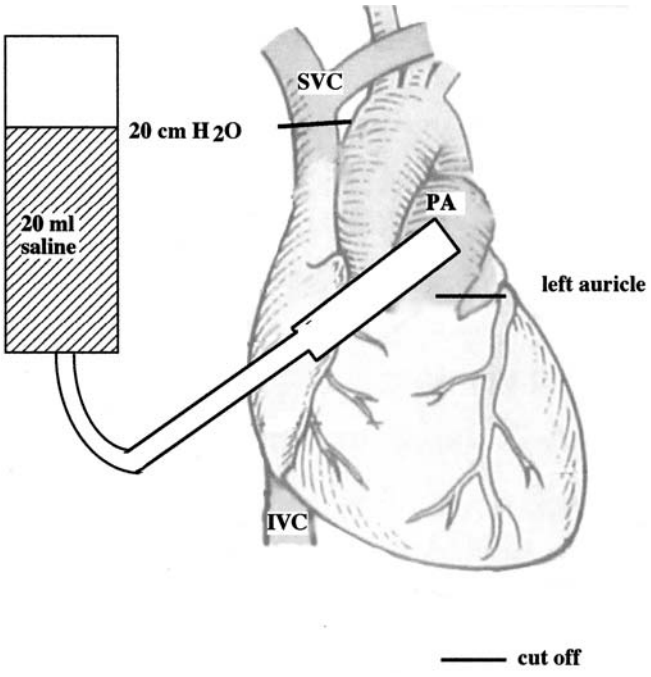

Fig. 2. Method to wash out the lungs. The pulmonary artery (*PA*) was cannulated. The left auricle and superior vena cava (*SVC*) were cut off, and the lungs were infused with 20 ml saline at a pressure of 20 cmH_2O, with the heart still beating, to wash out residual blood and Evans Blue from the pulmonary vascular bed. *IVC*, inferior vena cava

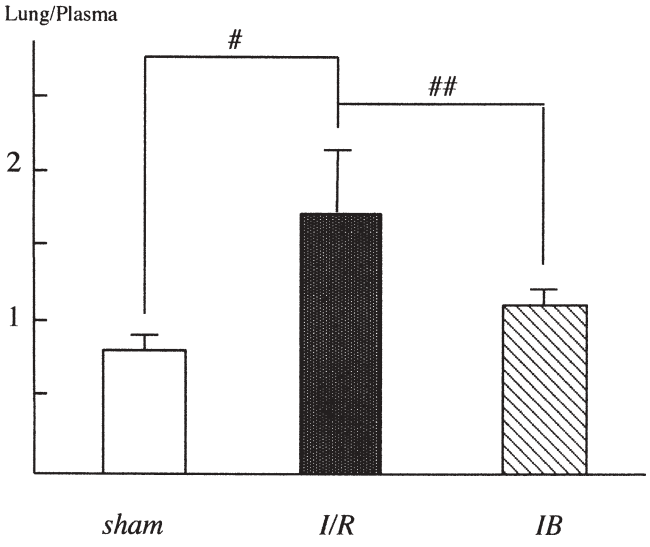

Fig. 3. Lung permeability. Lung permeability, as indicated by the lung/plasma ratio using Evans Blue; i.e., Lung permeability = [lung Evans blue (μg/g dry weight tissue)/plasma Evans blue (μg/ml)], was 1.732 ± 0.48 in the ischemia–reperfusion group (*I/R*) compared with 0.761 ± 0.132 in the sham group (*sham*) ($^{\#}P < 0.001$). The increase in lung permeability following intestinal ischemia and reperfusion significantly ($^{\#\#}P < 0.01$) decreased in the intestinal bag group (*IB*) (1.051 ± 0.225)

concentration of Evans Blue extracted in formamide was determined by spectrophotometry at a wavelength of 620 nm using a Hitachi U-1100 spectrophotometer. The results were plotted on a standard curve of Evans Blue (0.5–2.5 μg/ml). The Evans Blue content of each lung sample was expressed as the Evans Blue μg/g dry weight of tissue. The expression of our results as a function of the dry weight of tissue avoided an understimation of the changes due to edema formation. The Evans Blue content of the plasma was about 30 μg/ml in each group. Lung permeability was described as [lung Evans Blue (μg/g dry weight tissue)/plasma Evans Blue (μg/ml)].

Kinetics of Cytokine

Cytokines (IL-1β, TNF-α, IL-8) in plasma and ascites were measured by an enzyme-linked immunosorbent assay (ELISA). Interleukin-1β and IL-8 (GRO/CINC-1) were measured by a Sandwich Enzyme Immunoassay kit for rat (IBL, Gunma, Japan). Tumor necrosis factor α was measured by an ELISA kit for rat TNF-α (Genzyme, Framingham, MA, USA). Next, a 2-ml blood sample was taken from the inferior vena cava 30 and 90 min after ischemia, and 30, 90, and 180 min after reperfusion. The rats were killed by an overdose of anesthetics once the blood samples were obtained.

Statistical Analysis

The results are presented as the mean ± SD in the text and figures. Significance was tested by a one-way analysis of variance, and an unpaired Student's *t*-test value of $P < 0.05$ was considered to be significant.

Results

Lung Permeability (Fig. 3)

Three groups of rats underwent intestinal ischemia (90 min) and reperfusion (180 min). This treatment was fatal to 100% of the rats at 24 h postreperfusion. Although lung edema was less severe in the IB group, the rats in this group also died within 24 h. The concentrations of Evans Blue in the lung and plasma were measured at the end of reperfusion as an indicator of lung permeability. There was an increase in lung permeability after 90 min of ischemia and 180 min of reperfusion (I/R), as indicated by the lung/plasma ratio of Evans Blue: 1.732 ± 0.48 in I/R ($n = 7$) compared with 0.761 ± 0.132 in the sham group ($n = 7$), $P < 0.001$. The increase in lung permeability following intestinal ischemia and

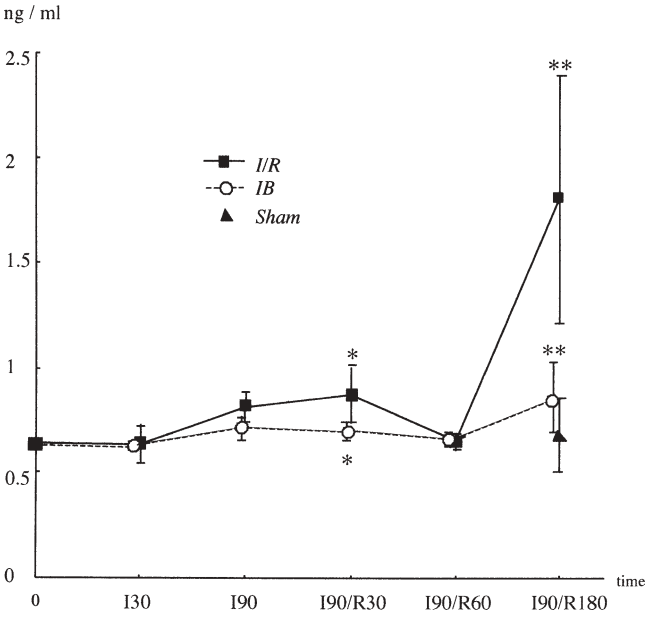

Fig. 4. Plasma interleukin (IL)-1β. At 180 min after reperfusion, the plasma concentration of IL-1β in the I/R group was about 2.5 times higher than in the sham group. This increase in plasma IL-1β thus substantially decreased in the IB group (**$P < 0.005$). The I/R group also showed a moderate increase during late ischemia and early reperfusion which was suppressed in the IB group (*$P < 0.05$). *I90/R180*, 90 min intestinal ischemia and 180 min after reperfusion

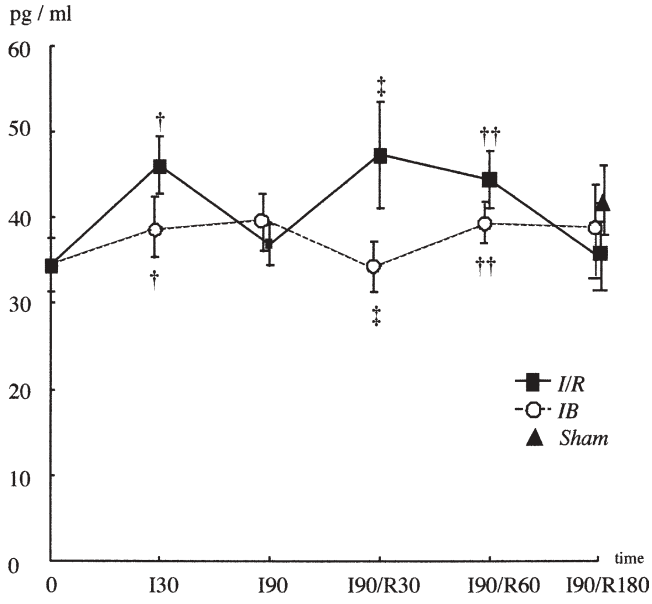

Fig. 5. Plasma tumor necrosis factor (TFN) α. Plasma TNF-α in the I/R group rose at 30 min after the start of ischemia and fluctuated during ischemia and reperfusion. In the IB group it was significantly lower than that in the I/R group at 30 min after ischemia ($^{\dagger}P < 0.05$), and at 30 min ($^{\ddagger}P < 0.005$) and 60 min ($^{\dagger\dagger}P < 0.05$) after reperfusion. *I90/R180*, 90 min intestinal ischemia and 180 min after reperfusion

reperfusion significantly ($P < 0.01$) decreased when the intestine was placed in a bag during treatment (IB) (1.051 ± 0.225, $n = 5$). At 180 min postreperfusion, the lungs were edematous, but the difference in the wet/dry ratio of the lungs in each group was not statistically significant (data not shown). Lung edema in the IB rats tended to be less severe, but a quantitative assessment of lung edema by histology proved to be difficult (data not shown).

Plasma IL-1β (Fig. 4)

The plasma IL-1β concentrations did not change significantly during ischemia. At 180 min after reperfusion, the plasma concentration of IL-1β in the I/R group was about 2.5 times higher than that in the sham group. This increase in plasma IL-1β decreased substantially when the intestine was placed in a bag ($P < 0.005$). The I/R group showed a moderate increase in plasma IL-1β late during ischemia and early during reperfusion, a reaction which was suppressed in the IB group ($P < 0.05$).

Plasma TNF-α (Fig. 5)

The plasma TNF-α in the I/R group rose 30 min after the start of ischemia and fluctuated during ischemia and reperfusion. The plasma TNF-α in the IB group remained relatively low during ischmia and reperfusion, and was significantly lower than that in the I/R group 30 min after ischemia ($P < 0.05$), and 30 ($P < 0.005$) and 60 min ($P < 0.05$) after reperfusion. The sham group also showed a slight increase in plasma TNF-α 180 min after reperfusion.

Plasma IL-8 (Fig. 6)

Plasma IL-8 was almost undetectable at the start of the experiment, but rose sharply in the I/R group 30 min after reperfusion and remained high until the end of the experiment. The intestinal bag caused a significant reduction of the rise in plasma IL-8 at 30 min ($P < 0.05$) and 60 min ($P < 0.005$) after reperfusion.

Cytokines (IL-1β, TNF-α, IL-8) in Ascites (Table 1)

After 180 min of reperfusion, the cytokine levels in the intestinal bag fluid were about three times as high as those in the plasma.

Discussion

Interleukin-1, TNF, and IL-8 are cytokines that play an important role in acute lung injury. Recent studies have

Table 1. Cytokine concentration in ascites

	IL-1β (ng/ml)	TNF-α (pg/ml)	IL-8 (ng/ml)
Plasma IB	0.863 ± 0.174**	37.673 ± 8.556	659.74 ± 146.306
Plasma I/R	1.956 ± 0.631**	33.967 ± 4.820	707.81 ± 116.778
Ascites IB	2.257 ± 0.797	95.208 ± 20.146	2358.2 ± 400.888

After 180 min of reperfusion, the cytokine concentration in ascites in the intestinal bag was about three times higher than in the plasma of the IB group rats
I/R, 90 min intestinal ischemia and 180 min of reperfusion; IB, 90 min intestinal ischemia and 180 min of reperfusion with intestinal bag; IL, interleukin; TNF, tumor necrosis factor
** $P < 0.005$

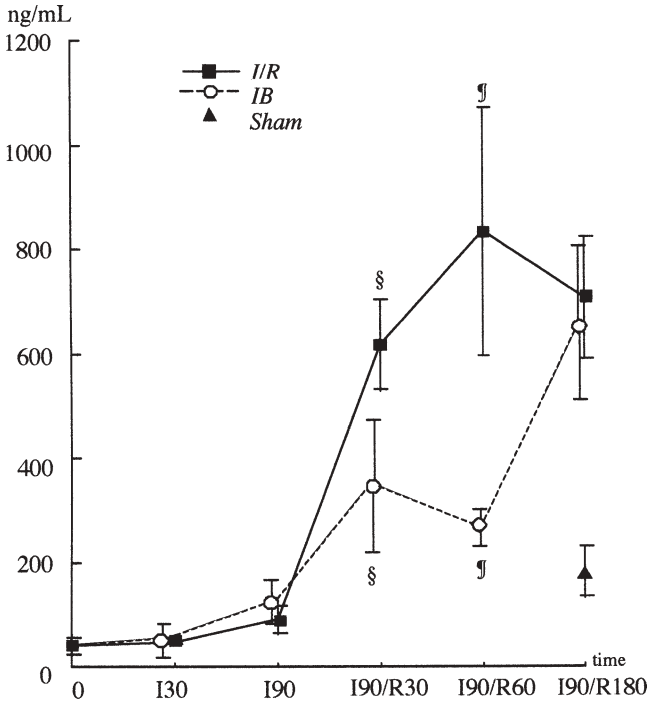

Fig. 6. Plasma IL-8. Plasma IL-8 was almost undetectable at the start of the experiment but rose sharply in the I/R group at 30 min after reperfusion, and remained high until the end of the experiment. Reperfusion with IB significantly reduced the increase in plasma IL-8 at 30 min ($^{\S}P < 0.05$) and 60 min ($^{\P}P < 0.005$) after reperfusion. *I90/R180*, 90 min intestinal ischemia and 180 min after reperfusion

demonstrated that TNF and IL-1 induce not only hypotension and hemodynamic changes but also lung injury.[22] The present study suggests that these cytokines locally produced at the site of ischemia have a remote effect on the lung. It also raises the possibility that the local control of cytokines may help manage more intractable lung injuries.

Figure 3 shows that the increase in lung permeability following intestinal ischemia and reperfusion significantly ($P < 0.01$) decreased when the intestine was placed in a bag during treatment (IB). The plasma cytokine concentrations were lower in the IB group than in the I/R group. The cytokine concentrations in the intestinal bag fluid were extremely high. These results support the previously published finding that cytokines are important in bowel ischemia-induced lung injury and also suggest that locally produced cytokines may have a remote effect directly on the lung. These hypotheses need to be confirmed by blocking experiments using antibodies to the cytokine as well as experiments to discover whether the fluid in the bag can reproduce lung injury in the rats without intestinal ischemia.

The kinetics of the plasma cytokine changes differed with each cytokine. The plasma TNF-α in the I/R group rose at 30 min after the start of ischemia and fluctuated during ischemia and reperfusion (30 min after intestinal reperfusion). Sorkine et al. studied the time course of the changes in serum TNF after intestinal reperfusion, and reported the serum TNF concentration to peak at 30 min after reperfusion and then return to baseline values within 180 min.[6] In a preliminary study we examined the lung histologically after intestinal ischemia and reperfusion. We found that the longer the ischemic or reperfusion time, the more severe the lung injury (data not shown). However, at 180 min after reperfusion, the plasma TNF levels in the I/R rats were not significantly higher than those in the sham rats. In a recent study, serum TNF was measured in patients with adult respiratory distress syndrome (ARDS);[23] just as in the present study when serum TNF peaked, ARDS was not always present, and no relationship was observed between the serum TNF and the PaO_2/FiO_2 of ARDS patients. Our data therefore closely correlate with theirs.

Interleukin-1β in the plasma showed an increase which started later than that of TNF-α. Interleukin-8 increased gradually and peaked 180 min after reperfusion. These results also correlate with the cytokine kinetics observed after the administration of *Escherichia coli* as reported by Van Zee et al.[24] We reasoned that TNF is produced early during the inflammatory response and that together with other factors it may then stimulate the production of IL-1 and IL-8.

Lung injury after intestinal ischemia and reperfusion significantly decreased when the intestine was separated from the parietal peritoneum by placing it in a bag during ischemia and reperfusion. The cytokine levels in

fluid collected from the bag at the end of reperfusion were about three times as high as those in plasma. Moreover, the plasma levels were lower in rats that had their intestine enclosed in a bag than in those that did not. These results indicate that the reduction in lung injury when the intestine was bagged during ischemia and reperfusion may be due to a reduction in the absorption of locally produced cytokines via the parietal peritoneum. In our model, since the visceral peritoneum was in the bag, there may have been a difference in the absorption of cytokine between the parietal and visceral peritoneum. Alternatively, the absorption of cytokines via the visceral peritoneum may be reduced by ischemia. Peritoneal macrophage activation may also decrease by separating the macrophages from high-dose cytokines. These possibilities should be explored in future experiments.

In this study we demonstrated that locally produced cytokines are closely involved in remote organ injury. There are several investigational immunotherapies for ARDS and multiple organ failure using substances which modify the cytokine responses.[25,26] However, for lung injuries caused by local inflammation such as those observed in our model, the local control of cytokines may be important. In a clinical setting, the suppression of cytokine absorption by chemically reducing the permeability of the peritoneum may be a feasible modality to prevent fatal lung injury in patients with severe ischemic damage to the intestine. In conclusion, the isolation of the intestine during ischemia and reperfusion was found to reduce the degree of subsequent lung injury, possibly due to a reduced absorption of locally produced cytokines via the parietal peritoneum.

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