Increased Gut Permeability after Hemorrhage Is Associated with Upregulation of Local and Systemic IL-6

Weiyang Wang, Ph.D.,*† Nadia Smail, M.D.,*† Ping Wang, M.D.,*† and Irshad H. Chaudry, Ph.D.*†‡,1

*Center for Surgical Research, †Department of Surgery, and ‡Departments of Molecular Pharmacology, Physiology, and Biotechnology, Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island 02903

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Although intestinal barrier failure after hemorrhage is a well-documented event, the underlying mechanism is poorly understood. The aim of this study, therefore, was to determine whether altered intestinal permeability after hemorrhage is associated with upregulation of local and systemic interleukin-6 (IL-6). To study this, rats underwent laparotomy (i.e., trauma induced) and were bled to and maintained at a mean arterial pressure of 40 mm Hg until 40% of the shed blood volume was returned in the form of Ringer’s lactate. The animals were then resuscitated with four times the volume of shed blood with Ringer’s lactate over 60 min. At 1.5 h postresuscitation, an in vivo ligated loop of a distal small intestine was formed and the passage of 4-kDa fluorescein isothiocyanate-conjugated dextran (FD4) from the intestinal lumen into the portal vein and carotid artery blood was analyzed by fluorescence spectrometry. Samples from the portal vein and a carotid artery were collected and plasma IL-6 was assayed. Intraepithelial lymphocytes from a distal small intestine were isolated and cultured in vitro for 24 h with or without anti-rat CD3 monoclonal antibody stimulation. IL-6 activity in freshly isolated cells and its release by cultured lymphocytes were determined. Intestinal perfusion and portal blood flow were determined by radioactive microspheres in another set of parallel experiments. The results indicate that lumen-to-blood passage of FD4 through the wall of the small intestine increased significantly at 1.5 h after hemorrhage and resuscitation and was associated with decreased intestinal perfusion and portal blood flow. Plasma IL-6 levels in the portal vein and carotid artery markedly increased at 1.5 h after hemorrhage and resuscitation. In addition, a significant correlation was observed between plasma IL-6 and FD4 concentrations. Higher IL-6 activity in freshly isolated cells was found in hemorrhaged rats. Increased IL-6 release by cultured lymphocytes was also observed either with or without anti-rat CD3 monoclonal antibody stimulation. Thus, the increased intestinal permeability following trauma–hemorrhage and resuscitation appears to be associated with systemic and intestinal IL-6 upregulation.© 1998 Academic Press

Key Words: small intestines; permeability; intraepithelial lymphocytes; tissue perfusion; proinflammatory cytokine; trauma; resuscitation.

INTRODUCTION

Apart from the major function for digestion and absorption of nutrients, the intestines act as a barrier to prevent microorganisms and toxins contained within the lumen from spreading to distant tissues and organs. Severe trauma and hemorrhagic shock may initiate a cascade of events leading to septic complications, multiple organ failure, and ultimately death. One of the causes of this cascade of events may be the failure of intestinal barrier function, resulting in the increased intestinal permeability and subsequent translocation of bacteria and/or their toxins from the gut [1–4]. In addition, endotoxemia further increases intestinal permeability under such conditions [5]. While the intestinal barrier failure after trauma and hemorrhagic shock is a well-documented event [2, 3], the underlying mechanisms of this failure are poorly understood. It has been demonstrated both clinically and experimentally that the release of proinflammatory cytokines, in particular interleukin-6 (IL-6), increases after trauma and hemorrhagic shock [6–9]. It has also been suggested that an exaggerated IL-6 response is associated with the subsequent development of major complications such as sepsis and multiple organ failure. Studies in our laboratory have indicated that systemic IL-6 levels markedly increased after trauma–hemorrhage and crystalloid resuscitation [10, 11]. Furthermore, downregulation of circulating levels of IL-6 by pharmacological agents is associated with the improved hepatocellular function and hepatic blood flow under such conditions [11]. Moreover, J anu et al.
have recently reported that systemic IL-6 correlates with increased intestinal permeability in hemodynamically stable patients following severe trauma [12]. However, whether the increased intestinal permeability was associated with upregulated local production of IL-6 remained unknown in that study [12].

Intestinal intraepithelial lymphocytes are a subpopulation of the gut-associated lymphoid tissue (GALT) closest to the intestinal lumen [13, 14]. These cells are a unique subset of lymphocytes in the small intestine. Thymus-dependent intraepithelial lymphocytes express predominantly CD8αβ1 [15]. Thymus-independent cells express CD8ααββ and correspond to the majority of the CD8αγδ1, but also to a fraction of CD8αβ1 [15–17]. Since there is an abundance of intraepithelial lymphocytes in the small intestine, and since these cells are in intimate contact with epithelial cells which have direct contact with foreign antigens derived from gut lumen, it has been suggested that intestinal intraepithelial lymphocytes play an important role in mucosal immunity and also in disease states of the intestinal tract [18, 19]. However, the function of intestinal intraepithelial lymphocytes has not been clearly defined. Intestinal intraepithelial lymphocytes have been shown to mediate various forms of cytotoxicity and secrete multiple cytokines such as IL-6 in vitro [18, 19], which may contribute to the functional changes encountered following trauma and hemorrhagic shock. In view of this, the present study was designed to determine whether the alteration in intestinal permeability after trauma–hemorrhage and crystalloid resuscitation was associated with the increase in the circulating levels of IL-6 and the local intestinal intraepithelial lymphocyte production of IL-6. In addition, the alteration of intestinal perfusion was determined since intestinal blood flow may also contribute to the altered gut permeability following trauma and hemorrhage.

### MATERIALS AND METHODS

Model of trauma–hemorrhage and resuscitation. The model of trauma–hemorrhage and crystalloid resuscitation in the rat utilized here was described in detail previously [20]. Briefly, male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 275 to 325 g, were fasted overnight prior to the experimental procedure but allowed water ad libitum. The animals were anesthetized with methoxyflurane and a 5-cm ventral midline laparotomy was performed (i.e., trauma induced) before hemorrhage. The abdominal incision was closed in two layers and bathed with 1% lidocaine to provide analgesia throughout the experiment. After recovery from anesthesia, the animals were immediately bled to a mean arterial pressure of 40 mm Hg within 10 min. The rats were maintained at that pressure until 40% of the shed blood volume was returned in the form of Ringer’s lactate and then centrifuged to separate the plasma. The plasma was stored at −80°C until fluorescent dextran and cytokine determinations were performed. The distal small intestine was removed at this time point and intraepithelial lymphocytes were isolated as described below. Tissue blood flow was determined by utilizing radioactive microsphere injection technique [21]. The experiments described here were performed in adherence to the National Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital.

Determination of intestinal permeability using an in vivo ligated loop model. At 1.5 h after resuscitation, the small intestine was assayed for macromolecule permeability in vivo according to the method described by Tagesson et al. [22] and Otamiri et al. [23]. Briefly, after anesthesia with methoxyflurane, the abdomen was reopened along the midline incision. The cecum and the distal ileum were externalized, and a 10-cm segment of the distal ileum was prepared as an in situ loop. An incision, −5 cm proximal to the cecum, was made in the small intestine. Double ligature was made at both ends of the incision with a loosely applied ligature placed proximally. Another incision was also made −10 cm proximal to the first incision and double ligature was made again at both end of the incision with a loosely applied ligature placed distally. After removal of the undigested food in the observed segment of ileum with phosphate-buffered saline (pH 7.4), the distal ligature was tightened. Before tying the proximal ligature, a flexible cannula (i.d. 3.2 mm, o.d. 4.8 mm) connected with a syringe was put into the segment through an insertion proximal to the ligature. A solution of 4-kDa fluorescein isothiocyanate-conjugated dextran (FD4) (10 mg in 1 ml of phosphate-buffered saline, Sigma, St. Louis, MO) was injected into the intestinal segment which resulted in a very slight distention in the intestinal loop. The gut loop was wrapped in a gauze that was wetted with saline solution and covered with a plastic film and foil to prevent evaporation and direct light. After 30 min of incubation, blood samples from both portal vein and a carotid artery were taken and then centrifuged to separate the plasma. The plasma was stored at −80°C until FD4 measurement was performed. This incubation period was chosen because Otamiri and co-workers’ studies [23] had determined that, in a rat reversible intestinal ischemia model, fluorescence intensity in the portal blood appeared as early as 10 min and reached the maximum at 30 min after introduction of the dextran.

Fluorescence intensity measurement. The plasma samples (0.1 ml) in both portal vein and a carotid artery from sham-operated or trauma-hemorrhaged rats were mixed with 1.9 ml of phosphate-buffered saline (pH 7.4). FD4 concentration was analyzed by using fluorescence spectrometry. Fluorescence measurements were made with an FL500 fluorescence plate reader (Bio-Tek Instruments, Inc., Winooski, VT) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Standard curves to calculate FD4 concentration in the plasma samples were prepared from dilutions of FD4 in phosphate-buffered saline (pH 7.4) with bovine serum albumin (5 mg/ml, Sigma). Background fluorescence in the plasma sample was made from the plasma of normal rats and subtracted from all the fluorescence values of unknown plasma samples in sham-operated or trauma-hemorrhaged rats.

Determination of tissue perfusion. In another set of parallel experiments, the tip of the left carotid arterial catheter was advanced into the left ventricle by following the left ventricle pulse pressure tracing at 1.5 h after crystalloid resuscitation. The exact position of the catheter in the ventricle was verified at the autopsy. Microspheres labeled with 35Sr (15 μm diameter, specific activity 12.4 mCi/g, DuPont/NEN, Billerica, MA) were suspended in 10% dextran containing 0.05% Tween 80 surfactant to prevent aggregation and dispersed with a vortex shaker for 3 min before injection. A 0.3-ml suspension of microspheres was injected into the left ventricle over a period of 20 s. A reference blood sample was withdrawn from the femoral artery starting 10 s before the onset of microsphere injection and continuing for 90 s at a rate of 0.7 ml/min. After the microsphere injection, the left ventricular catheter was flushed with twice the volume of the withdrawn blood with normal saline over 60 s. Blood pressure was monitored before and after the microsphere injection to...
ensure that this procedure did not affect mean arterial pressure. At the end of experiment, the rats were killed with intravenous injection of sodium pentobarbital. After sacrificing the animals, the right and left kidneys, stomach, spleen, pancreas, and small and large intestines as well as mesentery were harvested, rinsed with normal saline, blotted, weighed, and placed in a 4-ml Bio-Vial (Beckman), and radioactivity was counted on Wallac gamma counter (Model 1470 Wizard, Gaithersburg, MD). The reference sample was transferred to a vial and counted. The organ blood flows (in ml/min/100 g tissue) were calculated using the following equation: Organ Blood Flow = (RBF × C) × 100, where RBF is reference blood sample withdrawal rate (0.7 ml/min), C is percent per minute (cpm) per gram tissue, and C is pg/ml in reference blood sample. Portal blood flow was calculated as the sum of the blood flow to stomach, spleen, pancreas, and small and large intestines as well as mesentery.

Isolation of intraepithelial lymphocytes of the rat small intestine. The intraepithelial lymphocytes of a distal small intestine from sham-operated or trauma-hemorrhaged rats were isolated according to the method described by Lyscom and Brueton [24] and Fangmann et al. [25]. Briefly, a segment of the distal small intestine proximal to the cecum was removed immediately after sacrificing the animals. The intestinal segment was washed with an ice-cold phosphate-buffered saline (pH 7.4) solution. The Payer’s patches were carefully dislocated out and the mesentery, adherent connective tissue, and fat were removed. The small intestine was opened longitudinally and cut into segments (0.5–1.0 cm). The gut segments were washed in citrate buffer containing 50 mM trisodium citrate, 96 mM NaCl, 8 mM KH₂PO₄, 5.6 mM Na₂HPO₄, 1.5 mM KCl, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25% bovine serum albumin, pH 7.2, at 4°C. The segments were then incubated in citrate buffer containing 1 mM dithiothreitol for 3 min at room temperature to remove mucus, after which they were thoroughly rinsed in citrate buffer at 4°C. This was followed by incubation of gut segments in citrate buffer for 15 min at 37°C with gentle agitation and then vortexing for 30 s to separate the basement membrane from the epitheliums and to release intraepithelial lymphocytes. The intraepithelial lymphocyte suspension was filtered through cotton gauze, and then freely passed through a 20-μl syringe containing loosely packed nylon wool (0.6 g per syringe, Polysciences, Inc., Warrington, PA) to remove clumps of epithelial cells. The cell suspension obtained was centrifuged at 200g for 10 min at 4°C. The cell pellet was resuspended in 10 ml of 40% Percoll (Sigma), which was carefully placed above 2 ml of 70% Percoll and below 2 ml of AIM V media (serum-free lymphocyte medium, Gibco BRL, Grand Island, NY) with 2-mercaptoethanol (2 × 10⁻⁵ M). The intraepithelial lymphocytes were further purified by discontinuous density gradient centrifugation at 600g for 20 min at 4°C. The purified intraepithelial lymphocytes were recovered from the 40% and 70% Percoll interface and washed with the above AIM V media. Finally, the intraepithelial lymphocytes were resuspended in an appropriate amount of AIM V media and cell viability was determined by trypan blue exclusion was >95%. An aliquot of the freshly isolated intraepithelial lymphocytes (2 × 10⁶/ml) was centrifuged at 12,000g at 4°C for 10 min. The pellet containing intraepithelial lymphocytes was immediately stored at −80°C until IL-6 determination. On the day of IL-6 bioassay, the frozen cells were thawed and sonicated for 10 s twice in 1 ml of phosphate-buffered saline. Samples were then centrifuged at 12,000g at 4°C for 45 min. IL-6 activity was measured in the supernatant samples by using bioassay as described below.

In vitro culture of intraepithelial lymphocytes of the rat small intestine. The freshly isolated intraepithelial lymphocytes (2 × 10⁶/ml) from sham-operated or trauma-hemorrhaged rats were cultured in AIM V media supplemented with antibiotics at 37°C in humid air with 5% CO₂. Cells were cultured in media alone or in the presence of saturating concentrations of purified anti-rat CD3 monoclonal antibody G4.18 (20 μg/ml, PharMingen, San Diego, CA) [26] in 24-well plates for 24 h at 37°C. An anti-rat CD3 monoclonal antibody was immobilized to 24-well plates by incubating 0.3 ml of optimal dilutions of the antibody in phosphate-buffered saline (pH 7.4) for 5 h at 37°C and then washing with phosphate-buffered saline three times before adding intraepithelial lymphocytes. Supernatants from the cultured intraepithelial lymphocytes of sham-operated or trauma-hemorrhaged rats collected, centrifuged at 7500g for 15 min at

FIG. 1. Passage of 4-kDa fluorescein isothiocyanate-conjugated dextran (FD4) from the intestinal lumen into the portal vein and carotid artery blood. Figure 1 shows the lumen-to-blood passage of FD4 through the wall of the small intestine at 1.5 h after trauma–hemorrhage and crystalloid resuscitation. Values are expressed as means ± SE of 6 animals in each group. *P < 0.05 compared with correspondent sham values by unpaired Student's t-test.

4°C to remove cellular debris and then stored at −80°C until cytokine determination was performed.

Interleukin-6 bioassay. The IL-6-dependent mouse B-cell hybridoma 7TD1 was a gift from Dr. J. Van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium) and maintained according to their direction [27]. Levels of IL-6 in plasma and supernatant samples were determined by assessing the 72-h proliferation of the IL-6-dependent murine hybridoma cell line 7TD1 as previously described [10]. Briefly, 7TD1 cells were incubated in the presence of plasma and supernatant samples were serially diluted with RPMI 1640 medium (Gibco BRL), supplemented with 10% fetal bovine serum. Plasma and supernatant dilutions started at 1:10 (sample medium) were diluted in threefold increments thereafter. After 72-h incubation at 37°C in humid air with 5% CO₂ atmosphere, the proliferation of the IL-6-dependent cells was determined by adding 3-(4,5-dimethylthiazol-2,4)-2,5-diphenyltetrazolium bromide tetrazolium (5 mg/ml, Sigma) and then measuring the amount of dark blue formazan crystal formation spectrophotometrically. The units of IL-6 activity were determined by a comparison of the curves produced from dilutions of the experimental plasma or supernatant to those generated by dilution of a recombinant human IL-6 standard (200 units/ml, Genzyme, Cambridge, MA). The limit of detection for IL-6 was 0.01 units/ml.

Protein assay. Protein plasma contents in both portal vein and a carotid artery from sham-operated or trauma-hemorrhaged rats were measured by the Lowry method using a commercial available kit (Bio-Rad Laboratories, Hercules, CA). Because of hemodilution after crystalloid resuscitation, plasma FD4 concentration and circulating IL-6 levels were corrected for the plasma protein contents by dividing the microgram or units with milligram plasma protein, respectively.

Statistical analysis. All results are expressed as means ± SE. The statistical significance of the mean values between groups was evaluated by unpaired Student's t test. The relationship between plasma FD4 concentrations and circulating IL-6 levels was determined by the calculation of Pearson correlation coefficient. P values <0.05 were considered significant.

RESULTS

Passage of 4-kDa fluorescein isothiocyanate-conjugated dextran from the intestinal lumen into the portal vein and carotid artery blood. Figure 1 shows the lumen-to-blood passage of FD4 through the wall of the small intestine at 1.5 h after trauma–hemorrhage and crystalloid resuscitation. There was a significant increase in the passage of FD4 from the intestinal lumen to the
portal vein and carotid artery blood following trauma
and hemorrhagic shock. However, there was no signif-
icant difference in plasma FD4 concentration between
the portal vein and carotid artery within each group.

Alteration in regional blood flow. The adequacy of
mixing and distribution of the injected microspheres
were verified by the absence of any significant differ-
ence in blood flow to the left and right kidney in either
sham-operated or trauma-hemorrhaged rats (Fig. 2A).
There was a significant correlation in renal perfusion
between the left and right kidney (r = 0.98, P < 0.05).
The results in Fig. 2B indicate that there was signifi-
cant decrease in small intestinal perfusion and portal
blood flow at 1.5 h after trauma-hemorrhage and crys-
talloid resuscitation. Values are expressed as means ± SE of 10
animals in each group. *P < 0.05 compared with correspondent sham
values by unpaired Student’s t test.

intestinal intraepithelial lymphocytes from trauma-
hemorrhaged rats displayed a higher level of IL-6 activity
compared with those from sham-operated animals.

Secretion of IL-6 from cultured intraepithelial lympho-
cytes. IL-6 secretion was assayed by determining the
cytokine concentration in supernatant collected during
24 h of in vitro culture of intraepithelial lymphocytes.
There was increased secretion of IL-6 by unstimulated
intraepithelial lymphocytes from trauma-hemorrhaged
rats compared with sham-operated animals (Fig. 6).
Moreover, anti-rat CD3 monoclonal antibody induced ad-
ditional release of IL-6 from intraepithelial lymphocytes
in sham-operated rats while no significant difference in
IL-6 release was observed between unstimulated and
stimulated intraepithelial lymphocytes from trauma-
hemorrhaged animals. In addition, IL-6 release from
stimulated intraepithelial lymphocytes increased signifi-
cantly in trauma-hemorrhaged rats compared to the lym-
phocytes from the sham-operated animals.

FIG. 2. Alterations of organ blood flow in the kidneys (A) as well
as the small intestine and portal vein (B) in trauma-hemorrhaged
and resuscitated (Hem) and sham-operated (Sham) rats at 1.5 h after
crystalloid resuscitation. Values are expressed as means ± SE of 10
animals in each group. *P < 0.05 compared with correspondent sham
values by unpaired Student’s t test.

FIG. 3. Alterations of plasma interleukin-6 (IL-6) from both por-
tal vein and a carotid artery in trauma-hemorrhaged and resusci-
tated (Hem) and sham-operated (Sham) rats at 1.5 h after crystalloid
resuscitation. Values are expressed as means ± SE of 6 animals in
each group. *P < 0.05 compared with correspondent sham values by
unpaired Student’s t test.

FIG. 4. Relationship between plasma 4-kDa fluorescein isothio-
cyanate-conjugated dextran (FD4) concentration and plasma interleukin-6
(IL-6) level from both portal vein and a carotid artery in trauma-
hemorrhaged and resuscitated (filled square) and sham-operated (filled
circle) rats at 1.5 h after crystalloid resuscitation by the calculation of
Pearson correlation coefficient.
compared with sham values by unpaired Student’s t test. Values are expressed as means ± SE of 6 animals in each group. *P < 0.05 compared with sham values by unpaired Student’s t test.

**FIG. 5.** Interleukin-6 (IL-6) activity in freshly isolated intraepithelial lymphocytes from a distal small intestine as determined by bioassay in trauma-hemorrhaged and resuscitated (Hem) and sham-operated (Sham) rats at 1.5 h after crystalloid resuscitation. Values are expressed as means ± SE of 6 animals in each group. *P < 0.05 compared with sham values by unpaired Student’s t test.

**FIG. 6.** Interleukin-6 (IL-6) secretion by 24 h in vitro cultured intraepithelial lymphocytes from a distal small intestine with or without stimulation of anti-rat CD3 monoclonal antibody as determined by bioassay in trauma-hemorrhaged and resuscitated (Hem) and sham-operated (Sham) rats at 1.5 h after crystalloid resuscitation. Values are expressed as means ± SE of 6 animals in each group. *P < 0.05 compared with correspondent sham values, #P < 0.05 compared with unstimulated sham values by unpaired Student’s t test.

**DISCUSSION**

The passage of FD4 from the intestinal lumen to the portal vein and carotid artery was found to be significantly increased at 1.5 h after trauma and hemorrhagic shock, indicating that the intestinal barrier function against macromolecules appears to be compromised under such conditions. The above time point, i.e., 1.5 h after the completion of hemorrhage and resuscitation, was chosen since we have carried out a large number of studies dealing with the alterations in cell and organ function in multiple tissue at this interval following trauma-hemorrhage and crystalloid resuscitation. Moreover, since the function of the small intestine was also depressed at 1.5 h after trauma-hemorrhage and resuscitation [28, 29], we used the same time point to determine whether intestinal barrier function (i.e., gut permeability) was impaired under such conditions.

An in vivo ligated intestinal loop model was used since it had anatomically intact blood and lymph supplies as well as autonomic innervation and allowed high concentrations of labeled macromolecules to be delivered to the mucosal surface. In addition, the concentration of the marker within the loop could be easily controlled and delivery of large amounts of labeled macromolecules to the small intestinal mucosa was independent of factors such as gastric emptying and peristalsis as seen with gavage feeding, which can be affected by trauma injury and shock. Gut permeability is not uniformly the same and it varies depending on intestinal regions examined, the direction of molecule passage across the intestinal mucosa assessed, and properties of the permeability marker molecules used, such as size, lipophilicity, and charges. The permeability determination method applied in this study, based on a lumen-to-blood technique described by Tagesson et al. [22] and Otagiri et al. [23], utilized FD4 as the macromolecule marker of intestinal permeability. FD4 is stable, nontoxic, inert, and measurable with great sensitivity, accuracy, and ease than radioisotopes [22, 30]. Moreover, the use of macromolecules as an index of mucosal integrity appears to be of more physiologic relevance than the commonly used markers of low molecular weight, i.e., mono- and disaccharides and $^{51}$CrEDTA, since macromolecule markers may more accurately reflect the passage of antigens and other bioactive molecules released by gut microbes. The distal ileum was chosen since it contains higher concentrations of potent stimulants of proinflammatory molecules, such as endotoxin [31], N-formulated-methionyl oligopeptides (i.e., F-Met-Leu-Phe) [32] or peptidoglycan–polysaccharides (i.e., PG–PS) [33] within the intestinal lumen as compared to the proximal jejunum. Whether the increased intestinal permeability to FD4 necessarily reflects an increased translocation of endotoxin and other bacterial products remains unknown. Nonetheless, our previous studies using a similar model of hemorrhage in mice [34] have not shown any evidence of endotoxin, which would suggest that significant amounts of bacterial products were not translocated in such a model.

The mechanisms underlying the increased intestinal permeability in the hypotensive state (ischemia or hemorrhage) are poorly understood. Studies have shown that total intestinal ischemia in itself produces the increased intestinal mucosal permeability in both lumen-to-blood [23] and blood-to-lumen directions [35, 36]. Hemorrhagic shock is known to result in severe splanchnic blood flow reduction [21]. In this study, we found that the regional blood flow to small intestines was reduced by 41% at 1.5 h after trauma-hemorrhage and crystalloid resuscitation (Fig. 2B), which is comparable to our previous results [21]. These findings therefore collectively indicate a decreased small intestinal perfusion under such conditions. The observation also implies that the decrease in small intestinal perfusion might be a mechanism responsible for the enhanced intestinal permeability after trauma-hemor-
rhage and crystalloid resuscitation. However, Kubes’ study indicated that a progressive decrease in intestinal blood flow to as low as 20% of control, through the inhibition of nitric oxide production by L-NAME, had absolutely no effect on mucosal permeability to $^{51}$Cr-EDTA [37]. The results of Kubes [37] suggest that the increase in intestinal permeability is not a direct result of the reduced intestinal blood flow. In the present study, we also found a significant reduction in the portal blood flow at 1.5 h after trauma–hemorrhage and crystalloid resuscitation. In view of this, the increased plasma FD4 concentrations in the portal vein and carotid artery at 1.5 h after trauma–hemorrhage and crystalloid resuscitation. This was associated with elevated plasma FD4 concentrations. We therefore suggest that this exaggerated circulating IL-6 response may be associated with increased intestinal permeability after trauma and hemorrhagic shock. However, less is known about the contribution of local (intestinal) IL-6 release.

The intestinal intraepithelial lymphocytes are mainly T cells. Both CD8-$\alpha$+ and CD8-$\gamma$+ T cells are present in the intestinal epithelium [15–17]. These T cells are located between columnar epithelial cells and are interspersed throughout the gastrointestinal tract. Since large numbers of intraepithelial lymphocytes are continuously exposed to a wide variety of lumens antigens and mitogens, it is generally thought that most intraepithelial lymphocytes are constitutively activated cells [16, 38]. In addition to the well-documented cytolytic activity, intraepithelial lymphocytes are programmed for cytokine production. Barrett and co-workers indicated that both TCR-$\alpha$+ and TCR-$\gamma$+ intestinal intraepithelial lymphocytes synthesized and secreted IL-6 when activated with an anti-CD3 monoclonal antibody in either a primary or secondary culture [18]. Beagley et al. also reported that low but detectable levels of IL-6 were found in culture supernatant of anti-CD3-stimulated intraepithelial lymphocytes as determined by bioassay [19]. Because of the fact that intraepithelial lymphocytes are located adjacent to epithelial cells, it is likely that this population may modulate epithelial barrier function. Although the mechanism of its action has not been completely identified, IL-6 appears to be one possible candidate mediator which may contribute to the modulation of gut barrier function. This notion is based on the findings that IL-6 is one of the cytokines secreted by small intestinal intraepithelial lymphocytes as described above [18, 19]. Also systemic IL-6 has been shown to correlate with increased intestinal permeability in trauma patients [12]. In this study, we found higher IL-6 activity in freshly isolated intraepithelial lymphocytes and more IL-6 release by in vitro unstimulated as well as stimulated intraepithelial lymphocytes from trauma-hemorrhaged rats compared with sham-operated animals (Figs. 5 and 6). These findings also imply that trauma–hemorrhage is a sufficient stimulus to activate these lymphocytes to produce IL-6 and that further stimulation (with an anti-rat CD3 monoclonal antibody) only slightly increases the production of this cytokine.

It should be noted that in this study, the relative amount of IL-6 detected in all the supernatant of primarily cultured intraepithelial lymphocytes isolated from sham-operated and trauma-hemorrhaged rats was lower than that observed in the plasma. One possible reason may be related to the soluble inhibitory molecule(s) present in culture which may have suppressed cytokine production/release [18]. Also much lower IL-6 activity in freshly isolated intraepithelial lymphocytes was observed in this study. These data may imply that intraepithelial lymphocytes are constitutively activated cells in vivo as indicated by other
investigators [16, 38], but also may not be a major source of in vivo IL-6 release following trauma-hemorrhage. We have previously reported that Kupffer cells may be such a possible candidate after trauma-hemorrhage and resuscitation [39]. However, the amount of IL-6 release by the intraepithelial lymphocytes in this study could in itself have significant effect if it were to act in a microenvironment on adjacent enterocytes.

In summary, we have demonstrated a significant increase in the passage of FD4 from the intestinal lumen to the portal vein and carotid artery after trauma and hemorrhagic shock. Such an increase in intestinal permeability to macromolecules appears to be associated with decreased intestinal perfusion, early exaggerated circulating IL-6 levels, and increased IL-6 production by intestinal intraepithelial lymphocytes. Although the data presented here do not provide a definitive answer as to the cause-to-effect pathway, a direction for future studies should be to elucidate the contribution of IL-6 from intraepithelial lymphocytes to epithelial permeability by using enterocyte-lymphocyte coculture system. Determination of the precise mechanism responsible for producing intestinal barrier dysfunction following trauma and hemorrhage may allow us not only to further understand but also to better manage the intestinal intake requirements of the traumatized host.

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