Modulation of Mesenteric Lymph Flow and Composition by Direct Peritoneal Resuscitation From Hemorrhagic Shock

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Hypothesis: Traditional clinical resuscitation from hemorrhagic shock that focuses on restoring central hemodynamic function does not adequately perfuse the gut. Intestinal hypoperfusion could stimulate ongoing organ failure and gut-derived systemic inflammatory response syndrome. Direct peritoneal resuscitation (DPR) that uses dialysis fluid improves perfusion and survival. We examined mesenteric lymph flow and proinflammatory constituents to determine whether DPR-stabilized interstitial compartment function could explain improved outcomes.

Design: A paired-control experimental animal study.

Participants: Mesenteric lymph fluid was continuously collected in 4 groups of rats (n=7 per group): sham group; hemorrhagic shock (50% mean arterial pressure for 30 minutes) and resuscitation (shed blood plus 2 volumes of isotonic sodium chloride for 30 minutes) group; hemorrhagic shock and resuscitation plus intraperitoneal saline (30 mL) group; and hemorrhagic shock and resuscitation plus DPR (30 mL of 2.5% clinical peritoneal dialysis fluid).

Interventions: Both DPR and saline were placed intra-peritoneally at the time of resuscitation.

Main Outcome Measures: Lymph composition was analyzed by enzyme-linked immunosorbent assay (ELISA) for hyaluronic acid, its ligand CD44, and cytokines.

Results: Hemorrhagic shock and resuscitation elevated lymph flow (peak mean [SEM], 20.6[5.6] µL/min at 60 minutes after resuscitation) and CD44 serum levels (peak mean [SEM], 140.0[12.9] ng/mL at 120 minutes after resuscitation) compared with the sham group (mean [SEM], 1.2[0.7] µL/min and 15.6[1.5] ng/mL), and DPR returned levels to baseline (mean [SEM], 4.4[0.5] µL/min and 15.4[0.3] ng/mL). Hyaluronic acid levels were elevated in the hemorrhagic shock and resuscitation group (mean [SEM], 90.0[1.3] ng/mL) and the hemorrhagic shock and resuscitation plus intraperitoneal saline group (mean [SEM], 93.0[1.3] ng/mL) compared with the sham group (mean [SEM], 73.7[1.4] ng/mL) or DPR group (81.2[0.9] ng/mL). Interferon γ, interleukin 1β, interleukin 6, and interleukin 10 levels were also modulated by DPR.

Conclusions: Hemorrhagic shock and resuscitation increased lymph flow by altering capillary water transport and expanding interstitial volume. Increased lymph hyaluronic acid and inflammatory cytokines with traditional resuscitation were modulated to sham levels by DPR. In addition, DPR reduces these patterns presumably via an osmotic effect on capillary water transport. Adjunctive DPR might offer novel protection from systemic inflammatory response syndrome after hemorrhagic shock and resuscitation.

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SIRS, including increased proinflammatory cytokine release, oxygen radical production, upregulation of neutrophil adhesion molecules, and pancreatic enzyme digestion of gut barrier function. In trauma patients, the mediator of the gut inflammatory response remains elusive. Given the lack of clinical evidence for bacterial and endotoxin translocation, experimental and clinical focus has been on the gut lymphatic drainage as a pathway for SIRS, which leads to multiple organ dysfunction (MOD). Which specific inflammatory mediators are released into the lymphatic system and are responsible for end-organ inflammation is the current focus of experimental and clinical investigation. Because of the complexity of the pathogenesis of shock-induced end-organ tissue damage and MOD, a treatment protocol that can reverse the main course of the pathophysiology of shock to alleviate these end-organ changes and improve survival has been elusive.

In recent studies, we have shown that topical exposure of the small intestine to a commercially available direct peritoneal resuscitation (DPR) solution during resuscitation from hemorrhagic shock can prevent the vasocostriction and hyperperfusion commonly associated with conventional resuscitation. Direct peritoneal resuscitation produced a rapid and sustained vasodilation and hyperperfusion of the gut regardless of the timing of DPR initiation. Another series of studies examined the effects of adjunctive DPR with intraperitoneal instillation of 30 mL of a 2.5% glucose-based clinical peritoneal dialysis solution after hemorrhagic shock that evaluates whole organ blood flow, SIRS, and survival. Adjunctive DPR caused splanchnic hyperperfusion associated with a greater than 100% increase in lung blood flow. This splanchnic and distant organ hyperperfusion was associated with downregulation of the systemic inflammatory response and an increase in survival compared with conventional resuscitation therapies. Recent studies have noted that postresuscitation tissue neutrophils infiltration, which is tissue and time dependent, cellular edema, and capillary perfusion regulated by hydrogen and potassium membrane transport and water compartment distribution are all normalized by DPR with hypertonic peritoneal dialysis fluid.

In the current study, we examined the role of DPR on mesenteric lymph flow and lymphatic composition of inflammatory cytokines, CD44, and the structural glycoprotein hyaluronic acid after hemorrhagic shock and resuscitation. We hypothesized that DPR would downregulate the proinflammatory response and decrease gut lymphatic inflammatory mediators compared with conventional resuscitation. Furthermore, we thought that DPR would normalize lymph flow because of its osmotic effect on water capillary flow.

**METHODS**

**ANIMAL CARE**

Animals were maintained in a facility approved by the American Association for the Accreditation of Laboratory Animal Care. The research protocol was approved by the Institutional Animal Care and Use Committee, the Biohazard Safety Committee, and the Research and Development Committee at the Louisville Veterans Affairs Medical Center. Twenty-eight male Sprague-Dawley rats (200-225 g) were acclimated for 2 weeks and received standard rat chow (20 g/d) and water ad libitum before experimental use.

Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (50 mg/kg) with maintenance doses administered subcutaneously (25 mg/kg) as needed to maintain a surgical plane of anesthesia. A subcutaneous injection of sterile isotonic sodium chloride solution (2 mL) was given to maintain body fluid homeostasis during surgery and equilibration periods. A tracheotomy allowed animals to spontaneously breathe room air. A femoral artery was cannulated for measurement of mean arterial pressure and heart rate and for withdrawal of blood during the hemorrhage protocol. A femoral vein was cannulated for infusion of fluids and return of shed blood and isotonic sodium chloride solution during the resuscitation protocol.

**MESENTERIC LYMPH DUCT CANNULATION**

The mesenteric lymph duct was isolated and cannulated with silastic tubing (8 in; inner diameter, 0.30 mm; outer diameter, 0.64 mm) placed inside PE190 sheath (3 in) to prevent kinking. After a midline abdominal incision, the right flank was punctured with a 16-gauge needle so that the cannula assembly (PE190 and silastic) could be fed through the needle into the abdominal cavity. The root of the mesentery was exposed, and the mesenteric lymph duct was identified cephalad to the superior mesenteric artery and the accessory lymph duct caudal to the superior mesenteric artery. The accessory duct was then ligated with 6.0 silk sutures. The mesenteric lymph duct was dissected from surrounding structures, cannulated with the silastic tubing, and glued in place with cyanoacrylate cement. The PE190 sheath was slid over the silastic tubing and cemented in place. Successful mesenteric lymph duct cannulation was indicated by immediate lymph fluid drainage into the cannula assembly. Lymph fluid was continuously collected throughout the remainder of the protocol (baseline, hemorrhage, resuscitation, and after resuscitation), and the lymph flow rate was determined for each period.

**HEMORRHAGE AND RESUSCITATION PROTOCOL**

The experimental protocol is outlined in Figure 1. Hemorrhagic shock was 50% of baseline mean arterial pressure for 30 minutes. The resuscitation protocol was return of shed blood during 5 minutes followed by 2 equal volumes of isotonic sodium chloride solution infused for 25 minutes (total resuscitation time, 30 minutes). The shed blood was kept warm on the animal’s heating pad during the hemorrhagic shock protocol and minimally anticoagulated with heparin sodium (<10 IU in 6-8 mL of blood). Adjunct peritoneal resuscitation fluid was prewarmed to 37°C and placed in the abdominal cavity at the time of the return of shed blood. Either 30 mL of commercially available dialysis solution (2.5% Delflex; Fresenius USA Inc, Ogden, Utah; with a pH of 5-6 and an osmolality of 398 mOsm/L and containing glucose and lactate) or saline was used. Animals were randomly assigned to 1 of the following groups: (1) sham animals underwent identical handling as the other animals but no hemorrhagic shock or resuscitation was performed; (2) hemorrhagic shock and resuscitation; (3) hemorrhagic shock and resuscitation with intraperitoneal saline (IPS); or (4) hemorrhagic shock and resuscitation with adjunctive DPR. All groups that underwent hemorrhagic shock and resuscitation had intravenous infusion to return shed blood plus 2 equal volumes of IPS; groups 3 and 4 also received IPS or DPR, re-
Figure 1. Experimental time line of the study protocol. With the exception of the sham rats, all animals underwent the hemorrhage protocol, which consisted of mean arterial blood pressure held at 50% of individual baseline values for 30 minutes. All hemorrhagic shock groups received standard fluid resuscitation of the shed blood plus 2 equal volumes of intraperitoneal saline (IPS) delivered during a total of 30 minutes. The hemorrhagic shock and resuscitation plus IPS and hemorrhagic shock and resuscitation plus direct peritoneal resuscitation groups received IPS at the time of the return blood infusion. Mesenteric lymph fluid was collected throughout the protocol, and the lymph flow rate was calculated.

Figure 2. Central hemodynamics. Mean arterial pressure (A) and heart rate (B) responses in the 4 groups. These values were restored to baseline or suprabaseline levels during the resuscitation period in the animals of each hemorrhagic group. Animals in the hemorrhagic shock and resuscitation alone group developed tachycardia at the 160-minute postresuscitation time point despite stable mean arterial pressure. DPR indicates direct peritoneal resuscitation. Error bars indicate SEM. * P < .05 vs sham group. † P < .05 vs hemorrhagic shock and resuscitation alone.
sisted through the hemorrhagic shock period and re-
for 30 minutes. This resulted in tachycardia that per-
creased to 50% of baseline pressure and held constant
orrhagic shock protocol, the mean arterial pressure de-

**ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND COMPLETE METABOLIC PANEL (CMP)**

A blood sample was drawn at 240 minutes after resuscita-
for analysis of complete metabolic panel (Abaxis VS2 Blood
Chemistry Analyzer; Abaxis, Union City, California) using its
complete diagnostic panel function (sodium, potassium, cal-
cium, phosphate, glucose, albumin, total protein, globulins,
total bilirubin, alkaline phosphatase, alanine aminotransfer-
ase, amylase, blood urea nitrogen, and creatinine). The final
lymph fluid sample was also analyzed using the same Abaxis
VS2 complete diagnostic panel. The collected lymph fluid and
the 240-minute postresuscitation serum sample were also ana-
lyzed via ELISA for CD44 (Abcam Inc; Cambridge, Massachu-
setts), hyaluronic acid (Echelon Biosciences Inc; Salt Lake
City, Utah), interferon-γ, tumor necrosis factor α, interleukin
(IL) 1β, IL-6, and IL-10 levels (R&D Systems Inc; Minneapo-
lis, Minnesota).

**STATISTICAL ANALYSIS**

All data are expressed as mean (SEM). The null hypothesis
was rejected a priori at \( P < .05 \). For mean arterial pressure,
heart rate, effective hepatic blood flow, and cytokine data,
2-way analysis of variance was performed and the Tukey-
Kramer honestly significant difference test was applied when
differences were found. For complete diagnostic panel data,
1-way analysis of variance was performed and the Tukey-
Kramer honestly significant difference test was applied when
differences were found.

**RESULTS**

The central hemodynamic responses observed are pre-
sented in **Figure 2**. In all groups that underwent the hem-
orrhagic shock protocol, the mean arterial pressure de-
creased to 50% of baseline pressure and held constant
for 30 minutes. This resulted in tachycardia that per-
tained through the hemorrhagic shock period and re-
turned to normal levels during resuscitation. The hem-
orrhagic shock and resuscitation group manifested
tachycardia during the postresuscitation period, which
was absent in the hemorrhagic shock and resuscitation
plus IPS or the hemorrhagic shock and resuscitation plus
DPR groups.

The lymph flow rates for these groups are shown in
**Figure 3**. Baseline lymph flow was 7(1) µL/min in all
groups. Lymph flow was not significantly altered during
the hemorrhagic shock period, but all resuscitation pro-
tocols resulted in increased lymph flow rate (21 [6]). In the
hemorrhagic shock and resuscitation group, the flow rate
decreased during the postresuscitation period and re-
mained elevated compared with sham lymph flow rate
throughout the postresuscitation period. The addition of
adjunct DPR modulated the mesenteric lymph flow rate to
near sham levels, whereas the addition of IPS resulted in
flow rates that were slightly higher than hemorrhagic shock
and resuscitation alone.

At 1 hour after resuscitation, hyaluronic acid levels in
the mesenteric lymph fluid were significantly elevated in
the hemorrhagic shock and resuscitation and hemor-
hagic shock and resuscitation plus IPS groups compared
with the sham or hemorrhagic shock and resuscitation plus
DPR groups (**Figure 4**). However, serum levels of hyal-
uronic acid at 4 hours after resuscitation were elevated in
all hemorrhagic shock and resuscitation groups. CD44
lymph fluid levels were elevated at 2 hours after resuscita-
tion in the hemorrhagic shock and resuscitation alone
group compared with the other 3 groups, and CD44 se-
rum levels at 4 hours after resuscitation were significantly
higher in the hemorrhagic shock and resuscitation and hem-
orrhagic shock and resuscitation plus IPS groups com-
pared with the sham and hemorrhagic shock and resuscita-
tion plus DPR group.

Hemorrhagic shock and resuscitation increased the pro-
tein levels of the cytokines IL-1β, IL-6, and interferon-γ
in the mesenteric lymph fluid as shown in **Figure 5**. These
levels were fully or partially abrogated by the addition of
adjunctive peritoneal resuscitation. The observed levels of
these cytokines in the serum at 4 hours after resuscitation
were not significantly different in any group (data not
shown). No significant differences were found in mesen-

**Figure 3.** Mesenteric lymph flow rate. Flow rate increased significantly
during the resuscitation period in all hemorrhagic shock groups. The
addition of adjunct peritoneal resuscitation fluid modulated mesenteric
lymph flow toward sham levels, whereas the addition of an equal
volume of intraperitoneal saline (IPS) increased mesenteric lymph
flow. DPR indicates direct peritoneal resuscitation; error bars, SEM.
* \( P < .05 \) vs sham group, † \( P < .05 \) vs hemorrhagic shock and resuscitation alone, ‡ \( P < .05 \) vs hemorrhagic shock and resuscitation
plus IPS by 2-way analysis of variance and the Tukey-Kramer honestly
significant difference test.
teric lymph fluid levels of tumor necrosis factor α in any group. Figure 6 depicts the anti-inflammatory cytokines IL-10 and transforming growth factor β in the lymph fluid at 60 minutes (IL-10) or 120 minutes (transforming growth factor β) after resuscitation and in the serum at 240 minutes after resuscitation. Hemorrhagic shock and resuscitation significantly elevated these levels, and again the addition of peritoneal resuscitation also modulated the levels of these compounds. Finally, the Table provides the complete metabolic panel results for all groups. Hemorrhagic shock and resuscitation caused hyperkalemia and increased serum levels of creatinine, alkaline phosphatase, alanine aminotransferase, amylase, and globulins and decreased serum albumin levels. The addition of adjunctive peritoneal resuscitation prevented hyperkalemia and lowered creatinine and amylase levels and increased alkaline phosphatase and serum globulin levels. These metabolic panel results are consistent with the expected capillary fluid and electrolyte flux that occur during resuscitated hemorrhagic shock.

**COMMENT**

In the present study, we demonstrated that DPR down-regulates the cytokine-mediated proinflammatory response after hemorrhagic shock and resuscitation compared with controls. It is not clear whether the cytokine responses observed in our study reflect an early cause in the development of SIRS in this hemorrhagic model or merely an effect of altered interstitial water and electrolyte activity with compartmental fluid shifts to produce...
a “washout” effect from the mesentery. In addition, CD44 levels were reduced in the lymph fluid and the serum at 2 and 4 hours after resuscitation in the adjunct DPR group and, furthermore, hyaluronic acid levels were reduced in the lymph fluid at 1 hour after resuscitation. Given the importance of the hyaluronic acid–CD44 interaction in the sequestration of neutrophils in the liver, this might explain the previously shown survival benefits of DPR. In addition, we examined the role of DPR on the mesenteric lymph flow rate. Herein we showed that lymph flow initially increased in all resuscitation regimens compared with control. In addition, DPR modulated this increase at 2 hours after resuscitation compared with the other hemorrhagic shock and resuscitation groups. Presumably an increase in lymph flow reflects an expansion of the interstitial compartment past its compliance capacity. Previously we showed the timing of the administration of the DPR fluid corresponds with the intestinal vasodilation and improved gastrointestinal blood flow independent of the timing of the intravenous fluid replacement.17 Because the benefits in mesenteric lymph flow appear to depend on DPR rather than intraperitoneal volume infusion herein, we would expect the same relationship with regard to timing of DPR administration herein.

Senthil et al22 demonstrated lymph flow rates during hemorrhagic shock in a swine model of hemorrhagic shock. Lymph flow rates were decreased during hemorrhagic shock similar to our data. Movement of fluid between the vascular and interstitial compartments is primarily determined by a balance between capillary and interstitial pressures and osmotic gradients. During the shock period of hemorrhage, a shift of fluid from the interstitial compartment to the vascular space occurs presumably due to a decrease in capillary pressure.23 However, during intravascular volume replacement with crystalloid solutions, the transient increase in capillary pressure exceeds the osmotic intravascular pressure to cause a net shift of fluid toward the interstitial space and results in an increase in lymph flow as the interstitial volume becomes larger than its capacitance.

The route of inflammatory mediators from the ischemic gut to systemic circulation appears to be the lymphatic system.2,24 In the past the interstitial fluid compartment has been considered a passive entity that is merely a transport medium for nutrients and wastes. Recent studies25 have demonstrated the biologic activity of the mesenteric lymph fluid is far more diverse than first thought. These studies have led to the gut lymph hypothesis of MOD, which states that gut-derived factors are involved in the

Figure 5. Mesenteric lymph cytokine levels. Hemorrhagic shock and resuscitation increased levels of interleukin (IL) 1β (A), IL-6 (B), and interferon γ (IFN-γ) (D) in the mesenteric lymph, and these levels were modulated by adjunct peritoneal resuscitation with commercially available dialysate. Tumor necrosis factor α (TNF-α) (C) was not affected by hemorrhagic shock in these experimental groups. None of these cytokines differed among groups in the serum at 4 hours after resuscitation (data not shown). DPR indicates direct peritoneal resuscitation; error bars, SEM. *P < .05 vs sham group. †P < .05 vs hemorrhagic shock and resuscitation alone. ‡P < .05 vs hemorrhagic shock and resuscitation plus intraperitoneal saline (IPS) by 2-way analysis of variance and the Tukey-Kramer honestly significant different test.
Figure 6. Lymph fluid and serum interleukin (IL) 10 and transforming growth factor β (TGF-β) levels. Levels of both IL-10 and TGF-β were elevated in the mesenteric lymph by hemorrhagic shock and resuscitation at 60 minutes after resuscitation (A) and 120 minutes after resuscitation (B), and the addition of adjunct peritoneal resuscitation prevented these changes in cytokine expression. Similar patterns were observed in the serum levels of these cytokines at 240 minutes after resuscitation (C) and 240 minutes after resuscitation (D). * P<.05 vs sham group. † P<.05 vs hemorrhagic shock and resuscitation alone.

Table. Summary of Complete Metabolic Panel Results at 240 Minutes After Resuscitation

<table>
<thead>
<tr>
<th>Component</th>
<th>Sham</th>
<th>Hemorrhagic Shock and Resuscitation</th>
<th>Hemorrhagic Shock and Resuscitation Plus IPS</th>
<th>Hemorrhagic Shock and Resuscitation Plus DPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, mEq/L</td>
<td>145.0 (0.8)</td>
<td>146.7 (0.7)</td>
<td>145.4 (0.8)</td>
<td>149.6 (1.5)</td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>4.30 (0.04)</td>
<td>5.31 (0.21)a</td>
<td>4.66 (0.17)a,b</td>
<td>4.71 (0.20)a,b</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dL</td>
<td>19.3 (3.3)</td>
<td>23.9 (1.4)</td>
<td>19.4 (2.7)</td>
<td>23.7 (2.1)</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.20 (0.01)</td>
<td>0.64 (0.04)a</td>
<td>0.31 (0.05)a</td>
<td>0.30 (0.03)a</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>152.8 (8.0)</td>
<td>155.7 (4.8)a</td>
<td>151.1 (7.1)</td>
<td>164.0 (9.2)</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>7.80 (0.08)</td>
<td>7.73 (0.15)</td>
<td>7.94 (0.10)</td>
<td>7.76 (0.16)</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>6.55 (0.17)</td>
<td>6.67 (0.27)a</td>
<td>6.67 (0.14)</td>
<td>6.50 (0.28)</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>2.30 (0.04)</td>
<td>2.10 (0.09)a</td>
<td>2.11 (0.06)</td>
<td>2.13 (0.16)</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>119 (5)</td>
<td>130 (7)a</td>
<td>130 (6)a</td>
<td>140 (8)a</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>61 (2)</td>
<td>192 (26)a</td>
<td>47 (5)a</td>
<td>63 (11)a</td>
</tr>
<tr>
<td>Amylase, U/L</td>
<td>715 (25)</td>
<td>807 (39)a</td>
<td>747 (25)</td>
<td>727 (31)b</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>0.23 (0.02)</td>
<td>0.24 (0.02)</td>
<td>0.23 (0.02)</td>
<td>0.20 (0.15)</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>4.30 (0.04)</td>
<td>4.29 (0.07)</td>
<td>4.13 (0.05)a</td>
<td>4.23 (0.15)</td>
</tr>
<tr>
<td>Total immunoglobulins, g/dL</td>
<td>2.00 (0.04)</td>
<td>2.19 (0.06)</td>
<td>2.04 (0.03)b</td>
<td>2.13 (0.08)a</td>
</tr>
</tbody>
</table>

Abbreviations: DPR, direct peritoneal resuscitation (Delflex; Fresenius USA Inc, Ogden, Utah; 2.5% glucose, 30 mL); IPS, intraperitoneal saline solution (30 mL).

SI conversion factors: To convert sodium to micromoles per liter, multiply by 1.0; potassium to micromoles per liter, multiply by 1.0; serum urea nitrogen to micromoles per liter, multiply by 0.357; creatinine to micromoles per liter, multiply by 88.4; glucose to millimoles per liter, multiply by 0.0555; calcium to millimoles per liter, multiply by 0.25; albumin to grams per liter, multiply by 10; alkaline phosphatase to microkatals per liter, multiply by 0.0167; alanine aminotransferase to microkatals per liter, multiply by 0.0167; amylase to microkatals per liter, multiply by 0.0167; total bilirubin to micromoles per liter, multiply by 17.104; and total protein to grams per liter, multiply by 10.0.

a P<.05 vs sham.
b P<.05 vs hemorrhagic shock and resuscitation alone by 1-way analysis of variance and the Tukey-Kramer honestly significant difference test.
pathogenesis of MOD and are delivered to distant organs by the lymphatic as opposed to portal circulation. Rodent studies have supported this hypothesis by demonstrating that following hemorrhagic shock, mesenteric lymphatic duct ligation decreased lung neutrophil sequestration and downregulated neutrophil CD11b and CD18 expression. Other studies with splanchnic artery occlusion and mesenteric lymph duct ligation show a similar role for lymph ducts. Taken together, it appears that the interstitial fluid compartment is the primary conduit for gut-derived inflammatory mediators after hemorrhagic shock and resuscitation. Which lymphatic factors are specifically responsible for the increased neutrophil sequestration and improved survival after mesenteric lymph duct ligation is under investigation, but products of enzymatic digestion of the brush border have received attention.

Hyaluronic acid is a high-molecular-weight polysaccharide involved in regulation of tissue hydration. It is partially released from the interstitial fluid into the lymphatic circulation and eventually cleared by the liver reticuloendothelial cells. Liver dysfunction is associated with high levels of hyaluronic acid. Hyaluronic acid elimination rate has been used as a marker of graft function after liver transplantation, where poor early graft function was associated with low hyaluronic acid clearance. Early studies demonstrated patients with septic shock had significantly increased circulating levels of hyaluronic acid. In that study, serum hyaluronic acid concentrations from patients with severe infections, those with septic shock (survivors and nonsurvivors), and controls were compared, and levels of hyaluronic acid significantly increased with each gradation of infection. Studies examining the role of hyaluronic acid in hemorrhagic shock have found similar findings. Wang et al examined hyaluronic acid clearance in animals 24 hours after hemorrhagic shock. Hyaluronic acid levels were elevated during hemorrhage and 24 hours after resuscitation when compared with control animals. Two variables are thought to explain the early findings of increased levels of hyaluronic acid in sepsis and shock: an increase in release of hyaluronic acid in the interstitium and a decreased clearance by the liver endothelial cell as a result of decreased hepatic blood flow. The mechanism of release from the interstitial space is unknown but could reflect an increase in breakdown or simply enhanced washout.

Recent studies have demonstrated the role of organ neutrophil sequestration in the pathogenesis of hemorrhagic shock and sepsis. Migration of neutrophils into the liver during low-flow states appears to be independent of adhesion mediated by selectins as in other organs. It has been shown that endothelial binding of hyaluronic acid potentiates adhesion to leukocytes via an interaction with CD44. Using intravital microscopy, McDonald et al examined the specific role of hyaluronic acid and CD44 interactions in the liver sinusoids after an infusion of Escherichia coli. Hyaluronic acid was expressed in high concentrations in the liver vs other organs and specifically in the sinusoids during both basal and septic conditions. Animals treated with hyaluronase before the initiation of sepsis had significantly decreased sinusoidal adhesion compared with septic mice not receiving hyaluronase. Furthermore, CD44 knockout mice and those animals treated with anti-CD44 antibody had a 70% decrease in neutrophil sinusoid adhesion. The absence of the hyaluronic acid–CD44 complex was associated with reduced serum alanine aminotransferase levels and increased sinusoidal perfusion compared with controls. It was concluded that the hyaluronic acid–CD44 interaction in the liver was responsible for neutrophil migration in sepsis and that blocking or decreasing this interaction could be beneficial. Whether hyaluronic acid–CD44 is an active pathway during hemorrhagic shock and resuscitation for neutrophil migration through the interstitium is unclear, but our data indicate that DPR reduces its activity.

Recently, we have shown that DPR normalizes fluid compartments after hemorrhagic shock and resuscitation. Rats hemorrhaged to 40% mean arterial pressure for 60 minutes were used to estimate volume distribution in the liver, gut, and lung by quantitative autoradiography of isotope markers at 2 hours after resuscitation. Hemorrhagic shock decreased intravascular volume, which remained contracted despite restoration of central hemodynamic performance after intravenous fluid resuscitation. Hemorrhagic shock and resuscitation caused interstitial edema in the gut, liver, and lung. Direct peritoneal resuscitation prevented these volume shifts in the gut and lung and decreased them in the liver. Also, DPR normalized total tissue water as determined by wet-dry ratios. These findings correlate with our current findings of increased lymph flow after traditional resuscitation by altering capillary water transport and an expanded interstitial compartment volume results in increased lymph flow. Direct peritoneal resuscitation appears to normalize capillary water transport and thus global tissue edema and subsequent increased lymph flow. In conclusion, the current study supports the gut lymph hypothesis for SIRS after hemorrhagic shock. The concomitant increase in hyaluronic acid–CD44 and proinflammatory cytokines with traditional resuscitation in the lymph fluid was modulated to sham levels by DPR. Lymph flow rates were reduced after hemorrhagic shock and resuscitation by DPR compared with conventional resuscitation. Although the mechanisms by which DPR reduces SIRS after hemorrhagic shock remain to be elucidated, by the decrease of the production of inflammatory agonists or the subsequent administration of these factors to end organs, we suggest this novel method of resuscitation (DPR) may offer a new mechanism of protection from SIRS after hemorrhagic shock.

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Author Contributions: Study concept and design: Matheson, Mays, Hurt, Zakaria, and Garrison. Acquisition of data: Matheson, Mays, and Hurt. Analysis and interpretation of data: Matheson, Mays, Hurt, Richardson, and Garrison. Drafting of the manuscript: Matheson, Mays, Hurt, Richardson, and Garrison. Critical revision of the manuscript for important intellectual content: Matheson, Mays, Hurt, Zakaria, Richardson, and Garrison. Statistical analysis:
Matheson. Obtained funding: Zakaria and Garrison. Administrative, technical, and material support: Matheson, Mays, Hurt, and Garrison. Study supervision: Matheson, Mays, Hurt, Zakaria, Richardson, and Garrison. Financial Disclosure: None reported.

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Previous Presentation: This paper was presented at the 116th Annual Meeting of the Western Surgical Association: November 10, 2008; Santa Fe, New Mexico; and is published after peer review and revision. The discussions that follow this article are based on the originally submitted manuscript and not the revised manuscript.

REFERENCES

14. Zakaria R, Garrison RN, Spain DA, Matheson PJ, Harris PD, Richardson JD. cohorts have had a long-standing interest in mesenteric blood flow, and recently their efforts have centered on examining the ability of adjunctive resuscitation strategies, most notably direct peritoneal resuscitation (DPR), to favorably alter mesenteric perfusion and injury in hemorrhagic shock.
15. In the current study they used a rat model of severe, acute hemorrhagic shock to examine the utility of adjunctive DPR by the use of a clinically approved peritoneal dialysis solution. They showed that intraperitoneal DPR restored intestinal lymph flow and hyaluronic acid to sham levels, whereas standard resuscitation with shed blood with or without intraperitoneal saline line did not. They also reported that adjunctive DPR modulated cytokine levels after hemorrhage and speculate that the mechanism involves osmotic effects of DPR on capillary wall transport. They conclude that DPR might protect patients with severe hemorrhage from postresuscitative systemic inflammatory response syndrome (SIRS).
16. I have 3 questions:
1. It appears that DPR or intraperitoneal saline was given immediately after the 30-minute hemorrhagic shock interval, during the same 5-minute interval as the shed blood, while the remaining saline resuscitation was given over the next 25 minutes. Is it possible that the salutary effects of DPR were due to much earlier restoration of intravascular volume? Have you examined the acute impact of DPR on intravascular volume? How would the results compare if 30 mL of peritoneal dialysate was given intravenously at this earlier time?
2. The authors speculate that the benefits of DPR arise from direct osmotic effects. What is the osmolarity of the Deflex so-

Michael West, San Francisco, California: Dr Garrison and his coworkers have had a long-standing interest in mesenteric blood flow, and recently their efforts have centered on examining the ability of adjunctive resuscitation strategies, most notably direct peritoneal resuscitation (DPR), to favorably alter mesenteric perfusion and injury in hemorrhagic shock.

In the current study they used a rat model of severe, acute hemorrhagic shock to examine the utility of adjunctive DPR by the use of a clinically approved peritoneal dialysis solution. They showed that intraperitoneal DPR restored intestinal lymph flow and hyaluronic acid to sham levels, whereas standard resuscitation with shed blood with or without intraperitoneal saline line did not. They also reported that adjunctive DPR modulated cytokine levels after hemorrhage and speculate that the mechanism involves osmotic effects of DPR on capillary wall transport. They conclude that DPR might protect patients with severe hemorrhage from postresuscitative systemic inflammatory response syndrome (SIRS).

I have 3 questions:
1. It appears that DPR or intraperitoneal saline was given immediately after the 30-minute hemorrhagic shock interval, during the same 5-minute interval as the shed blood, while the remaining saline resuscitation was given over the next 25 minutes. Is it possible that the salutary effects of DPR were due to much earlier restoration of intravascular volume? Have you examined the acute impact of DPR on intravascular volume? How would the results compare if 30 mL of peritoneal dialysate was given intravenously at this earlier time?
2. The authors speculate that the benefits of DPR arise from direct osmotic effects. What is the osmolarity of the Deflex so-

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lution? Have the authors seen equivalent responses with alternative hyperosmotic solutions, for example, hypertonic saline?

3. The authors conclude that adjunctive DPR may favorably impact postresuscitative SIRS. To what extent have they been able to confirm this hypothesis in a chronic version of their animal model? And finally, the “million dollar question” (although perhaps in the current economic environment a million dollars is too small an amount)—have they examined the impact of this approach in patients?

I thank the Association for the privilege of discussing this important paper.

Dr Garrison: Dr West, thank you for your questions. The DPR solution was started at the beginning of the intravenous resuscitation rather than at the time of the hemorrhage. The resuscitation was then carried out during the ensuing 30 minutes. The vasodilatory effect of the peritoneal dialysis fluid is almost instantaneous, and the microvessels maximally dilate. If you topically apply nitroprusside, the vessels will not dilate further. We have done other studies focused on osmotic control of the dilation effect. For example, mannitol will have the same effect. We have used peritoneal dialysis fluid because it is available in the clinical setting. The mechanism of the osmotic effect appears to prevent the sodium and water transport across the membrane in response to cellular acidosis of the shock state. We have looked at a more severe model of shock with a focus on outcome, and DPR improves survival by about 30%. We have done experiments in a model of severe decompensated shock.

The osmolarity of the 2.5% solution that was used in these studies is almost 400. You asked about our clinical experience with this technique. We have used it in 12 patients with various degrees of acute abdominal compartment syndrome or in shock patients where damage control laparotomy was used. Several patients in this experience died as a result of severe injury early in care or from head injury. This small experience does not allow an analysis as a function of severity of injury or degree of blood loss, but we have noted that bowel edema was minimal at the time of reexploration and pack removal, and we were able to close the abdominal fascia.

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