A CD18 monoclonal antibody reduces multiple organ injury in a model of ruptured abdominal aortic aneurysm

A. J. Boyd, B. B. Rubin, P. M. Walker, A. Romaschin, T. B. Issekutz, and T. F. Lindsay

Division of Vascular Surgery, Department of Surgery, The Toronto Hospital (General Division), Faculty of Medicine, University of Toronto, Toronto, Ontario M5C 2C4; and 2Izaak Walton Killam-Grace Health Centre, Division of Microbiology and Immunology, Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada B3J 3G9

A CD18 monoclonal antibody reduces multiple organ injury in the model of ruptured abdominal aortic aneurysm. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H172–H182, 1999.—The role of CD18 antibody (anti-CD18) in remote and local injury in a model of ruptured abdominal aortic aneurysm repair was investigated. Rats were divided into sham, shock, clamp, and shock + clamp groups. Shock + clamp animals received anti-CD18 or a control mononal antibody. One hour of hemorrhagic shock was followed by 45 min of supramesenteric aortic clamping. Intestinal and pulmonary permeability to 125I-labeled albumin was determined. Myeloperoxidase (MPO) activity, F2-isoprostane levels, and transaminases were also measured. Only shock + clamp resulted in statistically significant increases in pulmonary and intestinal permeability, which were associated with significant increases in MPO activity and F2-isoprostane levels. Treatment with anti-CD18 significantly decreased intestinal and pulmonary permeability in shock + clamp animals. These reductions were associated with significantly reduced intestinal and hepatic MPO activity and pulmonary F2-isoprostane levels and reduced alanine and aspartate aminotransferase levels; however, anti-CD18 had no effect on intestinal or hepatic F2-isoprostane levels or on pulmonary MPO activity. These results suggest CD18-dependent and -independent mechanisms of local and remote organ injury in this model of ruptured abdominal aortic aneurysm.

rupture of an abdominal aortic aneurysm (RAAA) is a lethal event in 90% of patients (10). In-hospital mortality rates of 50–70% account for a large proportion of these deaths and are due primarily to multiple organ failure after successful repair (7, 18, 24). Despite this excessive mortality rate, there has been relatively little investigation into the pathophysiological mechanisms initiated by rupture and repair of an aortic aneurysm that lead to local and remote organ injury and failure. An understanding of the pathophysiological mechanisms by which the combination of shock and aortic clamping results in organ failure is critical to the development of intervention strategies designed to reduce the mortality associated with RAAA.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
pentobarbital sodium (50 mg/kg ip). A tail vein and the right carotid artery were cannulated with 22-gauge Angiocaths and sutured into place. The tail vein was used to administer supplemental doses of anesthetic, \(^{125}\)I-labeled albumin (\(^{125}\)I-albumin), monoclonal antibodies, and Ringer lactate solution for reinfusion of shed blood. The carotid artery cannula provided continuous monitoring of mean arterial pressure (MAP) and was used to hemorrhage animals.

After a midline laparotomy, the abdominal aorta was isolated at the superior mesenteric artery and just proximal to the iliac bifurcation. Intestinal permeability was used as an index of intestinal injury (6, 13) and was measured based on a modification of the method of Beck et al. (3). A 5-cm segment of jejunum, \(~10 \text{ cm from the ligament of Trietz,}\) was isolated and cannulated at its proximal end with an input cannula and at its distal end with an output cannula. The cannulas were exteriorized via two incisions made in the right abdominal wall. The abdomen was then sutured closed. The cannulated intestinal segment was then flushed with Ringer lactate solution until the output was devoid of solid particles. The intestinal segment was then perfused with 37°C Ringer lactate solution at a rate of 0.3 ml/min with an infusion pump (model AVI 480, 3M, St. Paul, MN) throughout the duration of the experiment. For the determination of intestinal and pulmonary permeability, animals then received \(^{125}\)I-albumin (\(~1 \mu\text{Ci}\)) via the tail vein catheter and were allowed to stabilize for 30 min to establish postoperative equilibrium. During the stabilization and experimental periods, intestinal perfusate was collected every 10 min. Throughout the experimental perfusion, samples of blood (0.3 ml) were withdrawn at \(~1\text{-h intervals.}\) The blood samples were used for the measurement of total albumin concentration and the specific activity of \(^{125}\)I-albumin for the calculation of intestinal albumin loss. A portion of the plasma from the blood samples was also assayed to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT) content. To determine myeloperoxidase (MPO) activity and \(^{125}\)I-albumin content, the intestinal segment was excised, washed in ice-cold saline, and rapidly frozen in liquid nitrogen and stored at \(~70°C until analyzed for MPO and \(^{125}\)I-isoprostane content. To assess structural tissue injury, the remainder of the right lung, liver, and intestine were placed in buffered 4% formalin and prepared for routine hematoxylin-and-eosin histology.

Determination of pulmonary permeability. The heart and lungs were excised in toto, the left lung was lavaged three times with 3.5 ml of Ringer lactate solution, and the effluent bronchoalveolar lavage (BAL) was collected. Blood and BAL fluid were weighed and counted for \(^{125}\)I content, and lung permeability index (LPI) was calculated from the following formula: \(\text{LPI} = \frac{\text{BAL} \ 125\text{I} \ (\text{cpm/g})}{\text{blood} \ 125\text{I} \ (\text{cpm/g})}\).

Determination of intestinal permeability. To calculate intraluminal intestinal albumin loss, all 10-min eluent collections from the intestinal perfusion were weighed, and a 1-ml sample of each was assayed for \(^{125}\)I-albumin with a gamma counter. Each blood sample drawn during the experimental procedure was centrifuged at 10,000 rpm, and 100 \(\mu\)l of plasma were removed for determination of albumin content and \(^{125}\)I-albumin activity. The level of \(^{125}\)I in the blood samples was regressed against time, and the slope of this regression was used to determine the activity of this isotope in whole blood. This was used to determine the specific activity of \(^{125}\)I per microgram of total albumin to calculate intestinal albumin loss in milligrams per gram dry weight of the perfused intestinal segment (3).

Determination of MPO activity. Tissues were assayed for MPO activity, an index of PMN sequestration, according to the method of Suzuki and co-workers (35). MPO activity was assessed at 37°C by monitoring the change in absorbance at 655 nm over a 3-min period in a Cobas FARA II centrifugal analyzer (Roche Diagnostic Systems, Montclair, NJ). The reaction mixture contained 16 mmol/l 3,3’,5,5’-tetramethylbenzidine dissolved in N,N-dimethylformamide in 0.22 mol/l phosphate buffer that contained 0.11 mol/l NaCl at pH 5.4. The reaction was initiated by the addition of 3 mmol/l hydrogen peroxide. One unit of activity was defined as a
one-unit change in absorbance per minute at 37°C. Protein content of pulmonary, hepatic, and intestinal samples was determined by the bicinchoninic acid protein assay system (Pierce, Rockford, IL). MPO activity was expressed as units per milligram of protein.

Determination of F_{2α}-isoprostane levels. F_{2α}-isoprostanes have been shown to be a sensitive and accurate marker of tissue lipid peroxidation (2, 28, 30, 31). F_{2α}-isoprostane levels were determined using an eicosanoid immunoassay (EIA) with acetylcholinesterase kit (Cayman Chemical, Ann Arbor, MI) according to the modification of the methods of Bligh and Dyer (4) and Morrow et al. (31). In general, samples of intestinal, hepatic, and pulmonary tissue (0.3 g) were spiked with 5,000 dpm of tritium-labeled prostaglandin F₂α ([^3H]PGF_{2α}). Samples were blade homogenized in 1 ml of Hanks’ buffered salt solution at high speed on ice. The 500-μl sample was then vortexed with 1 ml of 100% ethanol, allowed to stand at 4°C for 5 min, and then centrifuged at 1,500 g for 10 min. The supernatant containing F₂α-isoprostanes was then decanted, an equal volume of 15% KOH was added, and samples were incubated at 40°C for 1 h. After 1 h, samples were diluted to 5 ml with double-distilled water and the pH was lowered below 4 with HCl. Samples were then passed through preconditioned SPE-C₁₈ reverse-phase cartridges, followed by 5 ml of pure water and then 5 ml of Hexane. F₂α-isoprostanes were eluted with 5 ml of ethyl acetate containing 1% methanol. The ethyl acetate was then evaporated with nitrogen and 1 ml of EIA resuspension buffer was added, and samples were vortexed for 30 s and sonicated for 5 s. Samples were then separated for scintillation counting (250 μl) and EIA (100 μl) analysis.

The recovery factor was determined by dividing 4 × disintegrations per minute of sample by the amount of[^3H]PGF_{2α} added. F₂α-isoprostane levels in the extracted sample were determined by dividing the EIA (pg/ml) by the recovery factor. Total F₂α-isoprostane levels were determined by dividing the extracted F₂α-isoprostane levels by the volume (500 μl) of the sample used for purification. Protein content of samples was determined by the bicinchoninic acid protein assay system (Pierce), and all data are expressed as micrograms of F₂α-isoprostane per milligram of protein.

Determination of neutrophil counts and antibody saturation. Blood samples (1.5 ml) were also drawn into heparinized tubes just before termination of the experimental protocol. Blood samples (0.5 ml) were diluted 1:1 with cresol blue, and PMN counts were determined using a hemocytometer.

RESULTS

Pulmonary permeability. The LPI was 0.014 ± 0.005 in sham animals (Fig. 2A). In animals in the shock-only and clamp-only groups, LPI was 0.015 ± 0.002 and 0.022 ± 0.006, respectively, levels, which were not statistically different from levels in sham animals. However, in the control antibody-treated shock + clamp group, the LPI significantly increased to 0.047 ± 0.008 (P < 0.05). When treated with the CD18 monoclonal antibody, the LPI in shock + clamp animals decreased significantly to 0.025 ± 0.005 (P < 0.05).

Pulmonary MPO activity. Pulmonary MPO activity was 2.38 ± 0.33 U/mg protein in sham animals (Fig. 2B). Pulmonary MPO activity in shock animals remained at a similar level of 2.74 ± 0.47 U/mg protein, whereas clamp animals showed a slight but nonsignificant increase in pulmonary MPO activity to 3.86 ± 0.53 U/mg protein. Only in the shock + control animals treated with control antibody was the increase in pulmonary MPO activity to 5.19 ± 0.61 U/mg protein statistically significant compared with that in sham animals. Treatment of shock + clamp animals with anti-CD18 did reduce pulmonary MPO activity; however, this reduction was not significant (4.27 ± 0.23 U/mg protein).

Pulmonary F₂α-isoprostane levels. F₂α-isoprostane levels were 9.00 ± 15.0 pg/mg protein in sham animals (Fig. 2C). In the control antibody-treated shock + clamp group, F₂α-isoprostane levels significantly increased to 843.27 ± 231.24 pg/mg protein (P < 0.05). Treatment with anti-CD18 decreased F₂α-isoprostane levels to 396.6 ± 70.5 pg/mg protein (P < 0.05).

Intestinal permeability. The rate of intraluminal intestinal albumin loss remained stable throughout the entire experimental protocol in sham and shock animals (Table 1). In clamp animals the rate of intestinal albumin loss remained stable throughout the entire experimental protocol in sham and shock animals. Treatment of shock + clamp animals with anti-CD18 did reduce intestinal albumin loss; however, this reduction was not significant (6.65 mg/g dry intestine; Fig. 3A).

In the control antibody-treated shock + clamp group, intestinal albumin loss remained stable during the stabilization and clamp periods; however, it increased significantly to 0.395 ± 0.101 mg·min⁻¹·g dry intestine⁻¹ in the reperfusion period compared with 0.190 ± 0.047 mg·min⁻¹·g dry intestine⁻¹ in the preshock period (Table 1); however, only the first 30 min of reperfusion were associated with a significant increase in albumin loss in clamp animals (Fig. 3A).

In the control antibody-treated shock + clamp group, intestinal albumin loss remained stable during the periods before reperfusion (Table 1). On reperfusion, there was a statistically significant increase in intestinal albumin loss to 33.67 ± 9.90 mg/g dry intestine (P < 0.05 compared with preshock levels) in the first 30 min of reperfusion that remained at similar levels throughout the 120-min reperfusion period (Fig. 3A). Treatment with anti-CD18 failed to reduce intestinal protein loss in the first 30 min of reperfusion in shock + clamp animals (22.96 ± 6.65 mg/g dry intestine; Fig. 3A). However, anti-CD18 significantly decreased intestinal protein loss to 11.98 ± 4.34 mg/g dry intestine (P < 0.05) in each of the three remaining intervals of the
reperfusion period compared with shock + clamp animals treated with control antibody in the same time periods (Fig. 3A). The total mean rate of intestinal albumin loss during reperfusion was also significantly attenuated by anti-CD18 from 1.196 ± 0.237 to 0.491 ± 0.162 mg/g dry intestine (Table 1).

Intestinal MPO activity. Intestinal MPO activity in sham animals was 0.684 ± 0.16 U/mg protein (Fig. 3B). Shock or clamp alone resulted in significant increases in intestinal MPO activity to 1.91 ± 0.65 and 2.10 ± 0.498 U/mg protein, respectively. The combination of these two insults in control antibody-treated shock + clamp animals, however, resulted in a statistically significant increase in intestinal MPO activity to 4.10 ± 0.79 U/mg protein (P < 0.05). Treatment of shock + clamp animals with anti-CD18 decreased intestinal

Table 1. Mean rate of intestinal albumin loss

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Groups</th>
<th>MPO Activity (units/mg lung protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Shock</td>
</tr>
<tr>
<td>Preshock</td>
<td>0.159 ± 0.052</td>
<td>0.189 ± 0.080</td>
</tr>
<tr>
<td>Shock</td>
<td>0.188 ± 0.029</td>
<td>0.114 ± 0.039</td>
</tr>
<tr>
<td>Clamp</td>
<td>0.184 ± 0.034</td>
<td>0.245 ± 0.098</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>0.246 ± 0.071</td>
<td>0.152 ± 0.033</td>
</tr>
</tbody>
</table>

Values (in mg·min⁻¹·g intestine⁻¹) are means ± SE. Note that shock group animals were not subjected to clamp procedure; reperfusion actually started after infusion of shed blood (clamp period). *P < 0.05 compared with preshock period; †P < 0.05 compared with shock + clamp (control antibody).
MPO activity to 0.597 ± 0.159, a level equivalent to that in sham animals.

Intestinal F$_2$-isoprostane levels. F$_2$-isoprostane levels were 279 ± 74 pg/mg protein in the intestine of sham animals. In control antibody-treated shock + clamp animals, intestinal F$_2$-isoprostanes increased significantly to 1,182.3 ± 126.7 pg/mg protein (P < 0.05). Treatment of shock + clamp animals with anti-CD18 did not significantly reduce intestinal F$_2$-isoprostane levels (1,311 ± 135 pg/mg protein; Fig. 3C).

Hepatic MPO activity. Hepatic MPO activity in sham animals was 0.006 ± 0.0001 U/mg protein (Fig. 4A). The combination of shock and clamp in control antibody-treated animals resulted in a statistically significant increase in hepatic MPO activity to 0.117 ± 0.030 U/mg protein (P < 0.05). Treatment of shock + clamp animals with anti-CD18 significantly decreased hepatic MPO activity to 0.053 ± 0.010 U/mg protein (P < 0.05).

Hepatic F$_2$-isoprostane levels. Hepatic F$_2$-isoprostane levels were 84.13 ± 2.11 pg/mg protein in sham ani-
mals. Shock followed by clamp in control antibody-treated animals resulted in a significant increase in hepatic F2-isoprostanes to 189.36 ± 20.38 pg/mg protein ($P < 0.05$). Treatment of shock + clamp animals with anti-CD18 produced a nonsignificant decrease in hepatic F2-isoprostane levels to 137.95 ± 20.27 pg/mg protein (Fig. 4B).

Plasma ALT and AST levels. ALT and AST levels in sham animals were 25.0 ± 3.6 and 79.0 ± 10.6 U/l, respectively, at the end of the experimental protocol (Fig. 4, C and D). In the shock group, ALT and AST levels remained unchanged compared with those in the sham group at 30.7 ± 4.5 and 103.7 ± 16 U/l. In the clamp group, ALT levels remained unchanged from sham levels at 41.75 ± 5.69 U/l, whereas AST levels were significantly elevated at 150.0 ± 21.29 U/l. In the control antibody-treated shock + clamp group, ALT and AST levels increased significantly to 20- and 22-fold baseline at 493.28 ± 62.41 and 1,776.25 ± 194.15 U/l, respectively (Fig. 4, C and D). Treatment of shock + clamp animals with anti-CD18 significantly decreased ALT and AST levels to 115.0 ± 87.69 and
433.25 ± 193.93 U/l, respectively; however, these levels were still significantly elevated compared with sham, shock, or clamp groups.

Blood pressures and resuscitation requirements. In sham animals, MAP remained stable throughout the experimental procedure (Fig. 5A). In shock animals, MAP rapidly returned to baseline levels and remained stable at ≥100 mmHg throughout the reperfusion period (Fig. 5A), with little resuscitation fluid required (7.4 ± 2 ml of Ringer lactate; Fig. 5B). In clamp animals, MAP increased significantly from 128 ± 2.7 to 154 ± 5.3 mmHg after the clamps were placed on the aorta (Fig. 5A). After removal of the clamps, MAP was maintained at ≥100 mmHg by infusion of 27 ± 2 ml of Ringer lactate solution during the 120-min reperfusion period (Fig. 5B).

In control antibody-treated shock + clamp animals, on the other hand, MAP was significantly lower at the end of the clamp procedure: 143.7 ± 1.9 mmHg compared with 162.0 ± 3.0 mmHg in clamp animals (P < 0.05; Fig. 5A). Shock + clamp animals also required significantly more Ringer lactate solution than clamp animals: 53 ± 4 ml to maintain MAP at ≥100 mmHg during the reperfusion period (Fig. 5A). Treatment with anti-CD18 in shock + clamp animals resulted in significantly higher MAP during the clamping procedure than in shock + clamp animals treated with control antibody. In addition, anti-CD18-treated animals required only 31 ± 4 ml of resuscitation fluid during reperfusion (Fig. 5B).

Antibody saturation. In the blood of CD18-treated animals, CD18 antibody concentration was 22.67 ± 0.33 U/ml. In shock + clamp animals treated with control antibody, MAP was significantly lower at the end of the clamp procedure: 143.7 ± 1.9 mmHg compared with 162.0 ± 3.0 mmHg in clamp animals (P < 0.05; Fig. 5A). Shock + clamp animals also required significantly more Ringer lactate solution than clamp animals: 53 ± 4 ml to maintain MAP at ≥100 mmHg during the reperfusion period (Fig. 5A). Treatment with anti-CD18 in shock + clamp animals resulted in significantly higher MAP during the clamping procedure than in shock + clamp animals treated with control antibody. In addition, anti-CD18-treated animals required only 31 ± 4 ml of resuscitation fluid during reperfusion (Fig. 5B).

**Fig. 5.** A: MAP in sham, shock, and clamp animals and shock + clamp (S + C) animals treated with control and with anti-CD18. PS, preshock; S, shock; C, clamp; R, reperfusion. *P < 0.05, shock + clamp (control antibody) compared with shock + clamp (anti-CD18) or clamp alone at same time points. B: resuscitation fluid requirements in shock and clamp animals and shock + clamp animals treated with control and with anti-CD18. *P < 0.05 compared with shock. #P < 0.05 compared with shock + clamp (control antibody).
8.75 µg/ml plasma at the end of the experimental protocol, levels ≥5–10 times that required to saturate the receptors on the PMN in the blood (19, 26). Peripheral blood PMN counts in sham animals were 2.4 ± 1.3 × 10⁹/l. Shock followed by clamp in control antibody-treated animals resulted in a nonstatistical increase in PMN number to 5.25 ± 1.75 × 10⁹/l. Treatment with the monoclonal antibody directed against CD18 did not render animals neutropenic, inasmuch as their PMN counts were 8.31 ± 1.98 × 10⁹/l, which is not different from that in control antibody-treated animals.

Hepatic and intestinal histology. Intestinal tissues from sham animals (Fig. 6A) showed no evidence of injury, whereas intestines from animals treated with shock only (Fig. 6B) and clamp only (Fig. 6C) showed PMN infiltration and some blebbing of epithelial cells on some villi. Histological sections of intestine from shock + clamp control antibody-treated animals, on the other hand, showed significant denudation of intestinal villi, venous congestion in the submucosal and villus microvasculature, and significant PMN infiltration in all tissue layers (Fig. 6D), whereas those from anti-CD18-treated animals showed significantly less injury, with almost no denudation of intestinal villi (Fig. 6E).

Hepatic tissues from shock + clamp animals showed moderate PMN infiltration, sinusoidal and venous red cell engorgement, and swelling of hepatocytes, whereas hepatic tissues from anti-CD18-treated shock + clamp

![Fig. 6. Hematoxylin-and-eosin-stained intestinal histology in sham (A), shock (B), and clamp (C) animals, shock + clamp animals treated with control antibody (D), and shock + clamp animals treated with anti-CD18 (E). Magnification ×1,000. Arrows, blebbing of epithelial cells on intestinal villi.]
animals showed much less PMN infiltration and congestion.

**Discussion**

This study examined the effects of a monoclonal antibody directed against CD18 in an acute experimental model that simulates the sequence of events that occurs with repair of an RAAA. The events of RAAA were divided into two distinct ischemic phases: hemorrhagic shock and supramesenteric aortic clamping. We used capillary permeability to [125I]-albumin, PMN sequestration, lipid peroxidation, transaminase levels, and histological evidence as indexes of local and remote organ injury in this model.

In this RAAA model, hemorrhagic shock for 1 h at an MAP of 50 mmHg followed by 45 min of supramesenteric aortic clamping and 120 min of reperfusion resulted in significant pulmonary, hepatic, and intestinal injury. Intestinal injury in this model was associated with a significant increase in intestinal capillary permeability immediately after the release of the aortic clamps, an increase that persisted throughout the entire 2 h of reperfusion. At this time, intestinal MPO activity was also significantly elevated. Treatment with anti-CD18 after hemorrhage, but before clamp placement, significantly attenuated the increase in intestinal permeability and completely attenuated the increase in intestinal MPO activity, suggesting that CD18 mediates intestinal ischemia-reperfusion injury in this model. However, anti-CD18 did not attenuate albumin loss in the first 30 min of reperfusion, suggesting that there are two phases of intestinal injury in this model, with the early phase being CD18-independent. The fact that similar early intestinal permeability increases were also observed in clamp-only animals suggests that factors associated with supramesenteric aortic clamping result in early intestinal permeability changes that are unrelated to the shock component of the injury and may reflect oxygen radical-mediated (13, 14, 16), xanthine oxidase-mediated (15), or nitric oxide-mediated injury (36a).

F2-isoprostanes are compounds produced by the free radical-catalyzed peroxidation of polyunsaturated fatty acids independent of cyclooxygenase (31). F2-isoprostane levels have been shown to be excellent markers of tissue oxidative injury, inasmuch as their levels increase dramatically in animal models of oxidant injury, and these increases can be attenuated by the administration of antioxidants (29, 30). In terms of intestinal F2-isoprostane levels in this RAAA model, shock followed by aortic clamping resulted in a fivefold increase in the generation of these compounds, demonstrating the potent oxidative injury associated with this model. Although intestinal permeability and MPO activity increases were significantly attenuated by treatment with anti-CD18, intestinal F2-isoprostane levels remained unaffected. One possible explanation for this finding is our observation regarding the mucosal layer from the intestinal segment. In the period immediately after clamp removal in previously shocked animals, sections of intestinal mucosa were often observed to slough off directly into collection vials, attesting to the severity of this combination of ischemic insults. An examination of intestinal histology corroborated this observation (Fig. 6D), where almost complete denudation of the intestinal mucosa was observed in almost all histological sections from control antibody-treated shock + clamp animals. Histological sections of intestine from anti-CD18-treated shock + clamp animals, on the other hand, showed significantly reduced PMN infiltration and mucosal denudation (Fig. 6E). In our unpublished studies, loose ligatures placed around either end of a segment of intestine distal to the perfused one resulted in a 15-fold increase in intestinal F2-isoprostane levels in control antibody-treated shock + clamp animals. Therefore, it is possible that much of the F2-isoprostanes produced in the intestinal tissues of shock + clamp animals was in the sloughed mucosa. This implies that the majority of the lipid peroxidation occurs in the intestinal mucosa and that the loss of this tissue precluded accurate assessment of intestinal lipid peroxidation in these experiments.

In the lung, only the combination of hemorrhagic shock with aortic clamping resulted in significant increases in PMN sequestration, F2-isoprostane levels, and capillary permeability. Treatment with anti-CD18 decreased pulmonary oxidative injury, as evidenced by a reduction in F2-isoprostanes and pulmonary permeability, but anti-CD18 failed to reduce PMN sequestration in the lung. Similar results of pulmonary injury after intestinal ischemia-reperfusion injury were reported by Hill and co-workers (20): a monoclonal antibody against the CD11b component of CD18 reduced pulmonary permeability but had no effect on pulmonary PMN sequestration. Vedder et al. (37) also demonstrated that anti-CD18 was capable of reducing gastrointestinal but not pulmonary injury in hemorrhagic shock, suggesting that different organs sequester PMN by different mechanisms. Doerschuk and co-workers (9) also demonstrated that PMN adherence to the pulmonary endothelium may occur via CD18-dependent or CD18-independent mechanisms specific to the inciting stimulus. It is also possible that anti-CD18 decreased pulmonary injury in our model by preventing or attenuating PMN activation by cytokines or inflammatory mediators released in the vicinity of adherent cells (1, 8, 27).

Hepatic injury in shock + clamp animals is characterized by increased MPO activity, F2-isoprostane levels, and histological evidence of tissue injury. Although the aorta was clamped just proximal to the superior mesenteric artery in this model, the liver is considered a remote organ, since it still receives oxygenated blood from the hepatic artery during the clamp procedure. Treatment with anti-CD18 significantly decreased liver MPO activity and histological evidence of tissue injury, suggesting that anti-CD18 decreases tissue injury by preventing PMN adhesion to sinusoidal cells. The fact that anti-CD18 reduced, but did not significantly attenuate, increases in F2-isoprostane levels in this model suggests that other factors, e.g., xanthine oxidase, may...
also play a role in the generation of lipid peroxidation in the liver after aortic clamping (32).

In shock + clamp animals, hepatic injury was associated with a 20-fold increase in ALT and a 22-fold increase in AST levels. These increases demonstrate the significant hepatic injury associated with our RAAA model. Although ALT and AST are sensitive markers of hepatocellular damage, AST is present in many other tissues, including heart, skeletal muscle, brain, and kidney, and is thus somewhat less specific indicator of hepatocellular damage. Therefore, the increase in AST may also reflect widespread tissue injury in this model. Anti-CD18 significantly attenuated AST and ALT release, with similar percent reductions for both transaminases, suggesting that neutrophils mediate hepatic injury in this model via a CD18-dependent mechanism.

Throughout the ~5-h experimental procedure, sham animals maintained MAP at ≥100 mmHg with almost no Ringer lactate solution infused. In animals subjected to shock alone, MAP returned to prehemorrhage values immediately after reinfusion of shed blood, and animals subjected to shock alone, MAP returned to prehemorrhage levels, with similar percent reductions for both transaminases, suggesting that neutrophils mediate hepatic injury in this model via a CD18-dependent mechanism.

In the shock + clamp group, on the other hand, MAP during the clamp procedure was significantly lower than in the clamp-alone group. Furthermore, shock + clamp animals required an almost twofold increase in resuscitation fluid compared with the clamp-alone group to maintain their MAP at >100 mmHg during the reperfusion period. When shock + clamp animals were treated with anti-CD18, MAP during the clamp period was significantly higher than that in control antibody-treated animals and identical to that in the clamp-only group. This was the first observed difference between anti-CD18-treated and control antibody-treated shock + clamp animals. Furthermore, anti-CD18 treatment led to a twofold reduction in resuscitation fluid requirements during the reperfusion period compared with control antibody-treated animals. These results demonstrate that blockade of PMN adhesion reduces the diffuse increase in capillary permeability associated with this RAAA model and, in doing so, may preserve adequate intravascular volume to maintain MAP.

In conclusion, this model demonstrates that a synergy exists between the two phases of RAAA (hemorrhagic shock and supramesenteric aortic clamping) that results in significant intestinal, hepatic, and pulmonary injury characterized by increased capillary permeability, PMN sequestration, lipid peroxidation, and elevation of transaminases. These results support the “two-hit” hypothesis (11), which proposes that hemorrhagic shock followed by resuscitation renders animals more susceptible to injury by priming for an exaggerated response to a second stimulus.

This study also demonstrated that a monoclonal antibody against anti-CD18 significantly reduces organ injury in RAAA. Furthermore, the differences in the tissue protective effects of anti-CD18 in the three organ systems examined suggest that different PMN-mediated mechanisms are responsible for the local and remote injury observed in this model of RAAA. Further studies on the mechanism of CD18-independent aspects of this injury are needed. Finally, this study demonstrates that intervention strategies have the potential to reduce organ injury and mortality associated with RAAA repair.

This research was supported by Physicians of Ontario through Physical Sciences Incorporated. A. J. Boyd was awarded a Lifeline Foundation Fellowship (1997).

Address for reprint requests and other correspondence: T. F. Lindsay, Toronto Hospital (General Division), 200 Elizabeth St., Eaton 5-306, Toronto, Ontario, Canada M5G 2C4 (E-mail: tlindsay@torhosp.toronto.on.ca).

Received 9 November 1998; accepted in final form 8 March 1999.

REFERENCES


Anti-CD18 Reduces Injury in RAAA