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. A CD18 monoclonal antibody reduces multiple organ injury in a 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 monoclonal 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.

ischemia-reperfusion; F2-isoprostanes; neutrophils; myeloperoxidase

Elective abdominal aneurysm repair is characterized by ischemia and reperfusion of the lower torso during clamping and unclamping of the aorta. RAAA, in particular, is associated with the combined ischemic insults of hemorrhagic shock and lower torso ischemia. We previously demonstrated, in a rat model of RAAA, that shock paired with supramesenteric aortic clamping results in pulmonary injury characterized by increased capillary permeability and polymorphonuclear leukocyte (PMN) sequestration, whereas hemorrhagic shock or clamping alone in this model was insufficient to produce this injury (26). Similarly, animals subjected to shock paired with infrarenal aortic clamping also failed to develop pulmonary injury (26), suggesting that factors from the ischemic intestine play an intricate role in the development of pulmonary injury in this RAAA model.

PMN have been implicated in ischemia-reperfusion injury in various microvascular beds after local and hemorrhage-induced ischemia (5, 19, 33, 34, 37). Furthermore, PMN have been postulated to be mediators of remote tissue injury after local reperfusion in RAAA. In sheep, significant PMN infiltration and degranulation have been documented after lower torso ischemia-reperfusion (25).

Monoclonal antibodies directed against CD11b/CD18 adhesion molecules on PMN have been found to significantly protect against ischemia-reperfusion injury (5, 19, 37). It has been previously demonstrated that treatment with anti-CD11b or anti-CD18 significantly reduced pulmonary injury after intestinal ischemia (21, 22), suggesting that PMN may be mediators in the development of pulmonary injury after intestinal ischemia-reperfusion.

It was our hypothesis that PMN mediate local and remote organ injury after shock and aortic cross-clamping. To test this hypothesis, we examined the effect of a monoclonal antibody directed against CD18 on the generation of local (intestinal) and remote (pulmonary and hepatic) injury after the combined insults of shock and supramesenteric aortic clamping in an animal model simulating RAAA repair.

MATERIALS AND METHODS

Experimental design. Male Sprague-Dawley rats (370–475 g) were divided into four groups: 1) sham (n = 5), 2) shock (n = 7), 3) clamp (n = 7), and 4) shock + clamp (n = 14). Animals in the shock + clamp condition were further subdivided into anti-CD18-treated (n = 7) and control antibody-treated (n = 7) groups. All animals were anesthetized with...
The CD18 antibody (anti-CD18) is directed against monoclonal antibody (Wt.3, 4 mg/kg) was administered intra-arterially. In appropriate groups, shock was induced by blood withdrawal into a plastic heparinized syringe (500 U) to reduce and maintain MAP at 50 mmHg for 1 h. The shed blood was maintained at room temperature on a tube rocker during the stabilization and experimental periods. Intestinal perfusate was collected every 10 min. Throughout the perfusion, samples of blood (0.3 ml) were withdrawn at 1-h intervals. The blood samples were used for the measurement of total albumin concentration and the specific activity of 125I-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 F₂-isoprostane levels, as well as histological tissue injury, an additional 5-cm segment of jejunum distal to the cannulated segment was used.

In appropriate groups, shock was induced by blood withdrawal into a plastic heparinized syringe (500 U) to reduce and maintain MAP at 50 mmHg for 1 h. The shed blood was maintained at room temperature on a tube rocker during the shock period. At 55 min of shock, control antibody [anticholinergic toxin antibody (3H11-B9Co), 4 mg/kg] or the IgG1 CD18 reaction mixture contained 16 mmol/l 3,3',5,5'-tetramethylbenzidine dissolved in N,N-dimethyformamide 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 unit of enzyme that catalyzes the conversion of 1 µmol of hydrogen peroxide to water and oxygen per minute at 25°C.

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 in the blood samples was assayed for 125I-albumin with a gamma counter. Each blood sample drawn during the experimental procedure was centrifuged at 10,000 rpm, and 100 µl of plasma were removed for determination of albumin content and 125I-albumin activity. The level of 125I in the blood samples was assayed 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 unit of enzyme that catalyzes the conversion of 1 µmol of hydrogen peroxide to water and oxygen per minute at 25°C.
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 F2-isoprostane levels. F2-isoprostanes have been shown to be a sensitive and accurate marker of tissue lipid peroxidation (2, 28, 30, 31). F2-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]PGF2α). Samples were sliced and homogenized in 1.0 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 F2-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 precondensed SPE-C18 reverse-phase cartridges, followed by 5 ml of elution. For purification. Protein content of samples was determined by the bicinchoninic acid protein assay system (Pierce), and all data are expressed as micrograms of F2-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 cresyl blue, and PMN counts were determined using a hemocytometer. The remainder of the 1.5-ml sample was centrifuged, and the plasma was removed and stored at −70°C for subsequent determination of antibody excess. Antibody levels were determined as described by Iss酋t and Wytketovicz (23), and data are expressed as micrograms of CD18 per milliliter of plasma.

Statistical analysis. Values are means ± SE, and differences between group means were considered statistically significant at P < 0.05. Data were analyzed by a one-way ANOVA, and the means of all groups were compared a posteriori using the Student-Newman-Keuls test for multiple comparisons.

Animal care guidelines. All animals used in this study were maintained in an accredited facility and cared for in accordance with the recommendations of the Canadian Council on Animal Care, the requirements of the Animals for Research Act of the Province of Ontario, and the regulations of the Animal Care Committee, the Toronto Hospital, and the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 86-23, Revised 1985].

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 F2-isoprostane levels. F2-isoprostane levels were 9.0 ± 15.0 pg/mg protein in sham animals (Fig. 2C). In the control antibody-treated shock + clamp group, F2-isoprostane levels significantly increased to 843.27 ± 231.24 pg/mg protein (P < 0.05). Treatment with anti-CD18 decreased F2-isoprostane levels to 396.6 ± 67.5 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 during the stabilization and clamp periods; however, it increased significantly to 843.27 ± 231.24 pg/mg protein (P < 0.05). Treatment with anti-CD18 decreased 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>
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<tr>
<th>Conditions</th>
<th>Groups</th>
<th>Mean Rate (mg·min⁻¹·g intestine⁻¹)</th>
<th>Mean Rate (mg·min⁻¹·g intestine⁻¹)</th>
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<tbody>
<tr>
<td></td>
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<td>Shock</td>
<td>Clamp</td>
<td>Shock + Clamp Control antibody</td>
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<tr>
<td>Preshock</td>
<td>0.159 ± 0.052</td>
<td>0.189 ± 0.080</td>
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<td>Shock</td>
<td>0.188 ± 0.029</td>
<td>0.114 ± 0.039</td>
<td>0.217 ± 0.037</td>
<td>0.231 ± 0.099</td>
</tr>
<tr>
<td>Clamp</td>
<td>0.184 ± 0.034</td>
<td>0.245 ± 0.098</td>
<td>0.246 ± 0.057</td>
<td>0.320 ± 0.075</td>
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<tr>
<td>Reperfusion</td>
<td>0.246 ± 0.071</td>
<td>0.152 ± 0.033</td>
<td>0.395 ± 0.101*</td>
<td>1.196 ± 0.237*</td>
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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-isoprostanes levels to 137.95 ± 20.27 pg/mg protein ($P < 0.05$ compared with sham and shock). 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

![Fig. 4. A: hepatic MPO activity in sham animals and shock + clamp animals treated with control and with anti-CD18. *$P < 0.05$ compared with sham. #}$P < 0.05 compared with shock + clamp (control antibody). B: hepatic F2-isoprostane levels in sham animals and shock + clamp animals treated with control and with anti-CD18. *$P < 0.05$ compared with sham. C: alanine aminotransferase (ALT) levels in plasma of sham, shock, and clamp animals and shock + clamp animals treated with control and with anti-CD18. *$P < 0.05$ compared with sham, shock, and clamp. #}$P < 0.05 compared with shock + clamp (control antibody). D: aspartate aminotransferase (AST) levels in sham, shock, and clamp animals and shock + clamp animals treated with control and with anti-CD18. *$P < 0.05$ compared with sham and shock. #}$P < 0.05 compared with shock + clamp (control antibody).
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 ±

![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.](http://ajpheart.physiology.org/)

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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 [32P]-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 gastro-intestinal 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 a 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 these animals also required minimal resuscitation fluid (~10 ml). In animals subjected to clamping alone, MAP was significantly elevated during the clamp procedure. After removal of the aortic clamps, MAP fell rapidly and animals required significant resuscitation fluid to maintain their MAP.

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 has 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.

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