Role of endotoxin in the expression of endothelial selectins after cecal ligation and perforation

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1Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130-3932; and 2Department of Physiology, University of Western Ontario and Vascular Biology Program, London Health Sciences Centre, London, Ontario, Canada N6A 4G5

Bauer, Philippe, Cameron W. Lush, Peter R. Kvietys, Janice M. Russell, and D. Neil Granger. Role of endotoxin in the expression of endothelial selectins after cecal ligation and perforation. Am J Physiol Regulatory Integrative Comp Physiol 278: R1140–R1147, 2000.—The objectives of this study were to determine 1) the changes in endothelial cell adhesion molecule expression that occur in a clinically relevant model of sepsis and 2) the dependence of these changes on endotoxin (lipopolysaccharide [LPS]). The dual radiolabeled monoclonal antibody technique was used to quantify the expression of E- and P-selectin in LPS-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice that were subjected to acute peritonitis by cecal ligation and perforation (CLP). At 6 h after CLP, the expression of both E- and P-selectin was increased in the gut (mesentery, pancreas, and small and large bowel) compared with the sham-operated and/or control animals, with a more marked response noted in LPS-insensitive mice. The lung also exhibited an increased P-selectin expression in both mouse strains. An accumulation of granulocytes, assessed using tissue myeloperoxidase activity, was noted in the lung and intestine of LPS-sensitive but not LPS-insensitive mice exposed to CLP. These results indicate that the CLP model of sepsis is associated with an upregulation of endothelial selectins in the gut vasculature and that enteric LPS does not contribute to this endothelial cell activation response.

E-selectin; P-selectin; endotoxin-resistant mice; sepsis; shock; myeloperoxidase

BECAUSE THE RELEASE of endotoxin [lipopolysaccharide (LPS)] from gram-negative bacteria is generally regarded as a key initiating event in the pathogenesis of sepsis, bolus injections of LPS have been frequently used to induce and simulate the inflammatory changes observed in the clinically septic patient (7). As a rate-determining inflammatory event that has been characterized extensively using LPS injections is the expression of endothelial cell adhesion molecules (CAMs), such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and the selectins (E- and P-selectin) (26). These CAMs are known to mediate the coordinated, sequential recruitment of leukocytes in postcapillary venules, where the leukocyte-endothelial cell interactions progress from rolling to firm adhesion and ultimately to transendothelial migration (emigration) (14). Bacterial toxins, such as LPS, are known to elicit the upregulation of endothelial CAMs that mediate each of these critical steps in the recruitment of leukocytes to inflammatory foci. The endothelial selectins, for example, which are required for leukocyte rolling, respond vigorously to LPS stimulation both in vitro and in vivo, with several-fold increases in cell surface expression noted only 3–5 h after LPS exposure (10). The pathological significance of this LPS-induced expression of selectins has been demonstrated using blocking doses of monoclonal antibodies (MAbs) directed against either P- or E-selectin (37) or selectin-deficient mice (10), all of which exhibit attenuated inflammatory and tissue injury responses to LPS.

Although LPS-activated endothelial cells have provided valuable insights concerning the molecular mechanisms that sustain the inflammatory response in the walls of blood vessels, the use of LPS in animal models of human disease, including sepsis, has been criticized on several grounds (7). First, this approach appears to inadequately replicate many key features of human sepsis, such as an initial hyperdynamic circulation. Second, the doses of LPS that are often administered to experimental animals generally produce blood levels that far exceed LPS levels detected in human disease. Finally, the tissue responses to these levels of LPS, such as cytokine production, can be orders of magnitude greater than that experienced in the clinical setting. As a consequence of these limitations of the LPS injection model, other experimental approaches have been developed to more precisely mimic the human disease process and these approaches have gained widespread use. One such model involves ligation and perforation of the cecum in either small or large animal species, which produce peritonitis, bacteremia, and signs of systemic sepsis (38).

The cecal ligation and perforation (CLP) model appears to more closely mimic both qualitatively and quantitatively the vascular and inflammatory events that are observed in human sepsis. Hence, there have been a number of studies that have focused on charac-
terizing the inflammatory responses and defining the mechanisms that contribute to the pathogenesis of sepsis in the CLP model. These studies have provided indirect evidence for an upregulation of endothelial CAMs in different regional vascular beds that is comparable to that observed with LPS injections. For example, mRNA levels for different endothelial CAMs (P- and E-selectin, ICAM-1 and VCAM-1) are elevated in the lungs of mice with abdominal sepsis (29). Furthermore, the inflammatory responses to abdominal sepsis are greatly reduced in animals receiving blocking doses of MAbs directed against endothelial CAMs (32) or in mice that are genetically deficient in E- or P-selectin (39). Inasmuch as endothelial selectins have been implicated in the pathogenesis of abdominal sepsis and there is no direct evidence of an increased expression of these CAMs in the vasculature of septic animals, a major objective of this study was to quantify the expression of P- and E-selectin in different vascular beds of mice subjected to CLP.

Although LPS has been implicated as a key mediator of the inflammatory changes associated with pathological conditions, such as sepsis and ischemia-reperfusion (34), there are little published data that bear directly on this issue. In a recent study from our laboratory (3), we observed no difference in the ischemia-reperfusion-induced upregulation of intestinal E-selectin between LPS-sensitive and LPS-insensitive mice, suggesting that LPS does not significantly contribute to this model of acute inflammation. Several therapeutic approaches have been used to address the role of LPS in the pathogenesis of CLP-induced sepsis, including anti-LPS antibodies (21), polymyxin-dextran, which binds LPS (9), and LPS-sensitive and/or -insensitive mice (2). Although all of these anti-LPS strategies are associated with a reduced mortality in animal models of sepsis, it remains unclear whether this protective effect reflects a comparable beneficial action on key inflammatory responses to sepsis, such as endothelial CAM expression. Hence, another major objective of this study was to determine whether the increased endothelial CAM expression that occurs in the CLP model of sepsis is dependent on LPS.

MATERIALS AND METHODS

MAbs. The MAbs used for the in vivo assessment of E- and P-selectin expression were 10E9.6, a purified binding rat immunoglobulin (IgG2a) against mouse E-selectin (36), RB40.34, a purified binding rat immunoglobulin (IgG1) against mouse P-selectin (33), and P-23, a nonbinding murine IgG1 directed against human P-selectin (Pharmacia-Ujoh; Kalamazoo, MI) (22). The binding (10E9.6 or RB40.34) and nonbinding (P-23) MAbs were labeled with 125I and 131I, respectively (Du Pont-New England Nuclear; Boston, MA) using the 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril (Iodo-Gen) method (12).

Briefly, iodogen (Pierce; Rockford, IL) was dissolved in chloroform at a concentration of 0.5 mg/ml, and 250 µl of this solution were placed in glass tubes and evaporated under nitrogen. A 250-µg sample of MAb was added to each iodogen-coated tube, and either 125I or 131I (1 µCi/µg protein) was added. The mixture was incubated on ice, with periodic stirring for 5–10 min. The total volume was brought to 2.5 ml by adding phosphate-buffered saline (PBS, pH 7.4). Thereafter, the coupled MAb was separated from free125I or 131I by gel filtration on a Sephadex PD-10 column (Pharmacia; Uppsala, Sweden). The column was equilibrated (50 ml) and then eluted (2.5, 2.5, 1, and 7 ml) with PBS containing 1% bovine serum albumin. Four fractions were collected, the second 2.5 ml of which contained the radiolabeled MAb. Absence of free125I or 131I was ensured by extensive dialysis of the protein-containing fraction. Less than 1% of the activity of the protein fraction was recovered from the dialysis fluid. Radiolabeled MAbs were stored at 4°C and used within 1 (131I-P-23) or 2 mo (125I-10E9.6 or 125I-RB40.34) after labeling.

Animal procedures. Eight- to ten-wk-old male LPS-sensitive (C3H/HeJ, n = 61) and LPS-insensitive (C3H/HeJ, n = 63) mice (Jackson Laboratory; Bar Harbor, ME), weighing 29.5 ± 0.4 g, were used for the radiolabeled antibody experiments. The mice were anesthetized subcutaneously with 150 mg/kg of ketamine and 7.5 mg/kg of xylazine. The right jugular vein and right carotid artery were cannulated with polyethylene tubing (PE-10). To measure E- or P-selectin expression, a mixture of 10 µg binding 125I anti-E- or anti-P-selectin MAb and a dose (0.5–5 µg) of nonbinding 131I MAb, adjusted to ensure a total 131I injected activity of 500,000–100,000 counts/min, were injected through the jugular vein catheter (total volume 200 µl). Mice were then heparinized with 50 IU sodium heparin in 0.2 ml of saline. Blood samples (200 µl) were obtained through the carotid artery catheter 5 min after injection of the MAb mixture for measurement of plasma 125I and 131I activity (50 µl). Thereafter, an isovolumic blood exchange was rapidly performed with bicarbonate-buffered saline (BBS, 6 ml) through the jugular vein catheter. Thoracic inferior vena cava was severed and flushed with BBS (15 ml) through the carotid artery catheter. All the organs were harvested and weighed for radioactivity measurements. These experimental procedures were performed according to the criteria outlined by the National Institutes of Health and approved by the Louisiana State University Health Sciences Center-Shreveport Committee on Animal Care and Use.

Calculation of E- and P-selectin expression. The method for calculation of E- and P-selectin expression has been described previously (10). In brief, the activities of 125I-labeled binding MAb (anti-E- or anti-P-selectin) and the 131I-labeled nonbinding MAb in different tissues and in 50 µl aliquots of cell-free plasma were counted in a 14800 Wizard 3 gamma-counter (Wallac; Turku, Finland), with automatic correction for background activity and spillover. The injected activity in each experiment was calculated by counting a 2-µl sample of the mixture containing the radiolabeled MAbs. The radioactivity remaining in the tube used to mix the MAbs and in the syringe used to inject the mixture was subtracted from the total calculated injected activity. The accumulated activity of selectin MAb in an organ was expressed as nanograms of MAb per gram of tissue. E- and P-selectin expression was calculated by subtracting the accumulated activity of the nonbinding 131I MAb (131I-P-23) from the activity of the binding 125I-labeled selectin MAb. Previous studies have shown that MAbs retain their functional activity after radiolabeling, as evidenced by a similar effectiveness of labeled and unlabeled MAbs to block leukocyte adherence in rat mesenteric venules (30). In addition, we have shown that constitutive and endotoxin-induced expression of E-selectin and P-selectin are not detectable in the small intestine and other tissues of mice that are genetically deficient in E- or P-selectin, unlike their wild-type counterparts (10).

Experimental protocols. Two experimental protocols were employed to address the specific study objectives outlined in...
the introduction. In the first experimental protocol, the dual radiolabeled MAb technique was used to quantify the expression of E- and P-selectin in the intestinal vasculature and other vascular beds of LPS-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice subjected to CLP. In a previous study (3) we had demonstrated that LPS-sensitive and -insensitive mice respond in a differential manner to LPS, Escherichia coli LPS (serotype 0111:B4, Sigma Chemical; St. Louis, MO) at 3 h after intraperitoneal administration and at doses ranging from 0.5 to 50 µg per animal. Because preliminary studies demonstrated that E- and P-selectin were already upregulated within 3 h but more dramatically expressed at 6 h after CLP, this study focused on the latter time point for measurement of E- and P-selectin expression.

CLP was performed in anesthetized (as described in Animal procedures) LPS-sensitive and -insensitive mice (3). In one group of mice subjected to CLP, a 2-cm midline laparotomy was performed and the cecum (with adjoining intestine) was exposed. The cecum was tightly ligated with 2-0 silk at its base without causing bowel obstruction and perforated three times with a 20-gauge needle (top, middle, and bottom). Then the cecum was gently squeezed to extrude feces, which were spread around the abdomen using a cotton swab. The incision was closed using 4-0 silk suture, and the mouse received 1 ml of saline subcutaneously for fluid resuscitation. A second group of mice subjected to sham CLP were anesthetized and, after the laparotomy the cecum was isolated but not occluded. The mice were reanesthetized 6 h after the sham or actual CLP procedure, followed by measurement of E- and P-selectin expression. A third group of mice served as controls and was not exposed to a laparotomy.

In the second experimental protocol, another series of 15 LPS-sensitive (C3HeB/FeJ) and 15 LPS-insensitive (C3H/HeJ) mice, weighing 28.9 ± 0.5 g, with each group consisting of mice exposed to either sham surgery (n = 7) or to CLP (n = 8) (no control group) was studied to assess CLP-induced changes in mean arterial pressure, plasma lactate concentration, circulating leukocyte count, and tissue myeloperoxidase activity.

Determination of plasma lactate. Lactate concentration in plasma was determined from the enzymatic conversion of lactate to pyruvate coupled to the reduction of nicotinamide adenine dinucleotide (NAD; Sigma Diagnostica). The stoichiometric formation of NADH + H+ was monitored spectrophotometrically at 340 nm on a Gilford 240 spectrophotometer with completion of the reaction ensured using hydrazine trapping of the formed pyruvate. Briefly, 100 µl of heparinized blood were collected and immediately deproteinized in a chilled centrifuge tube containing 100 µl 10% trichloroacetic acid for the sample. After second centrifugation, 50 µl test samples were mixed in a cuvette containing 1 ml glycine-hydrazine buffer (0.6 mmol/l, pH 9.2 at 25°C) and 100 µl NAD (15 mM). After duplicate baseline readings, the reaction was started by adding 3 µl lactate dehydrogenase (1,000 U/ml) into the cuvette followed by three 10-min readings with the reaction completed in 20 min at 25°C. The blank cuvette was prepared, substituting 3 µl of water for the sample. A standard cuvette contained 50 µl lactate standard (2.2 mM) with the reaction being linear over the concentration range of 0.1–4.4 mM. Sample lactate concentrations were calculated after subtracting the appropriate blanks, using the 2.2 mM lactate standard.

Circulating leukocyte count. The number of circulating leukocytes was determined from a 25-µl blood sample obtained from the carotid artery at 6 h after CLP. Leukocytes were stained by mixing the blood sample with 465 µl of 3% acetic acid and 10 µl of 1% crystal violet. Polymorphonuclear cells and mononuclear cells were counted with the aid of a Neubauer hematocytometer.

Tissue myeloperoxidase activity. Myeloperoxidase activity, which is widely used to quantify neutrophil accumulation in tissues, was assessed using the O-dianisidine method (40). Lungs and small and large bowel were harvested and immediately stored frozen (−78°C) at 6 h after CLP. The tissue samples were thawed, weighed, suspended (10% wt/vol) in 50 mM potassium-phosphate buffer (KPi), pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide buffer (0.1 g/20 ml KPi), and homogenized. One milliliter of the homogenate was sonicated three times for 10 s and microcentrifugated at 12,000 rpm for 10 min at 4°C. The reaction was started by mixing and incubating the supernatant (100 µl) at 20–25°C for 5 min with a solution composed of 2,900 µl of 50 mM KPi, 30 µl of 20 mg/ml O-dianisidine dihydrochloride, and 30 µl of 20 mM hydrogen peroxide. The addition of 30 µl of 2% sodium azide stopped the reaction. The change in absorbency was read at 460 nm at 5 min in a spectrophotometer (Hitachi U-2000, Hitachi Instruments; Dallas, TX), and myeloperoxidase activity was expressed as the amount of enzyme necessary to produce a change in absorbency of 1.0·min−1·g wet weight of tissue−1.

Statistics. The data were analyzed using a one-way analysis of variance with Scheffé’s (post hoc) test (StatView 4.02 for Macintosh computers). All values are reported as means ± SE. Statistical significance was set at P < 0.05.

RESULTS

CLP was associated with a significant reduction in mean arterial pressure, which was reduced to the same level in both LPS-sensitive and LPS-insensitive animals (Fig. 1). This occurred despite a lower mean arterial blood pressure in the LPS-insensitive sham group compare with the LPS-sensitive animals. A significant drop in blood leukocyte count for both mouse strains accompanied this reduction in blood pressure after CLP. This decrement in blood leukocyte count was

![Fig. 1. Mean arterial pressure 6 h after cecal ligation and perforation (CLP) in lipopolysaccharide (LPS)-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice. *P < 0.05 vs. sham-operated animals. £P < 0.05 between the 2 types of mice.](http://ajpregu.physiology.org/)
primarily due to a reduction in the number of circulating neutrophils (Fig. 2), although there was a slight but nonsignificant reduction in blood lymphocyte count as well. The blood neutrophil count was significantly higher in LPS-insensitive sham group than in LPS-sensitive animals. CLP also elicited a significant increase in plasma lactate concentration in both LPS-sensitive and -insensitive mice (Fig. 3). Lactate levels were slightly, albeit not significantly, higher in LPS-insensitive sham group than in LPS-sensitive animals.

The responses of E-selectin expression to CLP were noted in the mesentery (Fig. 4A) and small bowel (Fig. 4B) of LPS-sensitive and -insensitive mice. In both tissues CLP produced a significant increase in E-selectin expression relative to their control and sham-operated counterparts. This increase was also observed in the pancreas and the large bowel (Table 1). In the mesentery, the magnitude of the increment was more pronounced in LPS-insensitive animals. In the lungs E-selectin expression was much higher in the LPS-insensitive animals compared with their LPS-sensitive counterparts however, CLP did not alter E-selectin expression in the lungs of either group.

The changes in P-selectin expression induced by CLP in the mesentery (Fig. 5A) and small bowel (Fig. 5B) of LPS-sensitive and -insensitive mice are summarized. As noted for E-selectin, CLP elicited significant increases in P-selectin expression in both vascular beds, and the amplitude of the response was greater in LPS-insensitive animals. This hyper-responsiveness of P-selectin expression in LPS-insensitive animals was noted in most organs (Table 2).

The changes in tissue myeloperoxidase activity, an index of neutrophil accumulation, were noted in the lungs (Fig. 6A) and small bowel (Fig. 6B) of LPS-sensitive and -insensitive mice after CLP. Significant CLP-induced increases in tissue myeloperoxidase were noted in the lungs and the small bowel in LPS-sensitive animals. However, no difference was found between the two different strains of mice.

**DISCUSSION**

Sepsis is a systemic inflammatory disorder that is associated with endothelium-dependent vascular dysfunction in the arterial, capillary, and venous segments of the vascular tree. In arterioles, endothelial cell dysfunction is manifested as an impaired ability to regulate blood flow (15), whereas capillary endothelial swelling promotes the trapping of leukocytes, which further diminishes tissue perfusion (27). It is the endothelial cells in venules, however, that bear the brunt of inflammatory responses elicited by sepsis. These responses include increased leukocyte trafficking, which is mediated by adhesion glycoproteins expressed on the surface of activated endothelial cells (35), and an increased extravasation of plasma proteins and water, which results from a diminished barrier function of venular endothelial cells (5). A dependence of the endothelial barrier dysfunction on leukocyte trafficking is supported by reports describing diminished albumin leakage from inflamed venules after inhibition of leukocyte rolling with selectin-blocking MAbs (4, 20).

The present study focused on an early and rate-determining step in the progression of acute inflammatory states such as sepsis, i.e., the regulation of endothelial CAMs that mediate leukocyte rolling. The endothelial selectins are well recognized for their essential role in the recruitment of leukocytes to sites of inflammation (25, 28). The importance of these surface proteins in the trafficking of leukocytes is exemplified by the observation that mice that are genetically deficient in both E- and P-selectin do not exhibit leukocyte rolling and display a severe impairment of leukocyte extravasation during thioglycollate-induced peritonitis (13). Although these observations suggest that selectins are critical for the altered pattern of leukocyte trafficking that is associated with the abdominal sepsis, the relative changes in E- and P-selectin expression that occur in different regional vascular beds during sepsis were undefined before the present study.
**Table 1.** E-selectin expression in different organs

<table>
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<tr>
<th></th>
<th>C3HeB/FeJ</th>
<th>C3HeB/FeJ</th>
<th>C3H/HeJ</th>
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<tr>
<td></td>
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<td>Sham (11)</td>
<td>CLP (11)</td>
<td>Control (6)</td>
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<tr>
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<td>36 ± 18</td>
<td>37 ± 15</td>
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<td>1 ± 1</td>
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<td>Liver</td>
<td>694 ± 48</td>
<td>405 ± 109</td>
<td>409 ± 108</td>
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<td>260 ± 120</td>
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<td>1 ± 0</td>
<td>5 ± 1†</td>
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<tr>
<td>Mesentery</td>
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<td>1 ± 0</td>
<td>3 ± 1†</td>
<td>2 ± 1</td>
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<tr>
<td>Stomach</td>
<td>0 ± 0</td>
<td>1 ± 0</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Small bowel</td>
<td>6 ± 1</td>
<td>3 ± 1</td>
<td>21 ± 5†</td>
<td>5 ± 3</td>
</tr>
<tr>
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<td>1 ± 0</td>
<td>4 ± 1†</td>
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<tr>
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<td>37 ± 11</td>
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<tr>
<td>Brain</td>
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Values are means ± SE in ng/g tissue; nos. in parentheses = no. of mice. LPS, lipopolysaccharide; CLP, cecal ligation and perforation; C3HeB/FeJ, LPS-sensitive mice; C3H/HeJ, LPS-insensitive mice. *P < 0.05 vs. control. †P < 0.05 vs. sham-operated animals. §P < 0.05 between 2 types of mice.

**Fig. 4.** E-selectin expression in mesentery (A) and small bowel (B) 6 h after CLP in LPS-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice. mAb, monoclonal antibody. *P < 0.05 vs. control. †P < 0.05 vs. sham-operated animals. £P < 0.05 between the 2 types of mice; ns, no difference.

**Fig. 5.** P-selectin expression in mesentery (A) and small bowel (B) 6 h after CLP in LPS-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice. *P < 0.05 vs. control. †P < 0.05 vs. sham-operated animals. £P < 0.05 between the 2 types of mice; ns, no difference.
Table 2. P-selectin expression in different organs

<table>
<thead>
<tr>
<th>Organ</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sham</td>
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<tr>
<td>Lungs</td>
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<td>Heart</td>
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<td>Small bowel</td>
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<tr>
<td>Large bowel</td>
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<td>Thymus</td>
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</tr>
<tr>
<td>Brain</td>
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Values are means ± SE in ng/g tissue; nos. in parentheses = no. of mice. *P < 0.05 vs. control. †P < 0.05 vs. sham. §P < 0.05 between 2 types of mice.

Our results indicate that the CLP model of sepsis elicits significant increases in the expression of both E- and P-selectin within 6 h after the initiating insult (fecal contamination of the abdominal cavity). The increased selectin expression appears to be confined largely to the bowel wall (small and large intestine) and mesenteric vascular beds, with a significant increase in P-selectin also noted in the pulmonary circulation. The magnitude of the increases in E- and P-selectin expression (4- to 6-fold) that were noted in the small intestine at 6 h after CLP is comparable to the responses elicited in this vascular bed by ischemia-reperfusion or exogenous LPS administered at doses less than 500 ng per mouse (3). The fact that vascular beds of organs within the abdominal cavity responded more vigorously to CLP than systemic organs suggests that high local concentrations of some fecal contents mediate the response, rather than a circulating factor to which all endothelial cells are exposed to an equal concentration. Studies assessing the mortality of experimental animals exposed to CLP or similar models of abdominal sepsis have implicated a key role for endotoxin in this pathological process. For example, it has been reported that the anti-endotoxin MAb E5 reduces both the elevation in plasma tumor necrosis factor (TNF) and mortality rate in rats with intra-abdominal sepsis induced by cecal perforation (21). Similarly, there is evidence that endotoxin-sensitive mice have a significantly higher mortality after CLP than endotoxin-insensitive mice (2). The data derived using these and other (9) experimental approaches strongly implicate endotoxin as the cause of mortality in septic animals. However, these observations do not provide meaningful insight into the role of endotoxin in mediating the endothelial CAM expression and leukocyte infiltration that accompanies sepsis. To address that issue we compared the changes in CLP-induced expression of E- and P-selectin in different tissues of endotoxin-sensitive and -insensitive mice. If endotoxin, derived from enteric bacteria, was primarily responsible for the upregulation of endothelial selectins and consequent leukocyte accumulation after CLP, then one would expect significantly lower levels of selectins and myeloperoxidase in tissues of LPS-insensitive compared with LPS-sensitive mice. We did not observe the predicted pattern of responses that would support a role for LPS in CLP-induced inflammation.

In this study the sham-operated LPS-insensitive animals showed lower mean arterial blood pressure, higher neutrophils count, and higher (albeit not significantly) plasma lactate levels 6 h after CLP compared with the sham-operated LPS-sensitive animals. Although we cannot exclude other causes, these differences between sham-operated mice of LPS-sensitive and -insensitive strains may reflect differences in the ability of the two strains to tolerate surgical stress. The LPS-sensitive strain, which is constantly exposed to LPS from the enteric bacteria, may develop tolerance to subsequent LPS insults that are known to occur during surgical procedure (41).

Fig. 6. Myeloperoxidase (MPO) activity in lungs (A) and small bowel (B) 6 h after CLP in LPS-sensitive (C3HeB/FeJ) and LPS-insensitive (C3H/HeJ) mice. *P < 0.05 vs. sham-operated animals; ns, no statistical difference between the 2 types of mice.
The pattern of changes in E- and P-selectin expression observed after CLP was the same in both strains of animals. However, we observed a trend for LPS-insensitive mice to respond more vigorously to CLP relative to selectin expression (Figs. 4 and 5) than their LPS-sensitive counterparts. Neutrophil recruitment responses to CLP were only noted in LPS-sensitive animals, suggesting dissociation between CLP-induced selectin expression and neutrophil accumulation. In the lungs, this dissociation between selectin expression and neutrophil accumulation likely reflects the fact that neutrophil entrapment by pulmonary capillaries occurs in many models of lung inflammation (8), including endotoxemia (3) and gut ischemia-reperfusion (3, 6). However, an explanation for the inconsistency between CLP-induced selectin expression and neutrophil accumulation is more difficult to explain in the intestine. It is conceivable that the selectin expression that occurs in response to CLP in LPS-insensitive mice is of lesser quantitative importance than endothelial cell and/or leukocyte adhesion molecules in mediating leukocyte-endothelial cell adhesion. Our data also demonstrate that P-selectin expression in the lungs after CLP is independent of endotoxin, which corroborates previous studies demonstrating that TNF-α is not a prerequisite for CLP-induced tissue injury (11, 23, 24). Similarly, it has been shown that gut ischemia-reperfusion also produces lung injury that is independent of endotoxin (18).

Our inability to obtain evidence that implicates endotoxin as a mediator of the inflammatory responses elicited by CLP raises a question regarding what factor(s) is involved in this inflammatory process (1, 16). It seems likely that the presence of fecal material in the abdominal cavity (19) would promote the release of cytokines and other inflammatory mediators from mast cells, macrophages, and other cell types (29). Gut mucosal hypoperfusion is widely considered to have a role in the pathogenesis of sepsis and to contribute to the multiple organ dysfunction syndrome that accompanies this disease (31). The lower arterial blood pressure and elevated blood lactate levels observed in both LPS-sensitive and LPS-insensitive mice after CLP suggest that tissue hypoperfusion may indeed be a factor that initiates selectin expression on endothelial cells and leukocyte infiltration in this model. This possibility is consistent with a recent study from our laboratory that demonstrates comparable increases in E-selectin expression and myeloperoxidase activity in postischemic intestine of LPS-sensitive and -insensitive mice (3). The role of intestinal ischemia in the initiation of CLP-induced inflammatory responses warrants further attention.

Perspectives

Studies in the literature have implicated a role for endothelial selectins (P- and E-selectin) in the systemic inflammatory responses to sepsis. Diminished inflammatory responses and improved survival in septic animals that are genetically deficient in E- or P-selectin have led to the speculation that selectins are upregulated on the surface of activated endothelial cells and that these adhesion molecules mediate the recruitment of inflammatory cells that ultimately mediate the tissue injury that causes death (17). This study provides the first quantitative demonstration of increased selectin expression in different vascular beds of septic animals, supporting the long-held view that endothelial cells are activated in this systemic inflammatory disorder. However, our findings fail to support the general assumption that enteric bacteria-derived LPS mediates the inflammatory responses associated with sepsis. Furthermore, this study indicates that the magnitude of the upregulation of endothelial selectins associated with our model of sepsis, while substantial, is not sufficient to explain the neutrophil recruitment into tissues that are either in direct contact (intestine) or distant to the septic focus (fecal contents). These observations underscore the importance of other factors, possibly other bacterial products and endothelial CAMs, in the recruitment of activated, circulating leukocytes to different vascular beds in septic animals.

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