Protective role of synthetic sialylated oligosaccharide in sepsis-induced acute lung injury

PHILIP C. RIDINGS, SHARON HOLLOWAY, GEOFFREY L. BLOOMFIELD, M. L. PHILLIPS, BERNARD J. FISHER, CHARLES R. BLOCHER, HARVEY J. SUGERMAN, and ALPHA A. FOWLER III

Abstract

The accumulation of neutrophils at the site of injury is a hallmark of the inflammatory response. Although generally beneficial to the host, there is evidence that in some circumstances neutrophil accumulation also plays a detrimental role in inflammatory conditions such as occur in burn injury, ischemia-reperfusion injury, organ transplant rejection, and the adult respiratory distress syndrome (ARDS). Recent in vitro and in vivo studies have demonstrated that neutrophil sequestration in tissues is the result of a multistep tightly controlled mechanism between circulating neutrophils and the vascular endothelium. More than 100 years ago, using intravital microscopy, Connheim demonstrated that neutrophils leave the axial blood flow in postcapillary venules and commence a slow rolling process along the endothelium (margination). The rolling neutrophils then stop, flatten out over the endothelium, extrude a pseudopodium through intercellular junctions, and then migrate between the endothelial cells into the interstitium. More recently, studies have demonstrated that this process is dependent on the expression of cell adhesion molecules and their ligands on both neutrophils and endothelium. Leukocyte β2-integrins (CD11/CD18) are essential for neutrophil adhesion and subsequent transendothelial migration (20, 27) and interact with intercellular adhesion molecules 1 (ICAM-1) and 2 (ICAM-2). However, under physiological conditions of flow, another class of adhesion molecules, the selectins, must be expressed and engaged to induce leukocyte rolling along the endothelial surface (29). The selectins, characterized by a lectin-type domain at the NH2-terminal region, consist of L-selectin, expressed on leukocytes, and P-selectin and E-selectin, both expressed on endothelium. L-selectin is constitutively expressed on neutrophil surfaces but is rapidly shed after activation, a time when CD11/CD18 expression is concurrently increasing (18). Endothelial P-selectin molecules, stored in Weibel-Palade bodies, are expressed on endothelial surfaces within minutes of endothelial cell activation. E-selectin expression depends on transcription and de novo synthesis of the molecule with peak expression occurring 4–6 hr after in vitro stimulation, after which there is a rapid decline (3). The lectin domain of the selectin molecule appears to be of prime importance for selectin-mediated binding (7), whereas the other domains may possess a modulatory role (22). Recent research has focused on the determination of the physiological ligands for each of the selectins. The main body of opinion indicates that the ligand for E- and P-selectins may be an oligosaccharide that contains fucose and sialic acid termed sialyl Lewis-x (11, 25). Sialyl Lewis-x is expressed on neutrophil L-selectin and also on other glycoprotein structures (24). The endothelial ligand for neutrophil S-selectin is not yet determined but may also be a similar glycoprotein (19).

We hypothesized that the infusion of a synthetic sialyl Lewis-x analog, CY-1503, may attenuate neutrophil-dependent lung injury by binding to the lectin domain, thus preventing selectin engagement. This hypothesis was tested in a porcine model of sepsis-induced lung injury by pretreating the swine with CY-1503 before the onset of sepsis. The pathophysiology of lung injury in this model has previously been shown to be highly dependent on sequestration of activated neutrophils into the lung (30, 34). This study confirmed that CY-1503 did significantly attenuate the development of lung injury, suggesting a major role for...
selectins in the pathophysiology of this condition and indicating a potential novel therapeutic approach to ARDS.

MATERIALS AND METHODS
Animal Preparation and Conditioning

Yorkshire pigs (15–20 kg) were obtained from a commercial vendor and housed in the Virginia Commonwealth University vivarium for 3–5 days before study. The experimental protocol used for these studies was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and adhered to National Institutes of Health guidelines for the use of experimental animals.

Porcine Model

Swine were preanesthetized with intramuscular ketamine hydrochloride (25 mg/kg) and placed supine. Pentobarbital sodium (10 mg/kg) was then administered intravenously to induce anesthesia. Tracheostomy was performed, and the trachea was intubated with a cuffed endotracheal tube (Argyle). Mechanical ventilation was commenced by using a Harvard large-animal ventilator (Harvard Apparatus) employing 0.5 inspired O₂ fraction and 5 cmH₂O positive end-expiratory pressure. The ventilator was set to deliver a tidal volume of 15 ml/kg with a respiratory frequency adjusted in all animals to produce an arterial Pco₂ of 40 Torr at the beginning of each experiment. Throughout the period of study, anesthesia was maintained by continuous infusion of pentobarbital sodium (5 mg·kg⁻¹·h⁻¹). Indwelling catheters were placed in the left common carotid artery for systemic arterial pressure (SAP), monitoring, blood sampling, and arterial O₂ determination and in the left external jugular vein for infusion of saline and Pseudomonas organisms. The left internal jugular vein was cannulated for infusion of CY-1503. An indwelling balloon-tipped pulmonary arterial catheter was inserted via the right external jugular vein and positioned in the pulmonary artery via pressure monitoring for measurement of pulmonary arterial pressure (PAP), pulmonary arterial occlusion (wedge) pressure, central venous pressure, and thermodilution cardiac output (model COM1, Edwards).

Sialyl Lewis-x Analog

CY-1503 (Cytel) is an oligosaccharide analog of sialyl Lewis-x that was prepared by combined chemical and enzymatic synthesis as previously described (15).

Bronchoalveolar Lavage (BAL)

BAL was performed through the indwelling endotracheal tube by using a fiber-optic bronchoscope (model Olympus BF-4, Olympus) at 0 and 300 min in right and left lungs, respectively. The distal end of the bronchoscope was gently wedged into third- or fourth-order bronchi of the middle and lower lobes. Each lobe was lavaged with two aliquots of 25-ml sterile 0.9% NaCl. BAL fluid was centrifuged at 400 g, 4°C, for 10 min, and the supernatant was stored at −20°C. Cell pellets were resuspended in Dulbecco’s phosphate-buffered saline containing 0.01% bovine serum albumin. Cell counts were determined by using a hemacytometer, and slide-directed cytocentrifugation was performed (Shandon Southern Instruments). Differential counts were performed on 200 cells stained by using a modified Wright-Giemsa stain (Diff-Quik, Baxter Scientific). Neutrophil counts in the BAL are expressed as total number of polymorphonuclear neutrophils (PMN) in BAL. BAL protein was measured in the noncellular fraction by the bicinchoninic acid method.

Total White Blood Cell Counts

Arterial blood samples were drawn into sterile glass tubes containing 0.15% EDTA and kept at 4°C (Vacutainer, Becton-Dickinson). Small aliquots of blood were set aside for white blood cell counts and blood smear differentials, which were performed as described above. The remainder was centrifuged at 500 g, 4°C, for 20 min, and the resulting plasma was stored at −20°C.

Organ Myeloperoxidase Content

At 300 min, animals were killed by infusion of pentobarbital sodium (100 mg/kg), and the right lung was immediately excised. Multiple random samples from all lobes were obtained, weighted to a total of 1 g, and homogenized (model 5-45, homogenizer, Virtis) in 4 ml of 20 mM potassium phosphate buffer (pH 7.4). One-gram samples were also taken from the liver (middle lobe), placed in buffer, and homogenized. All homogenates were then centrifuged (40,000 g, 4°C, 30 min, Beckman LS-65 Ultracentrifuge, Fullertont, CA). The pellet material was resuspended in 4 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma Chemical) and frozen at −70°C. Before the assay was performed, batched samples were thawed, sonicated X 90 s, incubated for 2 h (60°C), centrifuged (1,000 g, 30 min, 4°C), and myeloperoxidase content was assessed by adding 50 µl of each sample to quadruplicate wells of a 96-well microplate. Fifty microliters of 0.025% dimethoxybenzidine (Sigma Chemical) in 50 mM potassium phosphate buffer containing hexadecyltrimethyl ammonium bromide were then added. The reaction was started by addition of 50 µl of 0.01% H₂O₂, and the optical density at 460 nm was measured at 1-min intervals for several minutes. The rate of the reaction (slope) is directly proportional to the myeloperoxidase activity of the sample. Results are expressed as myeloperoxidase activity per gram of tissue.

Immunophenotyping for Neutrophil Integrin Expression

Direct immunophenotyping was performed by using a monoclonal antibody (MAb 60.3, Oncogen) that recognizes a functional epitope on the CD18 adhesion receptor. The antibody was previously conjugated with fluorescein isothiocyanate. Arterial blood samples from study animals were drawn into polypropylene tubes containing 0.15% EDTA and 0.1% NaN₃ and immediately placed on ice. One hundred-microliter aliquots of blood were then incubated with an equal volume of MAb 60.3 or immunoglobulin 2a control for 30 min at 4°C. Erythrocytes were lysed with NH₄Cl buffer, and cells were resuspended in phosphate-buffered saline. Cells were shielded from light at 4°C before analysis. Analysis was performed on a flow cytometer with a 4-decade 1,024-channel logarithmic amplifier (FACScan, Becton-Dickinson). Neutrophils were gated according to forward angle and 90° light scatter characteristics. A minimum of 5,000 events were analyzed for each sample, and the mean channel fluorescence of gated neutrophils was calculated. Mean channel fluorescence, a logarithmic function, was converted to a linear scale by using fluorescent microbead standards and Quickcal soft-
ware (Flow Cytometry Standards). Results are expressed as molecules of equivalent soluble fluorochrome.

**Superoxide Anion Assay**

Superoxide anion production was determined by measuring the superoxide dismutase inhibitable reduction of cytochrome c by using a dual-beam spectrophotometer (Shimadzu). Neutrophils (1 x 106/ml) and cytochrome c (100 µM) were combined in a thermostat-controlled stirred cuvette (37°C). An identically prepared reference cell contained reaction products plus superoxide dismutase (300 U/ml). The reaction was started by adding phorbol myristate acetate (200 ng/ml) to each cuvette. The change in absorbance at 550 nm was continuously recorded for 10 min. Results are expressed as the peak rate of O2 production (nmol·106 PMN·min−1·nm−1) based on an extinction coefficient of Δ550 nm = 2.10 x 104·M−1·cm−1.

**Experimental Design**

Three groups of animals were studied. Baseline blood sampling, hemodynamic measurements, and BAL were performed before any intravenous infusions. Group 1 (control; n = 10) received a 60-min intravenous infusion of sterile saline. Group 2 (sepsis; n = 10) received a 60-min intravenous infusion of live Pseudomonas aeruginosa, PAO strain (5 x 108 colony-forming units/ml at 0.3 ml·20 kg·min−1). Group 3 (CY-1503 pretreatment; n = 7) received a 60 mg/kg bolus of CY-1503 immediately before commencing the infusion of P. aeruginosa followed by a 60 mg/kg infusion over 5 h. Computer models predicted a serum concentration of 50 µg·ml−1 at 15 min after the initial injection of CY-1503.

**Statistical Analysis**

Data are means ± SE. Differences between and within groups were analyzed by using analysis of variance with Tukey’s Studentized range test. Paired data were analyzed by using a paired t-test. Statistical significance was assumed for P < 0.05.

**RESULTS**

**Hemodynamics (Table 1)**

Untreated septic animals exhibited rapid development of pulmonary arterial hypertension after the onset of sepsis. After termination of the bacterial infusion, PAP declined but remained significantly elevated above control animals throughout the duration of the experiment. CY-1503-treated animals also developed similar pulmonary arterial hypertension that was not significantly different from untreated septic animals at any time point. Control animals exhibited no increases in PAP during the experiment.

SAP in untreated septic animals increased significantly compared with control animals during the bacterial infusion. SAP then declined rapidly, becoming significantly lower than control animals by 2 h, and hypotension persisted for the duration of the experiment. Treatment of septic animals with CY-1503 failed to prevent the development of significant systemic arterial hypotension.

In untreated septic animals, cardiac index (CI) exhibited a biphasic response to sepsis. After the onset of sepsis, CI significantly declined, corresponding to the acute rise in PAP. CI then recovered toward baseline levels; however, from 2 h after the onset of sepsis, a second progressive significant decline was observed. Again, animals treated with CY-1503 exhibited an identical pattern in CI as untreated septic animals. In one control animal, CY-1503 was infused to search for adverse consequences of the agent alone. No adverse hemodynamic, neutrophil kinetic, or BAL protein consequences were observed in this single animal.

**Arterial PO2 (PaO2)**

Control animals showed no changes in PaO2 during the experiment (Fig. 1). In contrast, PaO2 declined rapidly within 1 h after the onset of sepsis in the untreated animals. From 1 h onward, the rate of decline slowed; however, these animals still exhibited a further progressive decline in PaO2 during the remainder of the experiment. CY-1503-treated septic animals also exhibited a significant decline in PaO2 compared with control animals from 1 h onward. However, CY-1503-treated animals exhibited a significant improvement in

**Table 1. Hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic arterial pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>95 ± 4</td>
<td>95 ± 3</td>
<td>104 ± 4</td>
<td>110 ± 5</td>
<td>114 ± 5</td>
<td>108 ± 6</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>Septic</td>
<td>101 ± 5</td>
<td>119 ± 5*</td>
<td>89 ± 7</td>
<td>64 ± 6*</td>
<td>63 ± 6*</td>
<td>71 ± 5*</td>
<td>78 ± 6*</td>
</tr>
<tr>
<td>CY-1503</td>
<td>83 ± 5</td>
<td>113 ± 6*</td>
<td>96 ± 6</td>
<td>68 ± 5*</td>
<td>69 ± 5*</td>
<td>73 ± 5*</td>
<td>82 ± 6*</td>
</tr>
<tr>
<td><strong>Pulmonary arterial pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
<td>16 ± 2</td>
<td>16 ± 1</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Septic</td>
<td>16 ± 2</td>
<td>47 ± 4*</td>
<td>35 ± 4*</td>
<td>31 ± 2*</td>
<td>35 ± 4*</td>
<td>35 ± 4*</td>
<td>32 ± 3*</td>
</tr>
<tr>
<td>CY-1503</td>
<td>14 ± 1</td>
<td>50 ± 3*</td>
<td>41 ± 3*</td>
<td>33 ± 3*</td>
<td>33 ± 3*</td>
<td>33 ± 3*</td>
<td>31 ± 2*</td>
</tr>
<tr>
<td><strong>Cardiac index, l·min−1·m−2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.1 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Septic</td>
<td>3.2 ± 0.3</td>
<td>2.0 ± 0.2*</td>
<td>2.8 ± 0.2*</td>
<td>2.6 ± 0.3</td>
<td>2.2 ± 0.2*</td>
<td>1.9 ± 0.1*</td>
<td>1.6 ± 0.3*</td>
</tr>
<tr>
<td>CY-1503</td>
<td>3.4 ± 0.3</td>
<td>2.3 ± 0.3*</td>
<td>2.6 ± 0.1*</td>
<td>2.8 ± 0.2</td>
<td>2.3 ± 0.2*</td>
<td>1.8 ± 0.3*</td>
<td>1.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CY-1503-treated group exhibited hemodynamic derangements similar to those observed in untreated septic animals developing systemic arterial hypotension, declining cardiac output, and significant pulmonary arterial hypertension. *Significantly different compared with control values, P < 0.05.
PO2 compared with untreated septic animals from 30 min onward.

Lung Permeability Injury

BAL protein content was increased more than five-fold at 5 h compared with baseline in untreated septic animals (898 ± 129 vs. 162 ± 19 µg/ml) and compared with control animals at the same time point, indicating that lung permeability injury had occurred (Fig. 2). CY-1503-treated animals, however, exhibited no increase in BAL protein content at 5 h compared with baseline (237 ± 65 vs. 197 ± 39 µg/ml). A significant reduction in BAL protein content was also observed compared with untreated septic animals.

Neutrophil Transendothelial Migration

In untreated septic animals, a 15-fold increase in BAL protein content was observed at 5 h compared with baseline (16.8 ± 5.0 vs. 1.0 ± 0.2 × 106 total neutrophils in BAL fluid), indicating significant migra-

tion of neutrophils across the alveolar-capillary membrane into the alveolar spaces (Fig. 3). CY-1503 treatment significantly attenuated this transmigration compared with septic untreated animals. The BAL protein content was only slightly elevated at 5 h compared with baseline in the treated group (2.4 ± 0.6 vs. 1.0 ± 0.2 × 106 total neutrophils in BAL fluid).

Neutrophil Kinetics

Peripheral neutrophil count. In both untreated and CY-1503-treated septic groups, peripheral neutrophil counts declined dramatically after the onset of sepsis, indicating organ sequestration (Fig. 4). The neutrophil counts were significantly lower than control levels within 30 min and reached a nadir at 2 h. No significant recovery in neutrophil counts was observed from this point onward in either septic group, and no differences were observed between these two groups at any time point.
Organ myeloperoxidase content. Lung myeloperoxidase content, a measurement of neutrophil sequestration, was significantly increased in untreated septic animals compared with control animals (194 ± 21 vs. 46 ± 6 units; septic vs. control animals) (Fig. 5). CY-1503-treated animals also demonstrated a significant increase in lung myeloperoxidase compared with control animals, but a significant reduction was observed compared with untreated septic animals (101 ± 16 units).

In control animals, liver myeloperoxidase content was barely detectable, whereas both treated and untreated septic groups demonstrated significant liver neutrophil sequestration at 5 h (3 ± 1 vs. 147 ± 38 vs. 130 ± 34 units; control vs. septic vs. CY-1503).

Indexes of Neutrophil Activation

Neutrophil CD18 expression. Circulating neutrophils exhibited significant upregulation of CD18 expression during the experiment in both the CY-1503-treated and untreated septic groups from 1 h onward compared with control animals (Fig. 6). Peak upregulation occurred at 3 h and was followed by a slight decline, although both treated and untreated septic groups exhibited significant upregulation until the end of the experiment. No significant differences were observed at any time point between treated and untreated septic animals.

Neutrophil oxidant burst. Neutrophil priming for the oxidant burst was also significantly increased in both the treated and untreated septic animals (Fig. 7). In neutrophils isolated at 5 h from both of these groups, a significant increase in the peak rate of superoxide anion generation was observed compared with neutrophils isolated at baseline.

DISCUSSION

The past decade has witnessed a twofold increase in the incidence of sepsis, affecting up to 500,000 patients per year in the USA (4). Sepsis is a major cause of morbidity and is associated with an overall mortality rate of up to 50% (33). The development of acute lung injury (ALI) in patients with gram-negative sepsis is a frequent occurrence, affecting 25% of such patients, but is associated with a mortality rate of 80–90% (8). Improvements in our knowledge of the pathophysiology of sepsis and ALI have led to the development of novel therapeutic approaches such as the use of anti-endotoxin or anti-tumor necrosis factor monoclonal antibodies in septic patients. Unfortunately, in recent clinical trials these agents have failed to be effective except in small subgroups (9, 14). Attention has now turned to a key cellular mediator of end organ damage, the neutrophil (32). Sequestration of neutrophils in both lung and other nonpulmonary organs followed by extracellular release of reactive \( \text{O}_2^- \) intermediates and potent lytic enzymes are believed to be primary events resulting in the genesis of multiorgan dysfunction. Formation of an intercellular cleft between tightly adherent activated neutrophils and endothelium creates a microenvironment protected from circulating...
antiproteases and endogenous antioxidants into which neutrophils secrete proteolytic enzymes (i.e., neutrophil elastase) and reactive $O_2$ intermediates (i.e., hydrogen peroxide), producing damage to underlying endothelium (17). There is significant evidence to support a major role for neutrophils in the pathogenesis of ALI associated with sepsis. Radiolabeling of neutrophils in animal studies shows rapid sequestration of these cells into the lung after the onset of sepsis (16). In patients with ARDS, BAL fluid obtained during life and lung tissue obtained at autopsy both contain large numbers of neutrophils (28). High levels of neutrophil elastase $\alpha_1$-protease inhibitor complexes are present in BAL fluids in the same patients, suggesting that neutrophils that migrate into the alveolar spaces during ARDS are actively degranulating (10). In animal studies, functional manipulation of the neutrophil to impair the formation of a microenvironment between the neutrophil and endothelium (30) and to reduce the oxidant burst has attenuated the extent of sepsis-induced lung injury. Neutrophils, therefore, exhibit both adherence to and migration across pulmonary vascular endothelium in ARDS. A critical first stage in this complex sequence of events is the adhesion of neutrophils to endothelium (17). Several studies have implicated a role for selectins in sepsis by demonstrating upregulation of E-selectin in several organs, including the lung in models of sepsis (6, 26). Mulligan et al. (22) have observed protection against lung injury conferred by selectin blockade in models of lung injury induced by immune complex deposition or complement activation. The present study has directly implicated a clear role for selectins in the genesis of sepsis-induced lung injury by attenuation of lung injury using infusion of the soluble form of a proposed ligand for selectin binding. This study has also demonstrated a potential role for selectin blockade as a therapeutic strategy in sepsis.

Infusion of the sialyl Lewis-x analog CY-1503 did not provide protection against the development of hemodynamic derangements associated with sepsis. CY-1503-treated septic animals developed a diminished cardiac output and SAP similar to that observed in untreated septic animals, and CY-1503 failed to prevent the development of significant pulmonary hypertension. The derangements in systemic hemodynamics are likely mediated by cytokines such as tumor necrosis factor (34), products of the kallikrein-kinin system, and possibly nitric oxide. Decreased production of these mediators is not expected to occur as a result of selectin blockade. The development of pulmonary arterial hypertension primarily results from thromboxane release due to activation of phospholipase A$_2$, which would not be attenuated by CY-1503 infusion. The striking feature of these experiments was the significant protection against lung injury. Two indicators of pulmonary injury measured in this model were significantly improved. First, BAL protein content, an indicator of protein leak from the pulmonary vasculature, showed no increase at 5 h compared with baseline in CY-1503-treated and control animals. In untreated septic animals, however, we observed a fivefold increase in BAL protein at 5 h, indicating a significant increase in alveolar-capillary membrane permeability. Second, a significant improvement in PaO$_2$, associated with the reduction in lung injury observed in CY-1503-treated animals, occurred. In CY-1503-treated septic animals, PaO$_2$ declined acutely in the first hour of the experiment but then failed to fall further, unlike untreated septic animals that continued to exhibit a further significant decline in PaO$_2$. In our model, the early decline observed in systemic PaO$_2$ in septic animals is likely explained by acute ventilation-perfusion mismatching related to the development of abrupt and severe pulmonary arterial hypertension (31). Continued deterioration in oxygenation in untreated animals is likely produced by neutrophil sequestration and oxidant-induced lung injury (30). These results suggest that although it did not prevent ventilation-perfusion mismatch, CY-1503 treatment did produce a reduction in subsequent lung damage. Attenuation of pulmonary injury in this neutrophil-dependent model may be produced by one or more mechanisms such as a reduction in lung neutrophil burden, attenuated neutrophil oxidant production, or an alteration of microenvironment formation at the endothelium-neutrophil interface. The results of the lung myeloperoxidase assay confirmed that CY-1503 infusion reduced lung neutrophil sequestration. This agrees with a previous study that showed that similar oligosaccharides reduced neutrophil sequestration in an immune complex-induced model of lung injury (21). However, CY-1503 treatment in the model employed in the present study did not prevent significant neutrophil sequestration in the liver. The reasons for the differential effects of CY-1503 on neutrophil sequestration in the liver and lung are not clear. We hypothesize that failure of CY-1503 to prevent sequestration in liver may be a flow-related phenomenon. During the septic period the lungs of septic pigs maintain high flow and vascular resistance, whereas systemic (nonpulmonary) organ perfusion and vascular resistances declined significantly. Gaboury and Kubas (12) recently showed that stimulated neutrophils exhibit binding to endothelium through integrin-dependent selectin-independent mechanisms under conditions of low flow. In our studies of septic CY-1503-treated animals, we hypothesize that as arterial hypotension develops and organ perfusion decreases, abdominal visceral organ blood flow decreases to critical levels that permit selectin-independent integrin-dependent binding to occur. This hypothesis accounts for the observed neutropenia and increased myeloperoxidase content of the liver in CY-1503-treated septic animals.

Circulating neutrophils in untreated septic animals exhibited significant activation as indicated by CD18 upregulation and priming for the oxidant burst during the experiment. Neutrophils obtained from CY-1503-treated septic animals also exhibited similar levels of activation, thus maintaining their ability to produce endothelial injury but failed to do so. Prevention of formation of a microenvironment interface in pulmonary vasculature may, therefore, be a second major mechanism of protection observed in these experiments. The microenvironment theory of leukocyte-
mediated endothelial damage, first proposed by Harlan (17), has gained increasing acceptance over recent years. This theory is based on the observation that activated neutrophils, once adherent to endothelium, flatten out over the target cell providing a bridge between cells into which O$_2$ radicals and protease enzymes may be secreted, protected from natural circulating antioxidants and antiproteases. The creation of the microenvironment is dependent on tight neutrophil/endothelial adhesion mediated by CD11/CD18 and ICAM-1 (20, 27). As noted previously, effective integrin/ICAM binding requires prior selectin engagement. Selectin engagement slows the passage of circulating neutrophils through capillaries and venules via a rolling phenomenon. The dramatic reduction in velocity produced by selectin engagement permits integrin/ICAM-mediated binding. It is therefore likely that a second mechanism responsible for the pulmonary protection afforded by CY-1503 treatment is the prevention of significant neutrophil rolling within pulmonary vasculature. Disruption of pulmonary vascular rolling would thus sharply reduce tight binding of neutrophils to pulmonary endothelium, resulting in a consequent decrease in microenvironment formation. O$_2$ radicals and proteases released by activated neutrophils would therefore be displaced or inactivated on exposure to circulating antioxidants. Consequently, their damaging effects on endothelium would be attenuated. Further data to support this hypothesis come from the reduction in BAL neutrophil content observed in CY-1503-treated animals. Neutrophil migration across endothelium is dependent on CD11/CD18 receptor expression (1, 20). Neutrophils in CY-1503-treated animals maintained their ability to migrate across the alveolar-capillary membranes (i.e., upregulated CD11/CD18 receptors) but failed to migrate. This suggests that the opportunity to do so was aborted by inhibition of close neutrophil/endothelial apposition.

Finally, the data presented in the present study support a physiological role for sialyl Lewis-x as an important ligand for selectins. Although other related oligosaccharides are shown to bind selectins, i.e., the stereoisomeric forms sialyl Lewis-a (2), sulfated Lewis-x, and sulfated Lewis-a (lacking sialic acid) (13), a physiological role for these related oligosaccharides has yet to be determined. The present study does not indicate which selectin molecule has prime importance in the genesis of sepsis-induced lung injury. Although all three selectins may bind to sialyl Lewis-x (11, 23), it is unclear whether L-selectin recognizes this ligand in vivo. Mulligan et al. (22) have demonstrated differential importance of the three selectins in a variety of models of lung injury (22). Further studies that use specific selectin antibodies or inhibitors are therefore required in models of sepsis. In this way, further insight may be gained into the pathogenesis of sepsis-induced lung injury and may permit more specific targeting of individual selectin molecules as potential therapeutic interventions in patients with gram-negative sepsis.

In conclusion, this study has investigated the effects of infusion of sialylated oligosaccharides (ligands for selectin interactions) in a model of gram-negative sepsis. Significant protection against the development of lung injury was observed. No protection, however, was observed against the hemodynamic derangements associated with sepsis. These data indicate a significant role for selectins in the genesis of sepsis-induced lung injury and point to a novel potential therapeutic intervention for patients with the increasingly common syndrome of sepsis.

This work was supported by the United States Army Medical Research and Development Command under Contract DAMD17–93–C-3106.

Address for reprint requests: A. A. Fowler III, Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Box 980050, MCV Station, Richmond, VA 23298-0050.

Received 11 December 1995; accepted in final form 18 September 1996.

REFERENCES


affinity binding of the leucocyte adhesion molecule L-selectin to 3'-sulphated-Le(a) and -Le(x) oligosaccharides and the predomi-
nance of sulphate in this interaction demonstrated by binding 
studies with a series of lipid-linked oligosaccharides. Biochem. 

N. R. MacIntyre, G. Emmanuel, H. Chmel, R. B. Kohler, M. 
McCarthy, and J. Plouffe. A controlled clinical trial of E5 
murine monoclonal IgM antibody to endotoxin in the treatment 
of gram-negative sepsis: the XOMA Sepsis Study Group. J. Am. 

15. Halcomb, R. L., H. Huang, and C. H. Wong. Solution- and 
and solid-phase synthesis of inhibitors of H. pylori attachment 
and E-selectin mediated leucocyte adhesion. J. Am. Chem. Soc. 116: 

I. R. McDougall, and T. A. Raffin. Kinetics of leucocyte 
sequestration in the lungs of acutely septic primates: a study 
using 111In-labeled autologous leucocytes. J. Surg. Res. 48: 

17. Harlan, J. M. Leukocyte-endothelial interactions. Blood 65: 

Neutrophil Mac-1 and MEL-14 adhesion proteins inversely 

Henzel, C. Grimley, C. Fennie, N. Gillett, S. R. Watson, and 
S. D. Rosen. An endothelial ligand for L-selectin is a novel 

20. Lawrence, M. B., C. W. Smith, S. G. Eskin, and L. V. 
McIntire. Effect of venous shear stress on CD18-mediated 
neutrophil adhesion to cultured endothelium. Blood 75: 227– 
237, 1990.

Ward. Protective effects of sialylated oligosaccharides in 
immune complex-induced acute lung injury. J. Exp. Med. 178: 

22. Mulligan, M. S., M. Miyasaka, T. Tamatini, M. L. J ones, 
and P. A. Ward. Requirements for L-selectin in neutrophil 

Singhal, S. Hakomori, and J. C. Paulson. ELAM-1 mediates 
cell adhesion by recognition of a carbohydrate ligand, sialyl-Le(x). 

24. Picker, L. J., R. A. Warnock, A. R. Burns, C. M. Doeschuk, 
E. L. Berg, and E. C. Butcher. The neutrophil selectin LECAM-1 
produces carbohydrate ligands to the vascular selectins ELAM-1 

Singhal, S. Hakomori, and J. C. Paulson. CD62 and endothelial 
cell-leucocyte adhesion molecule 1 (ELAM-1) recognizes the 

26. Redl, H., H. P. Dinges, W. A. Buurman, C. J. van der Linden, 
J. S. Pober, R. S. Cotran, and G. Schlag. Expression of 
endothelial leucocyte adhesion molecule-1 in septic but not 
traumatic/hypovolemic shock in the baboon. Am. J. Pathol. 139: 

27. Smith, C. W., T. K. Kishimoto, O. Abbassi, B. Hughes, R. 
Rothlein, L. V. McIntire, E. Butcher, D. C. Anderson, and O. 
Abbassi. Chemotactic factors regulate lectin adhesion molecule 1 
(LECAM-1)-dependent neutrophil adhesion to cytokine-stimu-

28. Tate, R. M., and J. E. Repine. Neutrophils and the adult 
559, 1983.

Bargatze, K. E. Arfors, and E. C. Butcher. Two-step model of 
leukocyte-endothelial cell interaction in inflammation: distinct 
roles for LECAM-1 and the leucocyte β2 integrins in vivo. Proc. 

Fowler, and H. J. Sugerman. Anti-CD18 antibody attenuates 
neutropenia and alveolar capillary-membrane injury during 

Leeper-Woodford, G. J. J. esmok, E. F. Ellis, and A. A. 
Fowler. Monoclonal antibody to tumor necrosis factor alpha 
attenuates cardiopulmonary dysfunction in porcine gram-

32. Weiland, J. E., W. B. Davis, J. F. Holter, J. R. Mohammed, 
P. M. Dorinsky, and J. E. Gadek. Lung neutrophils in the adult 
respiratory distress syndrome: clinical and pathophysiologic 

33. Wenzel, R. P. The mortality of hospital-acquired bloodstream 
infections: need for a new vital statistic? Int. J. Epidemiol. 17: 

34. Windsor, A. C., C. J. Walsh, P. G. Mullen, D. J. Cook, B. J. 
Fisher, C. R. Blocher, S. K. Leeper-Woodford, H. J. Sug-
erman, and A. A. Fowler. TNF blockade prevents neutrophil 
CD18 receptor upregulation and attenuates acute lung injury in 
porcine sepsis without inhibition of neutrophil O2 radical genera-