Reduction of Hepatic Ischemia/Reperfusion Injury by a Soluble P-Selectin Glycoprotein Ligand-1

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Objective
The authors' goal was to determine the effects of specific binding and blockade of P- and E-selectins by a soluble P-selectin glycoprotein ligand-1 (PSGL-1) in rat models of hepatic in vivo warm ischemia and ex vivo cold ischemia. The authors also sought to determine the effect of selectin blockade on isograft survival in a syngeneic rat orthotopic liver transplant model.

Summary Background Data
Ischemia/reperfusion (I/R) injury is a major factor in poor graft function after liver transplantation, which may profoundly influence early graft function and late changes. It is hypothesized that I/R injury leads to the upregulation of P-selectin, which is then rapidly translocated to endothelial cell surfaces within 5 minutes of reperfusion of the liver, initiating steps leading to tethering of polymorphonuclear neutrophil leukocytes to the vascular intima. Local production by leukocytes of interleukin-1, tumor necrosis factor-alpha, or both induces P-selectin expression on the endothelium and continues the cascade of events, which increases cell adherence and infiltration of the organ.

Methods
To examine directly the effects of selectins in a warm hepatic I/R injury model, 100 μg of PSGL-1 or saline was given through the portal vein at the time of total hepatic inflow occlusion. The effects of PSGL-1 in cold ischemia were assessed using an isolated perfused rat liver after 6 hours of 4°C storage in University of Wisconsin (UW) solution, with or without the instillation of PSGL-1 before the storage. To evaluate the effect of selectin blockade on liver transplant survival, syngeneic orthotopic liver transplants were performed between inbred male Sprague-Dawley rats after 24 hours of cold ischemic storage in UW solution. A separate group of animals received two doses of 100 μg of PSGL-1 through the portal vein before storage and before reperfusion of the transplanted liver. Recipient survival was assessed at 7 days, and the Kaplan–Meier product limit estimate method was used for univariate calculations of time-dependent recipient survival events.

Results
In an in vivo warm rat liver ischemia model, perfusion with PSGL-1 afforded considerable protection from I/R injury, as demonstrated by decreased transaminase release, reduced histologic hepatocyte damage, and suppressed neutrophil infiltration, versus controls (p < 0.05). When cold stored livers were reperfused, PSGL-1 reduced the degree of hepatocyte transaminase release, reduced neutrophil infiltration, and decreased histologic hepatocyte damage (p < 0.05 vs. UW-only controls). On reperfusion, livers treated with PSGL-1 demonstrated increased portal vein blood flow and bile production (p < 0.05 vs. UW-only controls). In addition, 90% of the rats receiving liver isografts stored in UW solution supplemented with PSGL-1 survived 7 days versus 50% of those whose transplanted syngeneic livers had been stored in UW alone (p < 0.05).

Conclusions
Selectins play an important role in I/R injury of the liver. Early modulation of the interaction between P-selectin and its ligand decreases hepatocyte injury, neutrophil adhesion, and subsequent migration in both warm and cold rat liver ischemia models. In addition, the use of PSGL-1 before ischemic storage and before transplantation prevents hepatic injury, as documented by a significant increase in liver isograft survival. These findings have important clinical ramifications: early inhibition of alloantigen-independent mechanisms during the I/R damage may influence both short- and long-term survival of liver allografts.
Liver allograft function depends on multiple factors, including donor–recipient major histocompatibility complex disparity and the degree of preservation injury incurred by cold storage and reperfusion.1–3 The mechanisms of acute liver injury after ischemia and reperfusion (I/R) are thought to involve a complex interaction of immediate cellular damage induced by reactive oxygen radicals,4–8 followed by cellular responses to Kuffer cell-derived cytokines9–11 and subacute endothelial-leukocyte recruitment, which further facilitates liver injury.12–14 Although the clinical consequences of the hepatic I/R injury, which include liver allograft failure or multisystem organ failure, are well recognized,15,16 the exact mechanism that leads to the ultimate decline in liver function and eventual organ failure remains elusive. Numerous studies evaluating the therapeutic effects of free oxygen scavengers have suggested that reactive oxygen radicals may play a role in determining the fate of liver function after I/R damage.6,17–19 Alternative hypotheses suggest that aberrant neutrophil and endothelial cell interactions may be the primary pathophysiologic mediators of liver damage.20–22

The endothelium serves as a dynamic interface that actively regulates the movement of cells and intercellular signaling proteins across the vessel wall. Cellular traffic across endothelial beds is directed by chemotactic gradients and the binding of specific adhesion molecules. Cellular transmission occurs in stages, with each stage mediated by a group of complementary cell adhesion molecules. The cell adhesion molecules involved in neutrophil–endothelial cell interactions constitute three families: the selectins, the integrins, and the immunoglobulin supergene family.23,24

Under physiologic conditions, selectin–ligand interactions are primarily responsible for the initial tethering and rolling of leukocytes on the vascular wall. The selectins are lectin glycoproteins that are divided into three subclasses (P, E, and L) and are known to be important in neutrophil migration through the endothelium. Endothelial cells express P- and E-selectin, whereas L-selectin is found on neutrophils.25 Flowing neutrophils directly roll on and tether to an endothelial surface bearing P-selectin. P-selectin is normally present in Weibel-Palade bodies of the endothelium and is rapidly redistributed from storage granules to the cell surface after exposure to interleukin-1, histamine, thrombin, and tumor necrosis factor-alpha.24–26 L-selectin is constitutively expressed on neutrophils and is shed with cellular stimulation. The selectins mediate margination and rolling of neutrophils on the vascular endothelium, which necessarily precedes firm adhesion and infiltration.

Selectins bind to a variety of sialylated and fucosylated glycoconjugates and express high affinity to a small subset of appropriately modified glycoproteins. The glycoprotein ligand with the most extensively characterized function is P-selectin glycoprotein ligand-1 (PSGL-1), a homodimeric mucin expressed on the surface of most leukocytes that binds all three selectins.20 P-selectin binds to an NH2-terminal domain of PSGL-1. PL1, a mAb against the NH2-terminal region of PSGL-1, blocks tethering and rolling of neutrophils on P-selectin.22 Thus, PSGL-1 is an essential ligand for primary tethering and rolling of neutrophils on P-selectin.

This study was designed to assess the role of a soluble form of PSGL-1 in inhibiting P-selectin activity and to evaluate how this inhibition may affect the degree of I/R injury sustained by the nonsteatotic liver in three distinct and well-defined rat models: in vivo warm hepatic ischemia, ex vivo cold hepatic ischemia, and syngeneic orthotopic liver transplantation.

**MATERIALS AND METHODS**

Inbred male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 250 to 350 g were used for these experiments and cared for according to NIH guidelines. Animals were given a nutritionally balanced rodent diet (#5001, Purina Mills, St. Louis, MO) and water ad libitum.

Soluble PSGL-1316 (Genetics Institute, Cambridge, MA) is a recombinant soluble form of PSGL-1.21 The mammalian expression vector pED27 was ligated to a cDNA encoding sPSGL-1316, which comprises the mature extracellular domain of PSGL-1, truncated at the isoleucine molecule at position 316. This vector was stably transfected and amplified in a DHFR-Chinese hamster ovary cell line stably transfected with pMT4neo expressing both the cDNA encoding an α(1,3/1,4) fucosyltransferase28 and a cDNA encoding core 2 β-1,6-N-acetylglucosaminyltransferase.29 The secreted soluble PSGL-1316 was purified from serum-free Chinese hamster ovary cell conditioned medium by conventional chromatography methods, as previously described.22 Selectin binding activity was assessed in a cell binding assay.21

Three experimental rat models (in vivo warm ischemia, ex vivo cold ischemia, and syngeneic orthotopic liver transplant) were examined in detail with specific reference to the biochemical and histopathologic degree of hepatocyte injury, neutrophil infiltration, and syngeneic isograft transplant survival.

**In Vivo Warm Ischemia**

Rats were anesthetized with inhaled ether and underwent midline laparotomy and mobilization of the liver by dividing the ligamentous attachments. To prevent portal blood

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pooling, anticoagulation with sodium heparin (200 IU/kg) was administered through the penile vein. Total hepatic ischemia was produced for 45 minutes by the placement of a vascular microclip (Weck, Research Triangle Park, NC) across the hepatic artery, portal vein, and extrahepatic bile duct at the porta hepatis. Sham-operated animals received anesthesia, midline laparotomy, heparinization, and mobilization of the liver only. Control animals received 0.5 ml phosphate-buffered saline (the vehicle for PSGL-1 administration) as a bolus injection into the portal vein before placement of the vascular microclip. The experimental animals received 100 μg (0.4 mg/kg) of PSGL-1 in 0.5 ml phosphate-buffered saline as a bolus injection into the portal vein before microclip placement. After 45 minutes of total hepatic warm ischemia, the microclip was removed and the livers were reperfused for 6 hours, at which time the liver tissue was collected and either fixed in 10% buffered formalin solution or snap-frozen in liquid nitrogen and stored at −70°C.

**Ex Vivo Cold Ischemia**

Rats underwent ether anesthesia and systemic heparinization. After mobilization of the liver, the portal vein, bile duct, and supravascular vena cava were cannulated, and the liver was subsequently flushed with 10 ml University of Wisconsin (UW) solution through the portal vein. Control livers were stored for 6 hours at 4°C; experimental livers received an additional flush of 1 ml UW with 100 μg of PSGL-1 (0.4 mg/kg) through the portal vein cannula before 6 hours of storage. After cold storage, all livers were placed on an isolated pressure-controlled perfusion apparatus. Before liver reperfusion, the perfusion apparatus was primed with perfusate consisting of rat whole blood mixed with 5% glucose Krebs-Ringer bicarbonate medium diluted to a hematocrit of 15%. The perfusate was maintained at pH 7.40 by the addition of 1 M sodium bicarbonate as deemed necessary and oxygenated with a Hamilton Silastic tube lung; the PO₂ was maintained at >250 mmHg. The livers were then perfused for 2 hours while temperature, pH, and inflow pressures were kept constant. Portal vein blood flow and pressure were recorded at 15-minute intervals. Portal vein blood flow was constantly adjusted to maintain portal pressures between 13 and 18 cm H₂O. Blood from the supravascular vena cava was collected at 30-minute intervals and analyzed. After 2 hours of perfusion, sections of the liver were either snap-frozen or fixed in formalin as described above.

**Biochemical Analysis of Hepatocyte Injury**

Serum glutamic-oxaloacetic transaminase (SGOT) concentrations were measured from systemic blood samples obtained 6 hours after reperfusion in the warm ischemia model and 30, 60, 90, and 120 minutes after reperfusion on the isolated perfused rat liver (IPRL) apparatus in the cold ischemia model. Serum SGOT levels were determined using an autoanalyzer by ANTECH Diagnostics (Los Angeles, CA) with appropriate standards.

**Assay of Myeloperoxidase in Hepatic Tissue**

The presence of myeloperoxidase (MPO), an enzyme specific for neutrophils, was used as an index of neutrophil accumulation in the liver. The frozen tissue was thawed and weighed and placed in 4 ml iced 0.5% hexadecyltrimethylammonium bromide and 50 mmol potassium phosphate buffer solution with the pH adjusted to 5. Each sample was then homogenized for 30 seconds and centrifuged at 15,000 rpm for 15 minutes at 4°C. Supernatants were then mixed with hydrogen peroxide–sodium acetate and tetramethylbenzidine solutions. The change in absorbance was measured spectrophotometrically at 655 nm. One unit of MPO activity was defined as the quantity of enzyme degrading 1 μmol peroxide per minute at 25°C per gram of tissue.

**Histologic Analysis**

Liver specimens from sham, control, and experimental groups of rats undergoing warm and cold ischemia were fixed in a 10% buffered formalin solution and then embedded in paraffin. Sections were made at 4 μm and stained with hematoxylin and eosin. Blinded histologic assessment was performed on all samples, which were scored from 0 to 4 according to Suzuki’s classification.8

**Syngeneic Orthotopic Liver Transplant Survival**

To evaluate the effect of selectin blockade on liver transplant survival, syngeneic liver transplants were performed between inbred male Sprague-Dawley rats after 24 hours of cold ischemic storage in UW solution. Preserved livers were transplanted orthotopically in hepatectomized recipients without reconstruction of the hepatic artery by a cuff technique. Experimental animals received two doses of soluble PSGL-1 (100 μg, 0.4 mg/kg) through the portal vein before cold storage and immediately before reperfusion at the time of transplantation. Control animals received UW solution alone. All animals were then followed, and survival was assessed at 7 days after liver transplantation.

**Statistical Analysis**

All data are expressed as mean ± SEM. Treatment groups were compared statistically using an unpaired two-tailed Student’s t test. In the syngeneic liver transplant model, recipient survival was assessed at 7 days and the Kaplan–Meier product limit estimate method was used for univariate calculations of time-dependent recipient survival.
events; statistical comparisons between groups were performed with the Breslow test. A probability value <0.05 was considered statistically significant.

RESULTS

Biochemical Evaluation of Hepatocyte Injury

In both the in vivo warm and ex vivo cold rat ischemia models, we sought to determine the degree of hepatocyte injury after I/R by analyzing the release of SGOT into the serum. As determined by serum SGOT levels obtained 6 hours after 45 minutes of warm hepatic ischemia, PSGL-1 treatment significantly reduced the biochemical evidence of hepatocyte injury (p < 0.05 vs. control groups). The serum SGOT was 174 ± 20 IU/L in the sham-operated group, 1018 ± 61 IU/L in the control group, and 486 ± 72 IU/L in the PSGL-1 treatment group (Table 1).

The biochemical evidence of hepatocyte injury was also significantly reduced after 2 hours of perfusion on the IPRL apparatus in the livers stored in UW solution supplemented with PSGL-1 (p < 0.05 vs. UW only). The serum concentrations of SGOT were 198 ± 38 IU/L in the livers stored in UW solution alone and 97 ± 8.8 IU/L in the livers stored in UW with the addition of PSGL-1 (see Table 1). These data demonstrate that endothelial cell P-selectin binding via PSGL-1 significantly reduced the degree of biochemical hepatocyte injury sustained by the liver in both warm in vivo and cold ex vivo rat models.

Liver Tissue MPO Activity

To determine the amount of neutrophil infiltration after warm and cold ischemia, we assessed the amount of MPO activity in the animal groups. The MPO activity in the hepatic tissue of sham-operated rats was 0.85 ± 0.23 U/g. After a warm ischemic injury without PSGL-1 treatment, the MPO activity increased to 5.2 ± 0.4 U/g. The administration of PSGL-1 during the warm ischemic injury reduced the MPO activity to 2.7 ± 0.3 U/g (p < 0.05 vs. warm ischemia controls) (see Table 1).

The MPO activity in liver tissue after 6 hours of cold storage in UW solution and 2 hours of perfusion on the IPRL apparatus was 2.89 ± 0.23 U/g. In contrast, livers that were cold-stored for 6 hours in UW solution with PSGL-1 had a marked decrease in MPO activity (1.71 ± 0.3 U/g) after 2 hours of perfusion (p < 0.05 vs. UW-only controls) (see Table 1). Thus, selective competitive P-selectin blockade decreases the degree of neutrophil infiltration after I/R insult in both warm and cold liver ischemic models.

Histologic Analysis

To determine if PSGL-1 decreased the hepatocyte injury induced by warm and cold hepatic I/R, a blinded histologic assessment was performed according to Suzuki's classification. There was no evidence of sinusoidal congestion or necrotic cells in sham-operated animals (Suzuki severity score 0). Control animals revealed severe sinusoidal congestion and hepatocyte necrosis after 45 minutes of warm ischemia and 6 hours of reperfusion (Fig. 1; Suzuki severity score 3.03 ± 0.5). In contrast, the extent of sinusoidal congestion and hepatocyte necrosis was markedly diminished in animals treated with PSGL-1 (see Fig. 1; Suzuki severity score 1 ± 0.8; p < 0.05 vs. controls).

After 6 hours of cold storage in UW solution alone and 2 hours of perfusion on the IPRL apparatus, the livers demonstrated marked sinusoidal congestion and hepatocyte necrosis (Fig. 2; Suzuki severity score 2.5 ± 0.6). The presence of PSGL-1 during the 6 hours of cold storage in UW solution also decreased the degree of sinusoidal congestion and hepatocyte necrosis (see Fig. 2; Suzuki severity score 1.2 ± 0.63; p < 0.05 vs. UW-only controls). These histologic data are consistent with biochemical and neutrophil infiltration analyses indicating that early P-selectin blockade plays a crucial role in decreasing the injury sustained by the liver during both warm and cold I/R insult.

Portal Blood Flow and Bile Production

To determine if P-selectin blockade decreases resistance to portal blood flow and improves bile production, we analyzed portal vein blood flow during the 2 hours of perfusion on the IPRL apparatus and quantitated the bile flow.
production at the end of this period in livers stored for 6 hours either in UW alone or in UW supplemented with PSGL-1.

As shown in Figure 3, administration of PSGL-1 dramatically decreased the resistance to portal vein blood flow throughout the observation period versus livers stored in UW alone (p < 0.05). Bile production was also improved by the blockade of P-selectin. As shown in Figure 4, the quantity of bile produced throughout the perfusion experiment was significantly higher in livers stored in the presence of PSGL-1 than in UW-only controls (p < 0.05).

Syngeneic Orthotopic Liver Transplant Survival

Because our initial experiments indicated that competitive blocking of P-selectin results in decreased hepatic I/R injury, we next sought to determine the functional significance of such a blockade on the survival of liver transplants. To eliminate the effects of major histocompatibility complex incompatibility, we performed syngeneic nonarterialized liver transplants between inbred Sprague-Dawley rats. We initially determined that with 24 hours of cold storage in UW solution, 50% of the recipients would die of liver failure in the first 48 hours after transplantation. As shown in Figure 5, recipients of liver isografts stored before transplantation in UW solution alone had a 50% survival rate at 7 days; however, livers stored for 24 hours in UW solution...
supplemented with PSGL-1 had a 90% survival rate at 7 days (p < 0.05 vs. UW-only controls). Collectively, our results demonstrated that P-selectin blockade not only decreases the degree of I/R injury but also improves the survival of syngeneic liver transplants.

**DISCUSSION**

In this study, we examined the role of P-selectin blockade in the pathogenesis of experimental liver I/R injury in rat models. Our results document the importance of endothelial P-selectin expression during the I/R insult by demonstrating that early blockade of the interaction between P-selectin and its ligand ameliorates hepatocyte injury, decreases neutrophil adhesion and migration, and improves the survival of liver isografts.

Because the cellular and molecular dynamics of liver I/R injury are poorly defined, we first examined the effects of selectively blocking the early adhesion cascade in well-defined rat models. Periods of ischemia as brief as 30 to 40 minutes followed by reperfusion with oxygenated blood usually result in some degree of injury to the organ. The extent of injury may be moderate and reversible, or it may be severe and extensive enough to cause cell death, organ failure, and ultimately host death. Several hypotheses have been proposed to explain I/R injury. Free radical generation and attack of unsaturated lipids, proteins, and nucleic acids; increased turnover of membrane phospholipids; disturbances of calcium hemostasis; Kupffer’s cell-derived cytokines; and leukocyte–endothelial cell interactions have all been postulated to play a role in hepatic I/R.2

P-selectin is the key adhesion molecule involved in the earliest events in the adherence of circulating leukocytes to tissues in inflammatory states.32 It is constitutively present in the membranes of alpha granules of platelets and the Weibel-Palade bodies of endothelial cells and is translocated to the plasma membrane of these cells in response to various stimuli.33 Translocation of stored P-selectin to the plasma membranes of endothelial cells occurs early after activation. It mediates the adhesion of polymorphonuclear leukocytes (PMNs) or monocytes to activated platelets or endothelial cells34 and the rolling of leukocytes to activated endothelial cells.35 P-selectin also plays a role in inflammatory and thrombotic disorders, including rheumatoid synovitis,36 leukocyte adhesion to lung endothelial cells in rats after cobra venom factor infusion,37 and leukocyte accumulation in thrombogenic grafts.38 In addition, studies using blocking antibodies have shown that the binding of P-selectin to its ligand is important in modulating I/R injury.39

Among several ligands for the selectin families,15 P-selectin’s high-affinity counterreceptor, PSGL-1, is the most extensively characterized.20–22 Several in vitro studies have demonstrated that PSGL-1 can serve as a ligand for all three selectin family members (P, E, and L).40,41 To investigate the role of selectin molecules in liver I/R injury, we used a recombinant soluble form of PSGL-1 (sPSGL-1). The dosing protocol was designed to provide an adequate concentration of the blocking ligand during the ischemic period. Accordingly, a second dose of sPSGL-1 was added to the final perfusion of cold UW solution to bind any P-selectin upregulated on the hepatic endothelium during the ischemic interval. Because it is a specific ligand for selectins, sPSGL-1 presumably acts by inhibiting the binding between endothelial cells and PMNs or L-selectin–mediated PMN aggregation.40,41 Indeed, recent studies document the importance of an early inhibition of PMN–endothelial interactions in preventing hepatic I/R insult.23

The current knowledge on the role of selectin blockade in liver I/R injury is limited. In accordance with our data showing decreased I/R injury after P-selectin blockade in both warm and cold hepatic ischemia rat models, Misawa et al.23 demonstrated a significant decrease in I/R liver damage after administration of a sialyl Lewis X oligosaccharide, a soluble ligand that binds P-, E-, and L-selectin. Accord-

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**Figure 4.** Bile production at various time points throughout the perfusion was dramatically higher in livers stored in the presence of PSGL-1 than in UW-only controls (p < 0.05).

**Figure 5.** Animal survival at 7 days after syngeneic orthotopic liver transplantation, n = 10 per group. The Kaplan-Meier product limit estimate method was used, with statistical comparisons between groups performed with the Breslow test. Recipients of livers stored in UW alone had a 50% survival rate at 7 days; in contrast, livers stored in the presence of PSGL-1 had a 90% survival rate at 7 days (p < 0.05).
ingly, in a rat model evaluating 90 minutes of total hepatic ischemia by means of an extracorporeal shunt, perfusion with sialyl Lewis\(^x\) oligosaccharide decreased hepatocyte injury, as assessed by transaminase release, MPO activity, and histologic examination. Hamamoto et al.\(^{15}\) examined the putative role of the immunoglobulin superfamily, integrin family, and selectin family on microcirculatory disturbances in the early phase of hepatic reperfusion after cold preservation using an isolated liver perfusion model. Only the use of monoclonal antibodies to the selectin family and its ligand (sialyl Lewis\(^x\) and sialyl Lewis\(^x\)) improved the microcirculatory abnormalities on reperfusion. Similarly, Weyrich et al.\(^{42}\) reported that a specific monoclonal antibody to P-selectin produced significant endothelial preservation and cardioprotection in myocardial ischemia and reperfusion. Finally, Takada et al.\(^{45}\) examined the role of selectins in a warm rat kidney I/R model by using sPSGL-1 as a probe. As in our studies, PSGL-1 facilitated blockade of early PMN adhesion, prevented damage to the glomerulus and tubular cells, and protected the kidney from dysfunction resulting from I/R. Indeed, the expression of E-selectin remained at baseline, the sequestration of leukocytes in the injured organ was abrogated, and the elaboration of proinflammatory cytokines was significantly diminished. Moreover, PSGL-1-induced depression of major histocompatibility complex class II antigen expression decreased the immunogenicity of the transplant itself. This in turn may reduce host inflammatory responses and decrease the incidence of rejection in grafts with delayed function.

In conclusion, our data document the critical role of selectin expression in I/R injury of the liver. The early blockade of in vivo interactions between P-selectin and its PSGL-1 decreases hepatocyte injury, neutrophil adhesion, and subsequent migration in both warm and cold rat liver ischemia models. In addition, the use of sPSGL-1 before the ischemic storage and transplantation may prevent hepatic reperfusion insult, as demonstrated by a significant increase in the survival of rat liver isografts. Our findings implicating antigen-independent mechanisms in I/R damage may have clinical relevance because strategies targeted at selectin blockade may prevent early and long-term consequences of early I/R injury in patients receiving liver transplants.

References

P-Selectin Blockade Inhibits Hepatic I/R Injury


Discussion

DR. WILLIAM MEYERS (Worcester, Massachusetts): DR. LAWS, Dr. Copeland, Members, and Guests. It is a privilege to comment on this superb paper from what is perhaps the leading liver transplant center in the world today. This paper is a striking example of what is needed, a real physiologic insight into the factors that control liver viability before and after transplantation.

According to the data, the authors have found a molecule — in this case a ligand of a cell-adhesion molecule — that convincingly reduces ischemic damage in three models. The most convincing of the models is the isolated perfused liver system which studied cold ischemia damage. There are vagaries of studying transaminases or survival as the only end points in a limited number of animals. I don’t mean to minimize the results of the other two models, but in the isolated perfused system, we see direct effects and some additional end points, which include portal vein resistance and bile flow.

Our laboratory several years ago previously showed that bile flow may be the more sensitive measure of ischemic injury. In these studies the bile flows of the control animals are extremely low — actually, at the level at what we used to call a ducular sweat. In this species, which has less ductular secretion, it may be better termed “canalicular” sweat. This low level just about always correlates with a really ugly venously congested gross appearance of the liver. It means a dead or about-to-die liver.

I have several questions for the authors. What were the gross findings? By giving the ligand later after the liver begins to look bad, will it be like the free-radical scavengers where you have to give this well beforehand?

What about the question of a direct effect? In fact did you give the ligand into the portal vein in the liver alone just before the perfusion, or did you give it in situ in model number two?

And, finally, these data are so striking, they suggest that you’ve really found an answer. Are we going to be disappointed in the translation of these data? Would you speculate on the factors which might be involved in the clinical translation?

Thank you. [Applause]

DR. GORAN KJELTMALM (Dallas, Texas): DR. LAWS, DR. CopeLAND, Members and Guests. I appreciate the opportunity to read the manuscript and to attend this presentation.

Organ transplantation begins with the procurement of a donor organ in good enough condition that after preservation and reperfusion it has enough remaining function to sustain life while the organ recovers. The ischemia and subsequent reperfusion with its release of tumor necrosis factor, histamine, gamma-interferon, and others, are the most potent inducers we know of today — the HLA class II antigens to present themselves and thus, consequently, triggering allograft rejection.

In 1989 we showed in a study from Baylor in Dallas that the more preservation injury the liver was subjected to, the more likely was rejection. The present work by Dr. Busuttil is a beautifully executed study using the work by LeMasters, who has shown us that the epithelial cells are the primary targets of preservation injury.

By using a recombinant ligand to P-selectin expressing itself on the epithelial cell surfaces after stimulation, the investigators have conclusively shown in their studies that the normal neutrophil adhesion and the resultant cell injury is abrogated and fast recovery of the tissue damage and liver function ensues.

I have only a few questions. First, in the isolated perfusion model, you selected to keep the PO2 over 250 mm Hg. This high PO2 could have had additional toxic effects on the perfused liver. Why not a more physiologic PO2?

Second, can the lack of re-arterialization of the syngeneic orthotopic transplant have an impact on the result of that model? Thirdly, have you tried the PSGL-1 administration in the preservation of marginal liver grafts, for example, in fatty liver grafts as in the model we have described?