Hepatic Reticuloendothelial System Dysfunction After Ischemia-Reperfusion: Role of P-Selectin–Mediated Neutrophil Accumulation

Nobuaki Sakamoto, Zhaoli Sun, Matthew L. Brengman, Korsei Maemura, Michitaka Ozaki, Gregory B. Bulkley, and Andrew S. Klein

The relationship between hepatic ischemia-reperfusion (I-R) and subsequent injury through neutrophil accumulation is well described. Although alterations in reticuloendothelial system (RES) function (specifically Kupffer cell function) after I-R have been delineated, the degree to which discrete components of RES function (phagocytosis and killing) are independently modulated under these conditions has not been quantified. A hepatic segmental I-R model was established in mice, in which blood supply to the left lateral lobe of the liver was occluded for 45 minutes, the liver was reperfused, and the laparotomy incision was closed. Experimental animals were pretreated with either vinblastin (1.5 mg/kg) to induce neutropenia or anti–P-selectin monoclonal antibody (mAb; 50 μg/mice) 4 days and 5 minutes before ischemia, respectively. We previously reported that after intravenous injection of chromium 51 (^{51}Cr) and iodine 125 (^{125}I) double-labeled *Escherichia coli*, hepatic ^{51}Cr levels could be used to reliably quantify hepatic phagocytic clearance (HPC) of bacteria from blood, whereas the subsequent release of ^{125}I from the liver accurately paralleled hepatic bacterial killing efficiency (HKE). Using this double-label bacteria clearance assay, HPC and HKE were depressed after I-R, in association with hepatic neutrophil accumulation. Segmental I-R resulted in decreased HPC and HKE activity in both ischemic and nonischemic hepatic lobes. Depressions in HPC and HKE were attenuated by either vinblastin-induced neutropenia or blocking neutrophil adhesion to the hepatic endothelium with anti–P-selectin mAb. These findings support the hypothesis that I-R induces hepatic RES dysfunction, at least in part, through P-selectin–mediated neutrophil accumulation. (*Liver Transpl 2003;9:940-948.*)

The reticuloendothelial system (**RES**) is composed primarily of macrophages resident in the liver (Kupffer cells [**KCs**]), spleen, and lung (alveolar macrophages) and is an important component of host defense against invasive pathogens.[1] The liver is the principle anatomic site of RES activity and is responsible for approximately 80% of total-body RES function.[2]

During the past three decades, liver transplantation has evolved from an experimental attempt to salvage desperately ill patients dying of liver failure to preferred definitive therapy for a defined population of patients with acute or chronic liver disease. Despite advancements in preoperative, intraoperative, and postoperative patient management and refinements in immunosuppressive protocols that have resulted in improved patient survival, postoperative sepsis has been documented in the majority of liver recipients.[3] Infection remains the predominant cause of posttransplantation morbidity and mortality.[4-6] The risk for infectious complications after liver transplantation has been reported to be between 50% and 83%.[4-7] Proportionally, the incidence of septic events seems to be greater than in recipients of other types of transplanted organs (i.e., kidney or heart) who undergo similar or often more intensive regimens of systemic immunosuppression.

We previously reported that hepatic RES function, quantified by the liver's capacity to phagocytose and kill circulating bacteria or fungi, is altered after liver transplantation in the rat.[7] However, these experimental animal models have not attempted to discriminate the relative contributions of the components of the entire transplantation process, including hepatic ischemia-reperfusion (**I-R**), replacement of resident donor phagocytes by bone-marrow–derived monocytes from the host allograft, and rejection.[8] Using a murine model, segmental warm hepatic I-R experiments were designed to quantify alterations in hepatic RES function specifically related to warm I-R.

The relationship between hepatic I-R and subsequent liver injury (parenchymal cell and endothelial cell) is well described. A major component of I-R injury is caused by the generation of reactive oxygen intermediates (**ROIs**) and consequent neutrophil accumulation and activation, which induces secondary endothelial

From the Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.

Supported in part by grants no. 1-K08-DK02126 and DK31764 from The National Institutes of Health.

Address reprint requests to Andrew S. Klein, MD, Department of Surgery, The Johns Hopkins University School of Medicine, Harvey 611, 600 N Wolfe St, Baltimore, MD 21287-8611. Telephone: 410-955-5662; FAX: 410-614-2079; E-mail: aklein@jhmi.edu

1527-6465/03/0909-0007$30.00/0
doi:10.1053/jlts.2003.50182

cell injury.[9-11] Studies have shown that leukocyte accumulation is a critical determinant of hepatic I-R injury,[12-16] and P-selectin has an important role in leukocyte adhesion after hepatic I-R.[15,16] The leukocyte-endothelial cell interaction that occurs after I-R injury has been shown to be a causative factor in the development of microvascular dysfunction and release of such cytotoxic mediators as ROIs and a variety of proteases.[10,17-19] The importance of neutrophils in this pathophysiologic process is emphasized by the observation that neutropenia (induced with vinblastin and/or an antineutrophil monoclonal antibody [**mAb**]) results in markedly decreased hepatic cell damage after I-R.[20,21]

Alterations in hepatic RES function have been variably modulated under conditions of cold or warm I-R. Caldwell-Kenkel et al[22] reported increased KC activity after cold I-R.[23] Rao et al[23] suggested that the reperfusion injury to the liver that occurs after cold ischemic preservation damages endothelial cells and activates KCs, which, in turn, initate a self-perpetuating injury pathway). However, warm I-R has been associated with either the activation or depression of KC function.[24-26] These conflicting observations may be attributable in part to differences in the assay selected to quantify KC activity, as well as variability in the I-R model itself. As a consequence of the procurement/preservation/implantation process that occurs during liver transplantation, both cold and warm I-R events may have a role in the alteration of hepatic RES function. Our studies were limited to quantifying the effects of warm I-R, which also is highly relevant to a number of other clinical situations (e.g., vascular occlusion during hepatic resection and transient hepatic hypoperfusion during cardiogenic shock).

Recognizing that the KC is a pluripotent cell that not only functions to remove pathogens and cellular debris from the circulation, but also has an important role in the local and systemic inflammatory response, previous studies investigating hepatic RES clearance function under conditions of warm I-R have been limited to measuring phagocytosis as a sole estimate of KC activity. The double-labeled bacteria RES assay used in the experiments described here is capable of further resolving hepatic phagocyte function into its two distinct component processes: phagocyte clearance (phagocytosis) and degradation (killing).

We previously found that two components of the phagocytosis of circulating microorganisms (phagocytosis and killing) by the hepatic RES may be modulated independently under conditions of RES activation or suppression.[2] The present study is designed to: (1) discriminately quantify alterations in these two distinct components of hepatic RES function after segmental warm hepatic I-R in both the ischemic and adjacent nonischemic liver, and (2) determine the degree to which neutrophil-dependent mechanisms are responsible for this I-R–induced alteration in hepatic RES function.

Materials and Methods

Animals

Male AKR/J mice (body weight, 28 to 30 g) were purchased from The Jackson Laboratory (Bar Harbor, ME) and kept in a light-cycled vivarium with free access to food and tap water. All experimental protocols were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine (Baltimore, MD).

Experimental Model of Lobar Hepatic I-R

Under methoxyflurane inhalation anesthesia, mice underwent systemic anticoagulation using heparin (40 IU/100 g of body weight), and a midline laparotomy was performed. Blood supply to the left lateral lobe of the liver was occluded with an atraumatic clamp for 45 minutes, after which the clamp was removed, the liver was reperfused, and the laparotomy was closed.

Experimental Protocol

Sham-operated mice were subjected to the same surgical procedure, but the vessels were not occluded. Groups of I-R mice received no pretreatment, vinblastin-induced (Velban7; Eli Lilly & Co, Indianapolis, IN) neutropenia, or anti–P- selectin mAb (Pharmingen Inc, San Diego, CA). Anti–P-selectin mAb was injected at a dose of 50 μg intravenously 5 minutes before inducing ischemia.

Hepatic RES Assessment in Vivo

To measure hepatic RES function, we discriminately quantified hepatic phagocytic clearance and killing using the dual-labeled *Escherichia coli* clearance method previously described and validated.[2] Briefly, an ampicillin-resistant XLI-BLUE strain of *E coli* (American Type Culture Collection, Rockville, MD) grown on Mueller-Hinton agar were labeled with 0.1 mCi of 5-iodine 125 [^{125}I] iodo-2=-deoxyuridine (^{125}I-UdR, IM-355V; Amersham, Arlington Heights, IL) in Trypticase soy broth (Becton Dickinson, Cockeysville, MD). Cultures were incubated for 18 hours at 37°C, washed three times with normal saline, pelleted, and incubated with 0.05 mCi of sodium chromate ($Na_2{}^{51}CrO_4$; CIS-11; Amersham). Bacteria concentration in suspension was adjusted to an optical density of 0.6 (1.0×10^8 bacteria/mL) in a spectrophotometer at 600 nm. Chromium 51 (^{51}Cr) and ^{125}I remained bound to the bacteria (cytoplasm and DNA, respectively) for at least 4 hours in vivo, as well as in vitro, and contained less than 2% free ^{51}Cr or ^{125}I.[2]

Under methoxyflurane anesthesia, these double-labeled *E coli* (4H10[8]) were injected into the jugular vein of I-R or control mice 1, 4, or 24 hours after laparotomy. Ninety minutes after injection of bacteria, mice were killed by cervical dislocation. The liver was removed and ischemic and nonischemic hepatic lobes were separated. After each lobe was weighed, ^{51}Cr and ^{125}I content were determined by 1 minute of gamma-counting of samples, using 50- to 175-keV and 0- to 20-keV windows, respectively. As validated in previous experiments, hepatic ^{51}Cr content is an accurate indicator of bacterial phagocytic clearance. In contrast to ^{51}Cr, ^{125}I-UdR is released from the liver after bacterial cell death; thus, hepatic ^{125}I reflects the number of viable *E coli* remaining in the liver. After hepatic ^{51}Cr and ^{125}I content had been normalized by wet weight, hepatic phagocytic clearance (**HPC**) and hepatic killing efficiency (**HKE**) were determined as follows:

$$\text{HPC (\%)} = \text{hepatic } ^{51}\text{Cr/total injected dose of } ^{51}\text{Cr} \times 100$$

$$\text{HKE (\%)} = (\text{hepatic } ^{51}\text{Cr B hepatic } ^{125}\text{I})/\text{hepatic } ^{51}\text{Cr} \times 100$$

Hepatic Myeloperoxidase

Hepatic myeloperoxidase (**MPO**) activity was used to estimate neutrophil accumulation in the liver. In I-R experiments, MPO activity was measured in both ischemic and nonischemic lobes. MPO activity was determined spectrophotometrically in 50 mmol/L of phosphate buffer (pH 6.0) containing 0.165 mg/mL of *o*-dianisidine hydrochloride and 0.15 mmol/L of hydrogen peroxide. Change in absorption per minute was determined at 460 nm.

Hepatic polymorphonuclear leukocyte accumulation. As an additional indicator of hepatic neutrophil accumulation after warm I-R, polymorphonuclear leukocytes (**PMNs**) were quantified histologically in formalin-fixed paraffin sections of the liver obtained at each reperfusion-sampling time point. A commercially available kit (91-C; Sigma-Aldrich, St. Louis, MO) was used to stain for sinusoidal and postsinusoidal-sequestrated PMNs using the well-established napthol AS-D chloroacetate esterase procedure according to the manufacturer's directions. At least four random sections from each group were analyzed by viewing (blindly) 50 random high-power fields (HPFs; original magnification ×400) on each section. Results are expressed as number of PMNs/50 HPF.

Quantitative Measurement of P-Selectin Expression in Vivo

The technique and validation of this assay have been previously described.[27] Briefly, the binding mAb (RB40.34; Pharmingen Inc) was labeled with ^{125}I, and the nonbinding (control) mAb (R3-34, Pharmingen Inc) was labeled with ^{131}I using the chloramine T method.[28] Under methoxyflurane anesthesia, the jugular vein was cannulated with a P-10 catheter (Becton Dickinson, Sparks, MD), and a mixture of 10 μg of ^{125}I-labeled monoclonal antibody against P-selectin and an equal volume of nonbinding immunoglobulin labeled with ^{131}I were injected intravenously. After 5 minutes of circulation, the mice were killed and the liver was flushed with 20 mL of cold heparinized phosphate-buffered saline buffer solution through the portal vein. The liver was excised and divided into ischemic and nonischemic lobes. Samples of both ischemic and nonischemic liver were placed in cuvettes, and residual radioactivity was determined in a 14800 Wizard 3 gamma-counter (Packard Instrument Co, Downers Grove, IL). P-Selectin expression in ischemic and nonischemic lobes was calculated by subtracting ^{131}I from ^{125}I.

Analysis of Data

Data are expressed as mean ± SEM. Differences in means were evaluated for statistical significance by Student's *t*-test or one-way analysis of variance (**ANOVA**). One-way ANOVA was performed to assess differences in HPC and HKE among the three groups. Separate *t*-tests with Bonferroni correction were used to make pairwise comparisons of groups. *P* less than .05 is considered significant.

Results

Neutropenia Induced by Vinblastin

To determine the optimal dose of vinblastin, separate animals were administered an intravenous injection of 0.75, 1.5, or 3.0 mg/kg of vinblastin. After 4 days, peripheral blood was sampled and neutrophil counts were determined. Neutropenia was induced by vinblastin at doses greater than 1.5 mg/kg. However, pancytopenia was induced by 3.0 mg/kg (Fig. 1). Therefore, a vinblastin dose of 1.5 mg/kg was selected.

Segmental Hepatic I-R

Hepatic RES function. Hepatic I-R resulted in decreased whole liver HPC of *E coli*, reflected by decreased hepatic ^{51}Cr content at 90 minutes compared with the sham operation group. HPC decreased 1 through 4 hours after I-R, then returned to near-baseline values by 24 hours after I-R (Fig. 2A). To discriminate HPC in ischemic and nonischemic lobes, data for each were normalized per gram of tissue. There was no difference in mean lobar weights between the I-R group and sham-operated group (data not shown). HPC in ischemic and nonischemic hepatic lobes was depressed compared with levels observed in livers recovered from sham-operated mice. Maximal HPC suppression was noted in the ischemic lobe. In both ischemic and nonischemic tissue, phagocytic activity reached a nadir 4 hours after I-R and had returned to near-normal levels after 24 hours (Fig. 2B). One hour after reperfusion,

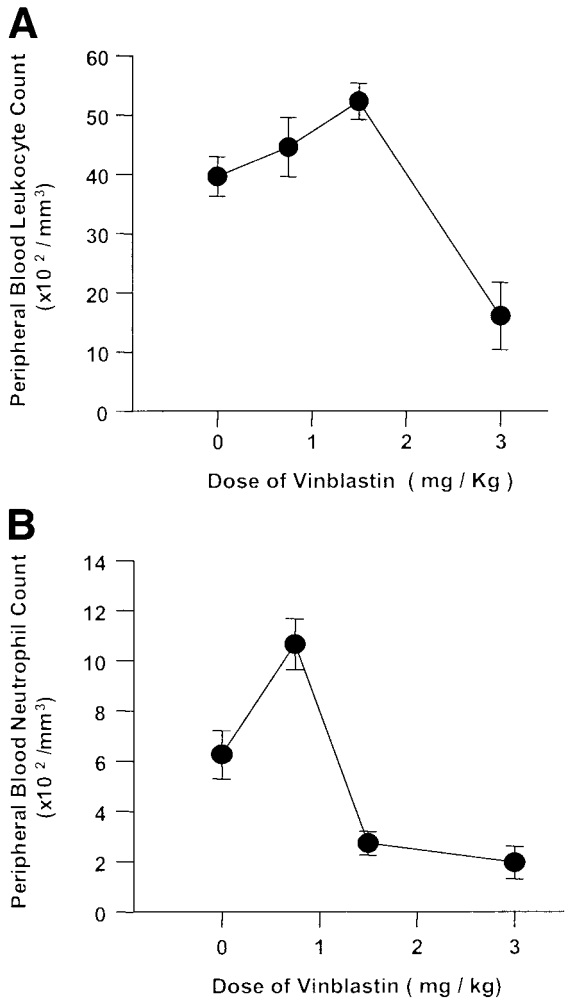

Figure 1. Peripheral-blood (A) leukocyte and (B) neutrophil counts 4 days after vinblastin treatment (mean ± SEM; n = 3 to 4 mice for each part).

HKE in ischemic and nonischemic lobes was markedly decreased compared with controls. Similar levels of HKE depression were observed in ischemic and nonischemic lobes 4 hours after I-R. By 24 hours after I-R, HKE in both lobes had returned to near-baseline levels (Fig. 2C).

Hepatic neutrophil accumulation. Neutrophil recruitment in postischemic liver was assessed by biochemical (**MPO** activity) and histological (**PMN** staining) anal-

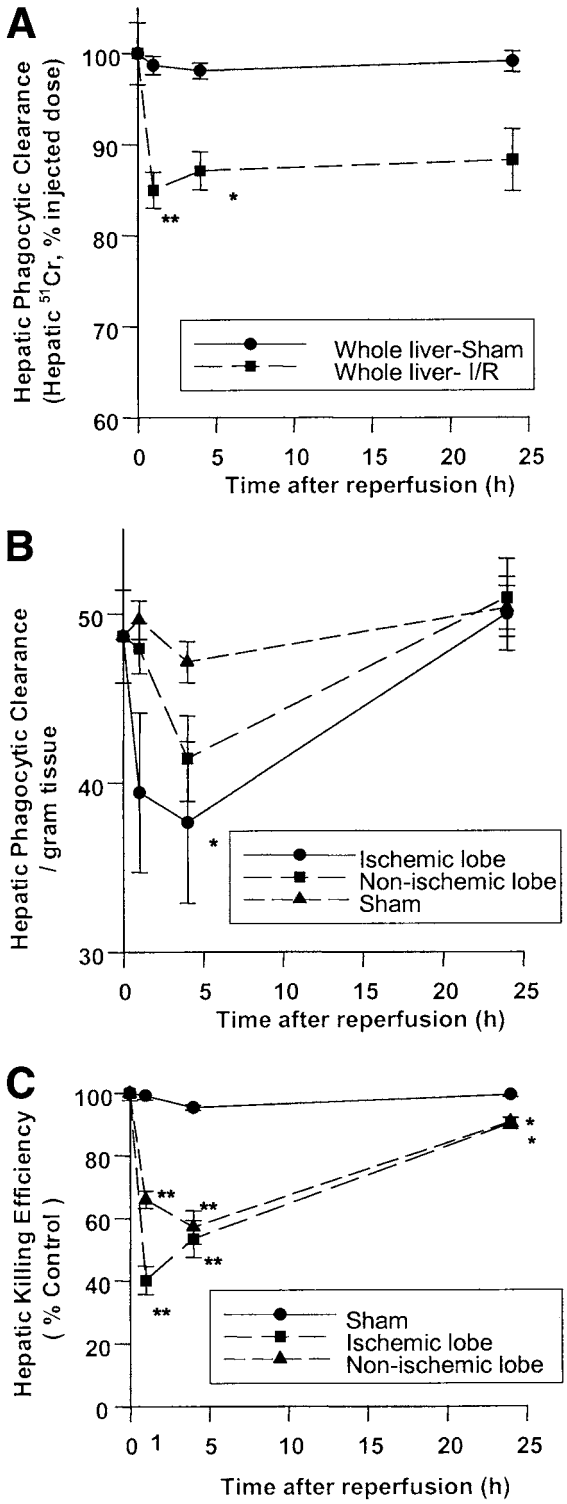

Figure 2. Depression of hepatic phagocytosis and killing after hepatic I-R. (A) Whole-liver HPC of *E coli* determined 90 minutes after injection. Data expressed as percentage of controls. (B) Lobar HPC of *E coli* (hepatic ^{51}Cr content per gram of tissue) for sham, ischemic, and nonischemic liver. (C) Lobar HKE for sham, ischemic, and nonischemic liver (mean ± SEM; n = 4 to 5 mice. **P* < .05 *v* sham. ***P* < .01 *v* sham).

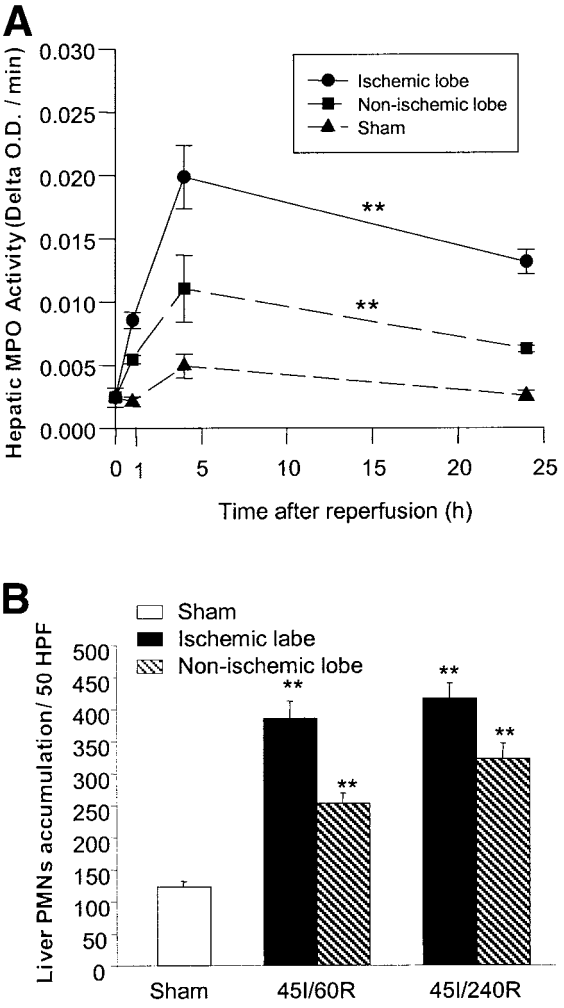

Figure 3. Hepatic neutophil accumulation after I-R. (A) MPO activity over time after hepatic I-R (mean ± SEM; n = 4 to 5 mice. **$P < .01$ *v* sham by ANOVA). (B) PMN staining. Livers were subjected to 45 minutes of ischemia (45I) and 60 or 240 minutes of reperfusion (60R or 240R) in vivo. Paraffin sections were specifically stained for PMNs as described in Materials and Methods, and PMNs were counted in 50 HPFs. (Mean ± SEM; n = 3 mice. **$P \leq .01$ *v* sham by ANOVA).

ysis. MPO activity in both ischemic and nonischemic lobes was increased at 1, 4, and 24 hours after I-R. Peak activity was noted at 4 hours, and for all times, MPO activity in the ischemic liver exceeded that of nonischemic liver (Fig. 3A). Similarly, PMN accumulation in livers in both ischemic and nonischemic lobes was significantly greater than in livers of sham-operated controls at 1 and 4 hours after I-R (Fig. 3B).

P-Selectin expression in the liver. There were two peaks of P-selectin expression occurring 30 minutes and 4 hours after reperfusion. Early increases in P-selectin expression were significantly greater than those in sham-operated controls only in the ischemic liver. However, at 4 hours, both ischemic and nonischemic liver had P-selectin levels substantially greater than control levels (Fig. 4).

Effect of Vinblastin and Anti–P Selectin mAb Treatment

Neutrophil accumulation. The elevation in MPO activity in the liver 4 hours after I-R was blocked by either vinblastin-induced neutropenia or pretreatment with anti–P-selectin mAb (Fig. 5A). Similarly, PMN accumulation in livers 4 hours after I-R in either vinblastin or P-selectin antibody-treated mice was significantly less than in livers of sham-control mice (Fig. 5B).

RES function. Vinblastin-induced neutropenia restored post-ischemic HPC activity to normal levels in I-R mice. Although vinblastin-induced neutropenia resulted in HKE suppression in the absence of ischemia, pretreatment of mice with vinblastin before I-R attenuated the suppression of HKE seen in untreated I-R mice. Similarly, HPC and HKE depression after I-R was attenuated by pretreatment with anti–P selectin mAb (Fig. 6). RES function in control mice was not affected by administration of the anti–P-selectin mAb (data not shown).

Discussion

In our previous studies, distribution of dual-labeled *E coli* in the liver, lung, and spleen was 75% to 80%, 3%

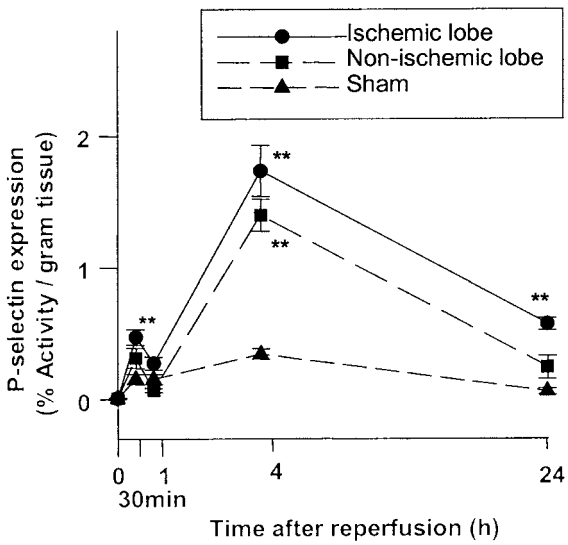

Figure 4. Lobar hepatic I-R results in increased P-selectin expression in both ischemic and nonischemic lobes (mean ± SEM; n = 5. **$P < .01$ *v* sham).

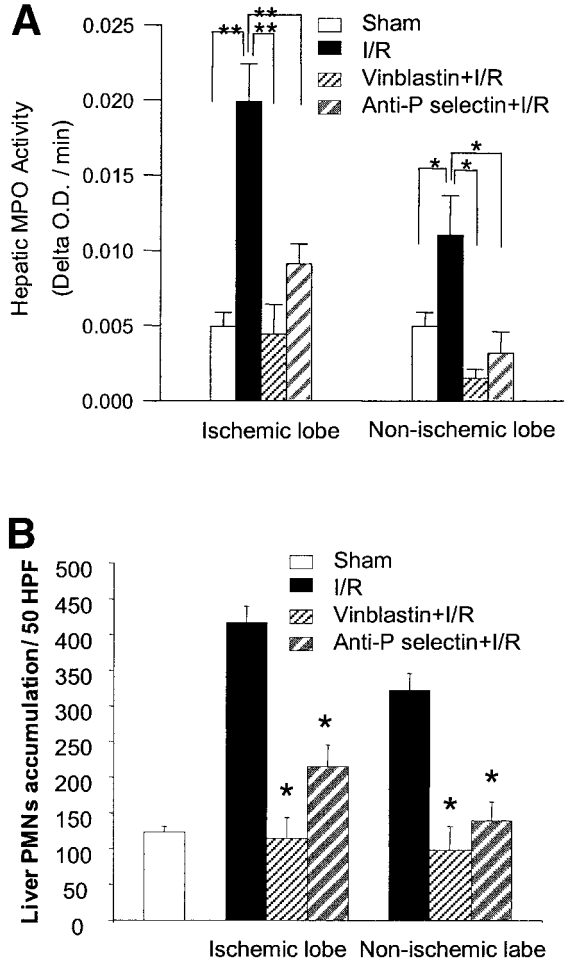

Figure 5. Effect of vinblastin and anti–P-selectin mAb treatment. (A) Elevation in liver MPO activity after I-R is blocked by pretreatment with vinblastin or anti–P-selectin mAb (mean ± SEM; n = 4 to 5 mice. *$P < .05$ *v* sham. **$P < .01$ *v* sham). (B) PMN sequestration at 4 hours after hepatic I-R was inhibited by pretreatment with vinblastin or anti–P-selectin mAb (mean ± SEM; n = 3 mice. *$P \leq .01$ *v* nontreatment I-R controls).

to 5%, and 5% to 10%, respectively, supporting the hypothesis that under normal conditions, clearance of this pathogen from systemic circulation is dependent largely on the phagocytosis and killing activity of resident hepatic macrophages (**KCs**).[2] We also reported that hepatic RES function, quantified by the liver's capacity to phagocytose and kill circulating bacteria or fungi, is altered after liver transplantation in rats.[7] However, the precise mechanism(s) responsible for RES dysfunction after liver transplantation has not been determined. These experiments therefore focused on alterations in hepatic RES function (phagocytosis and killing) specifically related to the process of warm hepatic I-R, one unavoidable component of liver transplantation.

Our data suggest that two distinct components of hepatic RES function, phagocytic clearance and killing of bacteria, are depressed after segmental warm hepatic I-R. Although alterations in hepatic phagocytic function after hepatic I-R have been reported by others, to our knowledge, suppression of phagocytic killing has not been described previously.[29-31] Importantly, our studies show that hepatic RES function decreased not only in the ischemic lobe, but also in the nonischemic lobe.

A variety of mechanisms and cytotoxic mediators have been implicated in I-R–dependent parenchymal cell and endothelial cell damage, including ROIs, proteases, cytokine release, adhesion molecule expression,

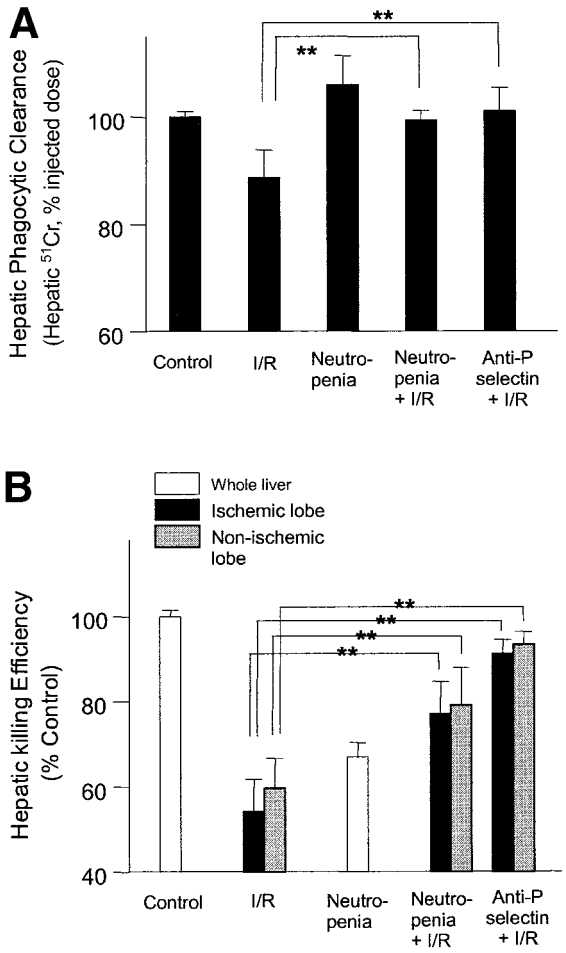

Figure 6. Attenuation of I-R–induced HPC and HKE depression by vinblastin or anti–P-selectin mAb. (A) Whole-liver HPC 4 hours after I-R. (B) HKE 4 hours after I-R in ischemic and nonischemic liver (mean ± SEM; n = 4 to 5. **$P < .01$ *v* I-R alone).

and neutrophil accumulation.[9,10,12-16,19-21,32-35] Infiltrating neutrophils have been implicated as key mediators of I-R injury associated with numerous organs, including the intestine, heart, brain, skeletal muscle, and liver.[21,36-39] The neutrophil is thought to have a central role, given the evidence that neutrophil elimination with vinblastin or blocking of neutrophil activity with mAb appears to be cytoprotective in the setting of I-R.[20,21] Our central hypothesis therefore was that RES dysfunction after I-R also was neutrophil dependent, at least to some degree. In this study, neutrophil accumulation in postischemic liver was assessed by both biochemical (MPO activity) and histological (PMN staining) analyses. Neutrophil accumulation increased from 1 to 4 hours after hepatic I-R. Strikingly, neutrophil accumulation in nonischemic hepatic tissue also increased with kinetics similar to those observed in ischemic liver. Moreover, peak MPO activity in ischemic and nonischemic lobes correlated with a nadir of hepatic RES (phagocytosis and killing) dysfunction. Finally, neutropenia attenuated I-R–dependent hepatic RES dysfunction in both ischemic and nonischemic lobes.

P-Selectin is an important adhesion molecule expressed on the surface of endothelial cells that, in turn, initiates rolling as an initial step in neutrophil accumulation.[40] Essani et al,[41] Zibari et al,[42] and others[43-46] have begun to define the role of hepatic P-selectin expression in the pathophysiological state of shock and I-R–induced injury. It has been reported that anti–P-selectin can protect against hepatic I-R injury.[47,48] Mice deficient for P-selectin showed a wide range of protective effects, including decreases in PMN infiltration and platelet sequestration, decreased release in serum transaminase levels, and improved animal survival after total hepatic ischemia.[15] We determined that after I-R, overall intrahepatic P-selectin expression was augmented in a biphasic pattern. Within 30 minutes of I-R, P-selectin expression was induced within the ischemic liver tissue, reaching levels significantly greater than in controls.

In many tissues, P-selectin is stored in Weibel-Palade bodies in endothelial cells and expressed on the cell surface rapidly (posttranslationally) after stimulation. However, liver sinusoidal endothelial cells have been reported to contain no Weibel-Palade bodies.[49] The first small peak of P-selectin expression may be caused by expression within the terminal hepatic venules and central collecting veins. To support this, similar kinetics of P-selectin expression in the liver has been shown to occur after exposure of the liver to lipopolysaccharide.[50] However, this study was not designed to address the specific intrahepatic localization of P-selectin expression (or of leukocyte accumulation). A second larger peak of P-selectin expression was seen 4 hours after I-R. At this time, P-selectin expression increased significantly not only in the ischemic lobe, but also in the adjacent nonischemic liver. This time course corresponds to the well-known transcriptionally regulated synthesis of new protein.[51]

One could speculate that upregulation of P-selectin expression at this time might be induced by a soluble mediator, such as tumor necrosis factor-α (TNF-α) and/or interleukin-1 (IL-1) through a transcriptional factor. Armstead et al,[52] Essani et al,[41] and others reported that TNF-α and/or IL-1 induced elevation of P-selectin messenger RNA levels in the liver. Furthermore, Scales et al[53] reported that after segmental hepatic ischemia tissue, TNF-α levels in nonischemic liver increased. Similarly, Zizzi et al[27] reported that P-selectin levels in the nonischemic right kidney increased significantly after isolated left renal I-R.

It was reported previously that blocking P-selectin by pretreatment with anti–P-selectin mAb or P-selectin glycoprotein-1 decreases hepatic PMN accumulation (MPO activity) and protects against hepatic I-R injury.[47,54] In murine models, neutrophil rolling in postischemic postsinusoidal venules is completely blocked by anti–P-selectin mAb.[42,55] Our data indicate that anti–P-selectin antibody substantially attenuates I-R–induced hepatic RES dysfunction. Moreover, vinblastin-induced neutropenia also blunted the I-R–induced RES dysfunction in this model.

Vollmar et al[55] reported that postsinusoidal (but not sinusoidal) neutrophil accumulation correlated with hepatic I-R injury. It therefore would be reasonable to suggest that anti–P-selectin mAb attenuates I-R–induced hepatic RES dysfunction through blocking of neutrophil accumulation, mainly within postsinusoidal venules. This is consistent with the findings of Steinhoff and Brandt,[56] who found variability in intravascular and interstitial expression patterns of adhesion molecules in human liver allografts. P-Selectin expression was upregulated after I-R–induced hepatic inflammation only on portal venous, central venous, and arterial endothelium. However, sinusoidal cells remained P-selectin negative under a variety of acute and chronic inflammatory conditions after liver transplantation.[56] Reported evidence suggests that leukocyte recruitment into inflamed liver sinusoids does not required selectins, with one notable exception: I-R.

Although our data are consistent with P-selectin–mediated neutrophil accumulation after hepatic I-R, it is arguable whether interventions directed against P-se-

lectin directly protect in any model of neutrophil-induced liver injury. A recent report by Kubes et al[16] provided an explanation for these contradictions. They convincingly showed that reduced neutrophil accumulation and tissue protection induced by P-selectin interventions in I-R models are secondary effects caused by gut protection, rather than directly related to mechanisms in the liver. Furthermore, they proposed that antiadhesion therapy is effective in liver I-R in sinusoidal and postsinusoidal venules, perhaps in part because of a beneficial effect at the level of the intestine.

Despite our observations that neutrophil accumulation impairs hepatic RES function after I-R, we recently reported that leukocytes appear to be important primarily to the phagocytic killing of systemically circulating bacteria by the normal hepatic RES under physiological conditions.[57] Interestingly, elimination of neutrophils has different effects on phagocytosis and bacterial killing in controls compared with animals subjected to hepatic I-R. The mechanism for these different disparate observations cannot be defined by this study, but could be attributed to either complex interactions between leukocytes and KCs or, more likely, greater neutrophil accumulation in the liver after I-R.

In summary, this study indicates that phagocytosis and killing of *E coli* within the liver is depressed by up to 50% after hepatic I-R, in association with hepatic neutrophil accumulation. Segmental I-R results in decreased phagocytic killing in both ischemic and nonischemic liver tissue. This I-R–dependent suppression of distinct components of RES function is attenuated by either vinblastin-induced neutropenia or blocking neutrophil adhesion to hepatic endothelium with anti–P-selectin mAb, indicating that I-R–induced hepatic RES dysfunction is caused, at least in part, by P-selectin–mediated neutrophil accumulation.

References

1. Saba TM. Reticuloendothelial systemic host defense after surgery and traumatic shock. Circ Shock 1975;2:91-95.
2. Klein A, Zhadkewich M, Margolick J, Winkelstein J, Bulkley G. Quantitative discrimination of hepatic reticuloendothelial clearance and phagocytic killing. J Leukoc Biol 1994;55:248-251.
3. Martin M, Kusen S, Allessiani M, Simmons R, Starzl TE. Infection after liver transplantation: Risk factors and prevention. Transplant Proc 1991;23:1929-1930.
4. Markin RS, Stratta RJ, Woods GL. Infection after liver transplantation. Am J Surg Pathol 1990;14:64-78.
5. Colonna JO, Winston DJ, Brill JE, Goldstein LI, Hoff MP, Hiatt JR, et al. Infectious complications in liver transplantation. Arch Surg 1988;123:360-364.
6. Kusne S, Dummer JS, Singh N, Iwatsuki S, Makowka L, Esquivel C, et al. Infection after liver transplantation: An analysis of 101 consecutive cases. Medicine 1988;67:132-143.
7. Klein AS, Zhadkevich M, Wang D, Margolick JB, Winkelstein JA, Bulkley GB. Discriminant quantitation of posttransplant hepatic reticuloendothelial function. Transplantation 1996;61: 1156-1161.
8. Gassel H-J, Engemann R, Thiede A, Hamelmann H. Replacement of donor Kupffer cells by recipient cells after orthotopic rat liver transplantation. Transplant Proc 1987;19:351-353.
9. Eppihimer MJ, Granger DN. Ischemia/reperfusion-induced leukocyte-endothelial interaction in postcapillary venules: Review article. Shock 1997;8:16-25.
10. Li X-K, Matin AFM, Suzuki H, Uno T, Yamaguchi T, Harada Y. Effect of protease inhibitor on ischemia/reperfusion injury of the rat liver. Transplantation 1993;56:1331-1336.
11. Scheridan FM, Douber IM, McMurty F, Lesnefsky EJ, Horwiz LD. Role of leukocytes in coronary vascular endothelial injury due to ischemic and reperfusion. Circ Res 1991;69:1566-1571.
12. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J 1990; 4:3355-3359.
13. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. Transplantation 1993;55:1265-1272.
14. Yadav SS, Howell DN, Gao W, Steeber DA, Harland RC, Clavien PA. L-Selectin and ICAM-1 mediate reperfusion injury and neutrophil adhesion in the warm ischemic mouse liver. Am J Physiol 1998;275:G1341-G1352.
15. Yadav SS, Sindram D, Perry DK, Clavien PA. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a caspase-dependent pathway. Hepatology 1999;30: 1223-1231.
16. Kubes P, Payne D, Woodman RC. Molecular mechanisms of leukocyte recruitment in postischemic liver microcirculation. Am J Physiol Gastrointest Liver Physiol 2002;283:G139-G147.
17. Iwabuchi K, Nagaoka I, Yamashita T. Modulation of neutrophil adherence to endothelial cells by platelet-derived adherence-inhibiting factor through interactions with selectin molecules. J Leukoc Biol 1998;63:500-508.
18. Harrison-Shostak DC, Lemasters JJ, Edgell CJ, Herman B. Role of ICE-like proteases in endothelial cell hypoxic and reperfusion injury. Biochem Biophys Res Commun 199724;231:844-847.
19. Jaeschke H, Smith CW. Mechanisms of neutrophil-induced parenchymal cell injury. J Leukoc Biol 1997;61:647-653.
20. Langdale LA, Flaherty LC, Liggitt HD, Harlan JM, Rice CL, Winn RK. Neutrophils contribute to hepatic ischemia-reperfusion injury by a CD18-independent mechanism. J Leukoc Biol 1993;53:511-517.
21. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J 1990; 4:3355-3359.
22. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Kupffer cell activation and endothelial cell damage after storage of rat livers: Effects of reperfusion. Hepatology 1991;13:83-95.
23. Rao PN, Liu T, Snyder JT, Platt JL, Starzl TE. Reperfusion injury following cold ischemia activates rat liver Kupffer cells. Transplant Proc 1991;23:666-669.
24. Lindert KA, Caldwell-Kenkel JC, Nukina S, Lemasters JJ, Thurman RG. Activation of Kupffer cells on reperfusion following hypoxia: Particle phagocytosis in a low-flow, reflow model. Am J Physiol 1992;262:G345-G350.
25. Vollmar B, Glasz J, Post S, Menger MD. Depressed phagocytic

activity of Kupffer cells after warm ischemia-reperfusion of the liver. J Hepatol 1994;20:301-304.
26. Jaeschke H, Farhood A, Bautista AP, Spolaries Z, Spitzer JJ. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol 1993;264: G801-G809.
27. Zizzi HC, Zibari GB, Granger DN, Singh I, Cruz LD, Abreo F, et al. Quantification of P-selectin expression after renal ischemia and reperfusion. J Pediatr Surg 1997;132:1010-1013.
28. Bocci V. Efficient labeling of serum proteins with ^{131}I using chloramine T. Int J Appl Radiat Isot 1964;15:449-510.
29. Suzuki S, Nakamura S, Sakaguchi T, Ochiai H, Konno H, Baba S. Alteration of reticuloendothelial phagocytic function and tumor necrosis factor-α production after total hepatic ischemia. Transplantation 1997;64;821-827.
30. Luzio NR, Olcay I, Holper K, Drapanas T, Trejo R. The relationship of reticuloendothelial dysfunction and endotoxemia to survival after hepatic ischemia injury in the baboon. Circ Shock 1975;2:77-85.
31. Lahnborg G, Bracher M, Hickman R, Terblanche J. Distribution of reticulo-endothelial function following experimental liver transplantation. Eur Sur Res 1989;21:129-136.
32. Lemasters JJ, Thurman BG. The many facets of reperfusion injury. Gastroenterology 1995;108:1317-1320.
33. Togashi, H, Shinzawa H, Yong H, Takahashi T, Noda H, Oikawa K, Kamada H. Ascorbic acid radical, superoxide, and hydroxyl radical are detected in reperfusion injury of rat liver using electron spin resonance spectroscopy. Arch Biochem Biophys 1994;308:1-7.
34. Ferguson DM, Gores GJ, Bronk SF, Krom RAF. An increase in cytosolic protease activity during liver preservation. Transplantation 1993;55:627-633.
35. Shito M, Wakabayashi G, Ueda M, Shimazu M, Shirasugi N, Endo M, et al. Interleukin 1 receptor blockade reduces tumor necrosis factor production tissue injury, and mortality after hepatic ischemia-reperfusion in the rat. Transplantation 1997; 63:143-148.
36. Hernandez LA, Grisham MB, Twohig B, Arfors KE, Harlan JM, Granger DN. Role of neutrophils in ischemia-reperfusion-induced microvascular injury. Am J Physiol 1987;253:H699-H703.
37. Korthuis RJ, Grisham MB, Granger DN. Leukocyte depletion attenuates vascular injury in postischemic skeletal muscle. Am J Physiol 1988;254:H823-H827.
38. Lucchesi BR, Werns SW, Fantone JC. The role of the neutrophil and free radicals in ischemic myocardial injury. J Mol Cell Cardiol 1989;21:1241-1251.
39. Prestigiacomo CJ, Kim SC, Connolly ES Jr, Liao H, Yan SF, Pinsky DJ. CD18-mediated neutrophil recruitment contributes to the pathogenesis of reperfused but not nonreperfused stroke. Stroke 1999;30:1110-1117.
40. Dore M, Korthuis RJ, Granger DN, Entman ML, Smith W. P-Selectin mediates spontaneous leukocyte rolling in vivo. Blood 1993;82:1308-1316.
41. Essani NA, Fisher MA, Simmons CA, Hoover JL, Farhood A, Jaeschke H. Increased P-selectin gene expression in the liver vasculature and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. J Leukoc Biol 1998;63:288-296.
42. Zibari GB, Brown M, Burney DL, Granger N, McDonald JC. Role of P-selectin in the recruitment of leukocytes in mouse liver exposed to ischemia and reperfusion. Transplant Proc 1998;30: 2327-2330.
43. Rivera-Chavez F, Toledo-Pereyra LH, Nora DT, Bachulis B, Ilgenfritz F, Dean RE. P-Selectin blockade is beneficial after uncontrolled hemorrhagic shock. J Trauma 1998;45:440-445.
44. Armstead VE, Minchenko AG, Campbell B, Lefer AM. P-Selectin is up-regulated in vital organs during murine traumatic shock. FASEB J 1997;11:1271-1279.
45. Sanders W, Wilson RW, Ballantyne CM, Beaudet AL. Molecular cloning and analysis of in vivo expression of murine P-selectin. Blood 1992;80:795-800.
46. Coughlan AF, Hau H, Dunlop LC, Berndt MC, Hancock WW. P-Selectin and platelet-activating factor mediate initial endotoxin-induced neutropenia. J Exp Med 1994;179:329-334.
47. Garcia-Criado FJ, Toledo-Pereyra LH, Lopez-Neblina F, Phillips ML, Paez-Rollys A, Misawa K. Role of P-selectin in total hepatic ischemia and reperfusion. J Am Coll Surg 1995:181: 327-334.
48. Singh I, Zibari GB, Zizz H, Granger DN, Cruz L, Gonsales E, et al. Anti-P-selectin antibody protects against hepatic ischemia-reperfusion injury. Transplant Proc 1998;30:2324-2326.
49. Irving MG, Roll FJ, Huang S, Bissell DM. Characterization and culture of sinusoidal endothelium from normal rat liver: Lipoprotein uptake and collagen phenotype. Gastroenterology 1984;87:1233-1247.
50. Brengman M, Wang D, Smith E, Klein AS, Bulkley GB. Endotoxemia depresses hepatic reticuloendothelial (RES) function and consequently redistributes the trapping of circulating bacteria to the lung. Gastroenterology 1998;114:A1446.
51. Eppihimer MJ, Wolitzky B, Anderson DC, Labow MA, Granger DN. Heterogeneity of expression of E- and P-selectins in vivo. Circ Res 1996;79:560-569.
52. Armstead VE, Minchenko AG, Campbell B, Lefer AM. P-Selectin is up-regulated in vital organs during murine traumatic shock. FASEB J 1997;11:1271-1279.
53. Scales WE, Campbell DA, Green ME, Remick DG. Hepatic ischemia/reperfusion injury: Importance of oxidant/tumor necrosis factor interactions. Am J Physiol 1994;267:G1122-G1127.
54. Dulkanchainun TS, Goss JA, Imagawa DK, Shaw GD, Anselmo DM, Kaldas F, et al. Reduction of hepatic ischemia/reperfusion injury by a soluble P-selectin glycoprotein-1. Ann Surg 1998; 227:832-840.
55. Vollmar B, Menger MD, Glase J, Leiderer R, Messmer K. Impact of leukocyte-endothelial cell interaction in hepatic ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 1994;267:G786-793.
56. Steinhoff G, Brandt M. Adhesion molecules in liver transplantation. Hepatogastroenterology 1996;43:1117-1123.
57. Brengman ML, Wang D, Wilkins KB, Sakamoto N, Arai T, Ceppa EP, et al. Hepatic killing but not clearance of systemically circulating bacteria is dependent upon peripheral leukocytes via Mac-1 (CD11b/CD18). Shock 2003;19:263-267.