Mechanism of hepatocellular dysfunction during hyperdynamic sepsis

PING WANG AND IRSHAD H. CHAUDRY

Shock and Trauma Research Institute, Departments of Surgery and Physiology, Michigan State University, East Lansing, Michigan 48824; and Center for Surgical Research, Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island 02903

Wang, Ping, and Irshad H. Chaudry. Mechanism of hepatocellular dysfunction during hyperdynamic sepsis. Am. J. Physiol. 270 (Regulatory Integrative Comp. Physiol. 39): R927–R938, 1996.—Because of its central role in metabolism and host defense mechanisms, the liver is thought to be a major organ responsible for the initiation of multiple organ failure during sepsis. It is, therefore, important to discuss whether hepatocellular dysfunction occurs during early sepsis and, if so, whether this occurs prior to hepatocellular damage as evidenced by elevation in serum enzyme levels. Because indocyanine green clearance has been demonstrated to be an early and extremely sensitive measure of active hepatocyte transport function, a technique for repeated measurement of hepatocellular function by in vivo indocyanine green clearance was developed in small animals, such as the rat. Studies have indicated that hepatocellular function is markedly depressed during early stages of polymicrobial sepsis despite the increased cardiac output and hepatic blood flow and decreased peripheral vascular resistance. The depression in hepatocellular function in early, hyperdynamic stages of sepsis does not appear to be due to any reduction in hepatic perfusion but is associated with elevated levels of circulating proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-6. Furthermore, administration of recombinant murine TNF-α at a dose that does not reduce cardiac output and hepatic perfusion produces hepatocellular dysfunction and increases plasma levels of IL-6. Thus upregulation of TNF and/or IL-6 may be responsible for producing hepatocellular dysfunction during early, hyperdynamic stages of sepsis.

SEPSIS, SEPTIC SHOCK, AND the ensuing multiple organ failure continue to be the most common causes of death in surgical intensive care units (4, 6), and statistics indicate that incidences of sepsis have increased by 137% over the past decade (9). This occurs despite improvement in the management of sepsis with novel techniques, such as antibodies against endotoxin, platelet activating factor antagonists, or interleukin (IL)-1 receptor antagonist. The increased morbidity and mortality of sepsis could be due to the fact that some of the subtle alterations in cellular functions that occur during the early stages of sepsis are not identified and, consequently, missed, leading to either inadequate or delayed treatment of the septic patient. This is further confounded by the difficulties encountered in appropriately assessing early alterations of cellular functions in the clinical arena. It is encouraging, however, that the complex pathophysiology of sepsis is becoming better understood as more studies are being reported (65, 85, 90, 99, 106). Through such studies, information might be forthcoming that will lead to a better understanding of the mechanisms responsible for cell and organ dysfunctions during sepsis and a better management of sepsis.

Hepatic failure is usually thought to be a late complication following pulmonary and renal failure under such conditions (5, 106). Hepatic failure is associated with decreased hepatic perfusion, hypoxia, acidosis, and increased circulating liver enzymes such as serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) (22). This may manifest itself eventually as hyperbilirubinemia, hypoglycemia, encephalopathy, and excessive bleeding secondary to inadequate synthesis of coagulation factors (5, 22, 106). It should be pointed out that the term hepatocellular dysfunction has been frequently used to
reflect hepatocellular damage (e.g., increased circulating levels of SGPT and SGOT). Because the elevated serum liver enzymes do not reflect early hepatocellular dysfunction but rather hepatocellular damage, it is critical to develop and use a sensitive technique for monitoring the early and subtle pathophysiological alterations of hepatocellular function following the onset of sepsis. In this regard, studies by Paumgartner et al. (80) and Rikkers and Moody (84) have indicated that indocyanine green (ICG) clearance is a good measure of hepatocellular function. ICG is cleared exclusively by the liver through an energy-dependent membrane transport process, and this technique has been utilized to study various human diseases (74, 86). By using this technique in a rat model of sepsis [i.e., cecal ligation and puncture (CLP)], Chaudry et al. (22) demonstrated that ICG clearance (as measured by the one-half time ($t_1/2$) of ICG in the circulation) decreased significantly at an early stage of sepsis when SGPT, SGOT, and reticuloendothelial function were not affected. These results suggest that the altered ICG clearance is an extremely sensitive early indication of hepatocyte abnormality (22, 110, 116). However, although ICG clearance technique could be used in small animals such as the rat (22, 84), the need for extensive blood sampling for determining plasma ICG concentration in vitro prevented its use for repeated measurement of hepatocellular function. To avoid this limitation, a technique for in vivo ICG measurement has been developed, using a fiber-optic catheter and an in vivo hemoreflectometer (49, 118). Such a technique allows one to perform repeated measurement of hepatocellular function without the need for blood sampling (110, 118). Moreover, with this technique, one can also measure cardiac output (117), effective hepatic blood flow (109), circulating blood volume (115), and oxygen saturation (48, 110).

A number of studies have been carried out to investigate and determine the alterations in various aspects of hepatocellular function during sepsis, such as protein metabolism and acute phase response (47), lipid synthesis and plasma membrane fluidity (126), altered calcium regulation and adrenergic receptors (54, 85, 87), hepatocyte and Kupffer cell interaction (8, 15), as well as hepatic insulin resistance (59). Instead of covering all aspects of hepatic function, the focus of this article is limited to discussing whether alterations in active hepatocellular function, i.e., energy-dependent ICG transport process in hepatocytes, occurs during early, hyperdynamic stages of sepsis. Before the alteration in hepatocellular function and the possible mechanism responsible for it during early sepsis are described, it is pertinent to first describe the model of progressive polymicrobial sepsis.

POLYMICROBIAL SEPSIS MODEL

Various animal models, such as endotoxemia and bacteremia, have been used to study the pathophysiology of sepsis, and such models have provided useful information for the understanding of pathophysiology of sepsis (21, 35, 36, 41, 93, 123). The models of endotoxemia and bacteremia, however, do not necessarily produce the progressive cardiovascular and hemodynamic alterations that occur in the septic patient in the usual clinical arena. Moreover, it remains unknown whether hepatocellular dysfunction occurs before the onset of hepatocellular damage and hepatic failure during endotoxic shock. In this regard, a number of investigators have used the model of CLP to produce progressive polymicrobial sepsis-peritonitis (18, 35, 101, 124, 125). Studies have demonstrated that CLP mimics many features of clinical peritonitis (21, 23, 123). This model (i.e., 2 punctures in the ligated cecum with an 18-gauge needle, followed by crystalloid resuscitation) produces polymicrobial sepsis-peritonitis, which is associated with an early hyperdynamic phase (characterized by increased cardiac output, tissue perfusion, and decreased total vascular resistance up to 10 h after the initiation of CLP), followed by a late, hypodynamic phase of sepsis (characterized by depressed tissue microvascular blood flow at 20 h after CLP) (110, 120, 123). The polymicrobial sepsis model of CLP in the rat was developed by Chaudry et al. (23, 123) and has been used in a large number of laboratories to study pathophysiological and immunologic alterations during sepsis. This sepsis model is associated with a mortality rate of 94% at 48 h after CLP (123). Moreover, studies have indicated that fluid resuscitation is required for hyperdynamic circulation to occur in early stages of polymicrobial sepsis (17, 111). Thus the sepsis model of CLP allows one to study alterations in cell and organ functions and the underlying mechanisms during hyperdynamic and hypodynamic stages of polymicrobial sepsis.

RELATIONSHIP BETWEEN HEPATIC HEMODYNAMICS AND CARDIOVASCULAR RESPONSES

Machiedo et al. (67) and Townsend et al. (96) have reported that effective hepatic blood flow decreased significantly during early sepsis (2–5 h after CLP), and their data suggest that the cellular abnormality observed early during sepsis may be due to flow-related phenomena (67, 96). In contrast, studies by Lang et al. (60, 61) have demonstrated that total hepatic blood flow and cardiac output, as determined by radioactive microspheres, increased significantly in hypermetabolic sepsis. Because the measurement of effective hepatic blood flow in the studies by Townsend et al. (96) and Machiedo et al. (67) was performed by using the galactose clearance technique (without assessment of hepatic extraction ratio for galactose) and because studies have shown that the transhepatic extraction of galactose in the rat has an upper limit of 0.7 (83), it appears that the above investigations may have underestimated hepatic blood flow during early sepsis (11). In this regard, Dahn et al. (27) reported that hepatic blood flow, as measured by galactose and ICG clearance with splanchnic extraction ratio for these indicators, increased significantly in septic patients. Moreover, extraction ratio for galactose and ICA was significantly lower in septic vs. nonseptic patients (27). The notion that hepatic perfusion is increased in early sepsis has been confirmed by recent
investigations (110, 120). These studies, taken together, would suggest a dissociation between the depressed hepatocellular function and increased hepatic perfusion during early sepsis.

Studies have indicated that cardiac output and organ blood flow increased during hypermetabolic sepsis (27, 61, 91). However, it is not known how early after the onset of polymicrobial sepsis hemodynamic parameters are altered. To study this, cardiac output and microvascular blood flow in various organs were determined at 0.5, 1, 1.5, and 2 h after the initiation of CLP. The results indicated that cardiac output, as measured by ICG dilution technique, increased and total peripheral resistance decreased significantly at 2 h but not at 0.5–1.5 h after CLP (114). Moreover, hepatic microvascular blood flow and small intestinal microvascular blood flow, as measured by laser-Doppler flowmetry, increased at 2 h after the onset of sepsis. Thus hyperdynamic circulation occurs as early as 2 h after the onsets of polimicrobial sepsis. In contrast, hepatic microvascular blood flow (Fig. 1A) and circulating blood volume (measured by in vivo ICG clearance technique) decreased markedly at 20 h after the onset of sepsis (late, hypodynamic sepsis) (110, 116). Although cardiac output decreased significantly at 20 h after CLP compared with the value at 5 h after CLP, it was not significantly lower than the values in sham-operated animals (Fig. 2A). Thus the model of sepsis, i.e., CLP, is characterized by hyperdynamic circulation at early stages and hypodynamic circulation at late stages. This appears to be a useful model for studying progressive changes in cell and organ functions during polymicrobial sepsis.

Fig. 1. Alterations in hepatic microvascular blood flow (HMBF, arbitrary units; A) and effective hepatic blood flow (EHBF, ml min⁻¹ 100 g body wt⁻¹; B) at 2, 5, 10, and 20 h after sham operation (Sham) or sepsis [i.e., cecal ligation and puncture (CLP)]. HMBF was assessed by laser-Doppler flowmetry (119), and EMBF was determined by in vivo indocyanine green (ICG) clearance (with adjustment for the appropriate hepatic extraction ratio) (110). Six animals were in each group at each time point, and data are presented as means ± SE and were compared by unpaired Student’s t-test. *P < 0.05 vs. corresponding sham-operated animals at each time point. [Modified from Wang et al. (110).]

Fig. 2. Alterations in cardiac output (CO, ml min⁻¹ 100 g body wt⁻¹; A) and total peripheral resistance (TPR: mmHg ml⁻¹ min⁻¹ 100 g body wt; B) at 2, 5, 10, and 20 h after sham operation or sepsis (CLP). CO was measured by dye dilution technique (117), and TPR was determined as mean arterial pressure divided by CO. Six animals were in each group at each time point, and data are presented as means ± SE and were compared by unpaired Student’s t-test. *P < 0.05 vs. corresponding sham-operated animals at each time point. [Modified from Wang et al. (110).]
Bacterial sepsis. Moreover, the increased hepatic blood flow to hyperdynamic sepsis, it was reported that hepatic perfusion increases at early stages of polymicrobial sepsis. This is associated with increased cardiac output and decreased total peripheral resistance. Moreover, there is a clear dissociation between the increased hepatic perfusion and depressed hepatocellular dysfunction (as shown in the following section) during early sepsis.

**HEPATOCELLULAR FUNCTION**

IGC clearance has been demonstrated to be an extremely sensitive and early indication of hepatocellular abnormality during sepsis (22, 110, 116) and following other adverse circulatory conditions such as trauma and hemorrhagic shock (118, 119). IGC is a tricarboxy-cyanine green that possesses several properties that make it particularly valuable in the assessment of hepatocellular function. This dye is bound to albumin in circulation and is cleared exclusively by the liver through an energy-dependent membrane transport mechanism and is nontoxic at low doses (62, 80). Paumgartner et al. (80) suggested that the capacity of liver to remove ICG has a maximal limit. Their studies also indicated that the classic Michaelis-Menten kinetics (with Lineweaver-Burk plot) could be applied to the initial ICG uptake in the rat and human livers. They also postulated that when all hepatocyte receptor/carry sites for ICG are occupied, removal capacity is at its maximum (80). Because saturation can theoretically be obtained despite fluctuations in hepatic blood flow and other variables and because it is not possible to determine ICG clearance at an extremely high dose of this agent, maximal velocity (V max) of ICG clearance can be determined from three or more submaximal doses of ICG; this appears to be an ideal method for evaluating active hepatocellular function following various adverse circulatory conditions. An in vivo ICG clearance technique for assessing active hepatocellular function in the rat without the need for blood sampling has been developed recently (110, 118). Briefly, three different doses of ICG (0.167, 0.333, and 0.833 mg/kg body wt) were administered intravenously, and ICG concentration was measured in vivo with a fiber-optic catheter and an in vivo hemoflectometer (Fig. 4A). The initial velocity of the clearance of ICG was calculated according to the mathematical constant e raised to a second-order polynomial

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[\text{ICG}] = a + bt + ct^2
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where a is ICG concentration ([ICG]) at time (t) 0, i.e., initial concentration of ICG; b is the initial velocity of ICG clearance; and c is a coefficient constant (49, 118). V max of ICG clearance (the number of functional ICG receptors/carriers on hepatocytes) and kinetic constant (K m; the efficiency of the active transport processes)
were determined from the Lineweaver-Burk plot (Fig. 4B) (118). Thus $V_{\text{max}}$ and $K_m$ of ICG clearance represent different aspects of active hepatocellular function.

A number of studies have been conducted to examine the alterations in hepatocellular function using the ICG clearance technique (22, 40, 44, 50, 110). The work of Heimburger et al. (50) has shown a decreased hepatocyte active transport following endotoxemia. Moreover, it has been reported that the $t_{1/2}$ of ICG clearance was prolonged in patients with multiple organ failure of septic origin (92). However, the relationship between changes in ICG clearance kinetics and elevation in circulating levels of SGPT and SGOT (i.e., hepatocellular damage) was not established in those studies. Accordingly, studies have been conducted to determine the time point at which hepatocellular dysfunction is evident in the CLP model of sepsis. The results indicate that the depression in hepatocellular function (i.e., the decreased $V_{\text{max}}$ and $K_m$ values of ICG clearance) occurs as early as 1.5 h after the onset of sepsis (Fig. 5, A and B) (114) and persists during the entire septic episode studied (i.e., up to 20 h after CLP), despite normal mean arterial pressure (Fig. 6, A and H) (110, 116). Furthermore, the depressed hepatocellular function in the early and late stages of polymicrobial sepsis cannot be corrected by simply doubling the volume of crystalloid resuscitation (i.e., 3 ml/100 g vs. 6 ml/100 g body wt normal saline) (108). Thus an increase in the volume of fluid resuscitation alone does not prevent or delay the occurrence of hepatocellular dysfunction during polymicrobial sepsis. In addition, circulating levels of liver enzymes (i.e., SGPT and SGOT) increased significantly only at 10–20 h after CLP (116). The fact that $V_{\text{max}}$ and $K_m$ of ICG clearance decrease much earlier than the elevation of SGPT and SGOT levels after the onset of sepsis indicates that in vivo ICG clearance is an extremely sensitive early indication of hepatocyte abnormality. Glutathione S-transferases (previously known as Y protein or ligandin) have been proposed as the cytosolic carriers for intracellular transport of ICG (56, 63). In view of this, any impair
ment of the transferases may be responsible for the decreased $V_{\text{max}}$ and $K_m$ values of ICG clearance during early stages of polymicrobial sepsis. Because there is little information concerning the biological nature of the ICG receptors and/or carriers on hepatocyte membrane, it is difficult to pinpoint the precise mechanism at the molecular level at present. Despite this limitation, in vivo ICG clearance technique offers various advantages for determining hepatocellular function. It allows repeated measurements of hepatocellular function in small animals, such as the rat, without blood sampling. Nonetheless, additional studies, such as determination of the ability of isolated hepatocytes to synthesize de novo proteins as a measure of hepatocellular function, need to be conducted to confirm the findings of ICG clearance technique.

Hepatocellular dysfunction observed during early stages of polymicrobial sepsis (i.e., 2–10 h after CLP) does not appear to be due to any reduction in hepatic blood flow or hepatic microcirculation. This conclusion is based on the findings that hepatic perfusion increases at 2–10 h after CLP (Figs. 1, A and B, and 3A) and that $V_{\text{max}}$ and $K_m$ values of ICG clearance decrease at the same time points after the onset of sepsis (Figs. 5, A and B, and 6, A and B). Although a number of studies have clearly indicated that hepatic blood flow increases significantly during early stages of sepsis (27, 60, 61, 108, 110, 111, 114, 120), a few investigators have reported that effective hepatic blood flow, as determined by galactose clearance technique, decreases under such conditions (67, 96). Because hepatic extraction of galactose is incomplete (83) and because galactose extraction ratio was not determined in those studies (67, 96), it appears that the blood flow values in those studies might have been significantly underestimated. Thus hepatocellular dysfunction is not a flow-related event during early stages of polymicrobial sepsis. In addition, because hyperdynamic circulation occurs at 2 h and hepatocellular function is depressed at 1.5 h after CLP (Fig. 5, A and B), it appears that hepatocellular dysfunction, observed in the very early stage of sepsis, is not due to hyperdynamic circulation or a hypermetabolism-related event (114). Fry et al. (38) reported that hepatic surface oxygenation (measured by an oxygen electrode) decreased to <10% of the control values at 6 h after CLP, whereas arterial PO$_2$ was unchanged. In contrast, studies by Dahn et al. (28) indicate that hepatic blood flow increased by 72% and splanchnic oxygen consumption increased by 60% in hyperdynamic septic patients. Despite the fact that hepatic blood flow increased more than splanchnic oxygen consumption in septic patients, hepatic venous oxygen tension was lower than in normal subjects (28). This would suggest that hepatic oxygen extraction increased significantly in septic patients. High hepatic oxygen extraction resulted in a 13% reduction in sinusoidal oxygen tension (28). Although hepatocytes may become more sensitive to hypermetabolism-induced “relative hypoxemia” during sepsis, the fact that hepatocellular dysfunction occurs even earlier than the onset of hyperdynamic circulation or hypermetabolism (114) strongly suggests that such a relative hypoxemia is not responsible for the initiation of hepatocellular dysfunction during polymicrobial sepsis.

Although tissue ATP contents decreased markedly soon after hemorrhagic shock or ischemia-reperfusion (19), studies have indicated that hepatic ATP was not different from that in sham-operated animals at 10 h after CLP (23). The normal energy status in the liver was associated with normal hepatic perfusion (23). Moreover, Hampton et al. (46) reported that hepatic energy charge was not statistically different from that in sham-operated animals at 5 h after CLP. Studies by Garrison et al. (40) indicated that hepatic mitochondrial function was not impaired even at 18 h after the onset of sepsis. In addition, hepatic energy charge or ATP levels were not reduced during *Escherichia coli* bacteremia (81) or reversible endotoxin shock (58). However, hepatic ATP decreased significantly during late stages of sepsis (i.e., 16–24 h after CLP) (23). These results, taken together, indicate that hepatocellular dysfunction observed during early sepsis does not appear to be due to any reduction in hepatic energy status or hepatic perfusion.

In summary, active hepatocellular function, as assessed by in vivo ICG clearance technique, is significantly depressed during early stages of polymicrobial sepsis despite the increased hepatic blood flow. Indeed, hepatocellular dysfunction occurs even earlier than the onset of hyperdynamic circulation under such conditions. The depression in hepatocellular function in early, hyperdynamic stages of sepsis does not appear to be due to any reduction in hepatic perfusion but may be associated with elevated levels of circulating proinflammatory cytokines, such as tumor necrosis factor (TNF) and IL-6.

### THE ROLE OF PROINFLAMMATORY CYTOKINES IN PRODUCING HEPATOCELLULAR DEPRESSION

Studies have indicated that circulating levels of proinflammatory cytokines such as TNF and IL-6 are elevated during sepsis (12, 57, 68, 69, 102). A large number of studies have suggested an inverse relationship between elevated levels of proinflammatory cytokines and the outcome of septic patients (16, 29, 42, 68, 69, 82, 102–104). Thus proinflammatory cytokines TNF and IL-6 are important mediators of hemodynamic, metabolic, and immunologic alterations in the host during sepsis, and sustained elevation in TNF and IL-6 are associated with the fatal outcome from sepsis. In this regard, studies by Ertel et al. (32) have indicated that circulating levels of TNF and IL-6 increased significantly at 2, 5, and 10 h after CLP (Fig. 7, A and B), which were associated with an elevation in plasma IL-1 and prostaglandin E$_2$ levels. In contrast to sepsis, circulating levels of TNF increased in a transient fashion during the first 3 h but were not detectable at 4 h after administration of endotoxin (37, 51, 93). Byerley et al. (13) reported that TNF-$\alpha$ mRNA levels in the liver and spleen peaked at 1–2 h after septic challenge. In addition, studies by Hadjimina et al. (45) have shown...
that peritoneal macrophage TNF mRNA levels increase significantly as early as 1 h after the onset of sepsis. The correlation between the elevated circulating levels of proinflammatory cytokines (32) and the occurrence of hepatocellular dysfunction (110, 114) during early, hyperdynamic sepsis may suggest a cause-and-effect relationship between these two events.

Blockade of TNF biological activity by anti-TNF monoclonal antibodies or its synthesis by pharmacological agents such as pentoxifylline has been shown to be beneficial during sepsis (10, 18, 31, 34, 105). Monoclonal antibodies to TNF have been found to attenuate cardiopulmonary dysfunction and to protect experimental animals from lethal outcome during sepsis (105). Pentoxifylline, which downregulates TNF synthesis by inhibiting TNF mRNA transcription via increasing intracellular adenosine 3′,5′-cyclic monophosphate levels, has been shown to have significant protective effects during sepsis (10, 18). Moreover, studies by Fong et al. (37) and Mullen et al. (75) have indicated that anti-TNF-α monoclonal antibodies attenuate plasma IL-6 levels during bacteremia or gram-negative sepsis. Thus blockade of TNF biological activity or its synthesis and release may be beneficial to cell and organ function during sepsis.

It has been demonstrated that Kupffer cells are a major source of proinflammatory cytokine release following adverse circulatory conditions (77). Kupffer cells, by virtue of their anatomic location in the mainstream of splanchnic blood flow, are strategically positioned to have a constant exposure to endotoxin and other antigens (20). Studies have shown that Kupffer cells are activated to express cytokine mRNAs (24, 39) and to release proinflammatory cytokines during sepsis (2, 15, 72, 73). Recent studies by Hoffmann et al. (52) have indicated that Kupffer cells are the only cells in the hepatic sinusoids of lipopolysaccharide-perfused liver to express TNF-α mRNA, as demonstrated by nonradioactive in situ hybridization. In light of these observations, it could be postulated that, although enhanced Kupffer cell cytotoxicity may be beneficial in the destruction of pathogens seen in the liver because of bacterial translocation (1, 30, 122), this same activity may also contribute directly or indirectly to hepatocellular dysfunction that is observed during sepsis (73, 107, 108, 110). Kupffer cell number, however, can be reduced experimentally by gadolinium chloride (14, 77, 94) or a liposome-mediated macrophage “suicide” technique (66), and such animals demonstrated significantly reduced proinflammatory cytokine levels following various adverse circulatory conditions (66, 77, 94). Recently, studies have shown that blockade of Kupffer cells by gadolinium chloride reduces lethality in endotoxemic animals (64). It has been suggested that TNF production by Kupffer cells requires protein kinase C activation and protein phosphorylation (3). In addition, activation of protein kinase C decreases hepatic blood flow and oxygen consumption and increases net lactate production, which is augmented by increased Ca2+ concentration (55). Moreover, suppression of macrophage hyperactivity has been shown to improve survival of experimental animals in a burn-sepsis model (78). These results, taken together, support the notion that Kupffer cell- or macrophage-derived proinflammatory cytokines, such as TNF, may be responsible for hepatocellular dysfunction during early, hyperdynamic stages of polymicrobial sepsis.

The findings that proinflammatory cytokines increase and hepatocellular function decreases early after the onset of sepsis suggest that the increased synthesis and release of these cytokines during early, hyperdynamic sepsis may be responsible for the depressed hepatocellular function. To test this hypothesis, low doses of recombinant murine TNF-α (1.2 x 10¹⁷ U/mg; 0.05 or 0.25 mg/kg body wt) were infused over 30 min in normal rats. Various parameters were determined at 1 and 4 h after the completion of TNF-α administration. The results shown in Fig. 8, A and C, clearly demonstrate that infusion of TNF-α at a dose of 0.25 mg/kg produces significant depression in hepatocellular function (i.e., Vmax and Km of ICG clearance) with a marked elevation in plasma IL-6 levels at 1 and 4 h after TNF-α administration (107). At this dose of TNF-α, however, cardiac output, mean arterial pressure, hepatic microvascular blood flow, heart rate, stroke volume, total peripheral resistance, and plasma glucose were not significantly altered (107). Thus infusion of recombinant TNF-α in normal animals, at a dose that does not suppress cardiovascular function, significantly depresses hepatocellular function. Similarly, administration of recombinant TNF-α at the dose mentioned above (0.25 mg/kg) depresses vascular endothelial cell function in vitro as well as in vivo (113). Because TNF-α infusion increases circulating levels of TNF without decreasing hemodynamic parameters, the de-
Although administration of TNF at a dose of 0.2 mg/kg caused significant hypotension, metabolic acidosis, tissue necrosis, and a mortality rate of 64%. TNF did not produce any lethality, administration of 1.8 mg/kg TNF (0.2-3.6 mg/kg body wt) into normal rats. TNF may be responsible for producing the depression in hepatocellular function observed in early sepsis. Thus it appears that the severe tissue damage and organ dysfunction observed under those conditions may be due to profound circulatory depression (97). Although injection of a lethal dose of endotoxin induces very high levels of circulating TNF similar to those administered intravenously with a large dose of TNF (97), plasma levels of TNF in early hyperdynamic stages of sepsis are much lower (32). This raises the question of whether the dose of TNF, which does not significantly alter systemic hemodynamics, produces cellular dysfunction. In this regard, recent studies have indicated that administration of a low dose of TNF (0.25 mg/kg) did not alter cardiac output or hepatic microcirculation but produced hepatocellular dysfunction, suggesting that TNF can directly or indirectly depress hepatocellular function (107).

Fig. 8. Alterations in $V_{\text{max}}$, clearance of ICG (mg·kg body wt$^{-1}$·min$^{-1}$; A), efficiency of its active transport ($K_m$, mg/kg body wt; B), and circulating levels of IL-6 (U/ml plasma; C) in animals that received either normal saline ($n = 6$), 0.05 mg/kg TNF-$\alpha$ ($n = 6$), or 0.25 mg/kg TNF-$\alpha$ ($n = 5$). All measurements were performed at 1 and 4 h after completion of recombinant murine TNF-$\alpha$ (1.2 $\times 10^7$ U/mg) infusion. Data are presented as means ± SE and were compared by 1-way ANOVA and Tukey’s test. *$P < 0.05$ vs. saline-infused group. [Modified from Wang et al. (107).]

Increased vascular endothelial cell function, observed during early stages of polymicrobial sepsis (112), may be due to the elevated circulating levels of TNF. Studies have indicated that TNF-$\alpha$ stimulates superoxide anion generation by Kupffer cells, contributing to hepatocellular dysfunction (7). Warren et al. (121) reported that TNF, given by intraperitoneal injection produced dose- and time-related increases in hepatic amino acid uptake and a pattern of endocrine hormone alterations characteristic of acute phase responses to tissue injury and sepsis. In addition, studies by Dahn et al. (26) have shown that TNF-$\alpha$ inhibits glucose and albumin production in the primary culture of rat hepatocytes. Thus TNF may be responsible for producing the depression in hepatocellular function observed in early sepsis. However, it remains to be determined whether high circulating levels of IL-6, also seen during sepsis (2, 32), can directly or indirectly produce any deleterious alterations in hepatocellular function.

Tracey et al. (97) injected different doses of recombinant TNF (0.2–3.6 mg/kg body wt) into normal rats. Although administration of TNF at a dose of 0.2 mg/kg did not produce any lethality, administration of 1.8 mg/kg TNF caused significant hypotension, metabolic acidosis, tissue necrosis, and a mortality rate of 64% (97). Thus it appears that the severe tissue damage and organ dysfunction observed under those conditions may be due to profound circulatory depression (97). Although injection of a lethal dose of endotoxin induces very high levels of circulating TNF similar to those administered intravenously with a large dose of TNF (97), plasma levels of TNF in early hyperdynamic stages of sepsis are much lower (32). This raises the question of whether the dose of TNF, which does not significantly alter systemic hemodynamics, produces cellular dysfunction. In this regard, recent studies have indicated that administration of a low dose of TNF (0.25 mg/kg) did not alter cardiac output or hepatic microcirculation but produced hepatocellular dysfunction, suggesting that TNF can directly or indirectly depress hepatocellular function (107).

Cardiovascular and hemodynamic responses to polymicrobial sepsis include an initial hyperdynamic state characterized by increased cardiac output, decreased systemic vascular resistance, and increased blood flow in various organs such as the liver. This is followed by late hypodynamic response characterized by reduced tissue microvascular perfusion. Hepatocellular func-
tion, however, is depressed at early stages of sepsis despite the increased hepatic perfusion. Indeed, hepatocellular dysfunction occurs even earlier than the onset of hyperdynamic circulation during polymicrobial sepsis. Hepatocellular dysfunction observed in early sepsis does not appear to be due to any reduction of hepatic blood flow but is associated with elevated circulating levels of proinflammatory cytokines such as TNF and IL-6. In addition, administration of recombinant TNF-α at a dose that does not reduce cardiac output and hepatic perfusion produces hepatocellular dysfunction and increases circulating levels of IL-6. Thus TNF and/or IL-6 may be responsible for producing hepatocellular dysfunction during early, hyperdynamic stage of polymicrobial sepsis.

Although it is now well established that hepatocellular dysfunction occurs during early sepsis despite increased cardiac output and hepatic perfusion and although it is known that the depressed hepatocellular function in sepsis appears to be primarily due to upregulation of TNF, the precise mechanisms responsible for TNF-induced hepatocellular dysfunction under such conditions should be ascertained. Efforts should also be directed toward examining whether other cytokines such as IL-6 and IL-1, which were found to be elevated during early sepsis, have any deleterious effects on hepatocellular function. To further confirm the cause-and-effect relationship between upregulation of TNF and hepatocellular dysfunction, studies should be conducted to determine whether neutralization of TNF biological activity prior to the onset of sepsis prevents the occurrence of hepatocellular dysfunction. Although studies have indicated that Kupffer cells are the main source of TNF release, it remains to be determined whether reduction of Kupffer cell numbers has any beneficial effects on hepatocellular function during sepsis. Further studies are required to identify and intercept hepatocellular dysfunction at an earlier time in the course of the septic syndrome and thereby prevent subsequent progression of hepatocellular dysfunction. In this regard, pharmacological agents, such as pentoxifylline and ATP-MgCl₂, have been demonstrated to downregulate TNF release. Studies examining whether administration of these agents inhibits proinflammatory cytokine production and subsequently prevents hepatocellular dysfunction during early stages of polymicrobial sepsis are also needed.

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Address for reprint requests: I. H. Chaudry, Center for Surgical Research, Rhode Island Hospital, Middle House II, 593 Eddy St., Providence, RI 02903.

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