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Review

The microcirculation during endotoxemia

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Abstract

The initial responses to endotoxemia are detectable in the microcirculation as a microvascular inflammatory response characterized by activation of the endothelium stimulating these cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for leukocytes and platelets. Concomitantly, arteriolar tone is lost and reactivity to a variety of agonists is modified. Tissue damage subsequently results not only from reduced perfusion of the exchange vessels, but also from injurious substances released from activated, sequestered leukocytes as well as activated endothelial cells, macrophages, and platelets. This is the result of endotoxins inducing activation and interaction of a number of effector cells, cascades, and acute-phase responses, such as the complement, coagulation, bradykinin/kinin, and hematopoietic systems accompanied by the release of a myriad of mediators. These include eicosanoids, cytokines, chemokines, adhesion molecules, reactive free radicals, platelet-activating factor, and nitric oxide. This paper briefly reviews the microvascular responses to endotoxemia and discusses some of the mechanisms involved.

Keywords: Microvascular flow; Microcirculation; Endotoxins; Macrophages; Endothelium; Platelets; Leukocytes

1. Introduction

In spite of improved intensive-care techniques and multimodal therapy, mortality from severe septic complications in trauma and surgical patients remains very high. Fatal outcome of sepsis usually results from cardiac dysfunction, progressive hypotension, coagulopathies and organ dysfunction leading to multiple organ failure and septic shock. A common pathophysiological denominator in sepsis, organ failure, and septic shock is a systemic inflammatory response which principally involves the microvascular system. The response is characterized by activation of the endothelium stimulating these cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for leukocytes and platelets. Tissue damage subsequently results not only from reduced perfusion of the exchange vessels, but also from injurious substances released from activated, sequestered leukocytes as well as activated endothelial cells, macrophages, and platelets. Various injurious and noxious substances as well as several microbacteria toxins (e.g., peptidoglycans from gram-positive bacteria) may initiate these reaction cascades. The most severe septic microvascular inflammatory responses, however, are observed with gram-negative bacteremia and can be reproduced by injection of endotoxin.

2. Endotoxin and the microvascular inflammatory response

Endotoxins are high-molecular-weight complexes of lipopolysaccharides (LPS) that are major components of the outer membranes of the cell walls of gram-negative bacteria which are shed from the bacteria when cell lysis occurs and to a lesser extent during active growth. The LPS component of the complex accounts for most of the toxic activity which is principally due to its lipid A moiety [1]. Endotoxemia can result both from local or systemic gram-negative infections and from translocation of bacteria or endotoxin from the gut into the circulation through increases in gut permeability due to stress, ischemia, burn

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injury, hemorrhage, trauma, abdominal surgery, and other lesions. Even under healthy conditions, small amounts of endotoxin can be detected in the portal blood [2–4]. Normally such gut-derived endotoxin is endocytosed by Kupffer cells in the liver which is the principal site for the clearance of endotoxin, thereby preventing its spillover into the systemic circulation [5,6]. Additional sites of clearance include macrophages in other organs and tissues as well as neutrophils and monocytes.

Once in the circulation, LPS may bind to plasma components of the blood such as high-density lipoproteins or LPS binding protein (LBP). The LBP–LPS complex interacts with CD14, a high-affinity receptor for LPS expressed on monocytes/macrophages, resulting in activation of these cells, or it interacts with sCD14 in the blood to stimulate endothelial cells which do not express CD14 [7,8]. Endotoxins are capable of inducing activation and interaction of a number of effector cascades and acute-phase responses, such as the complement, coagulation, bradykinin/kinin, and hematopoietic systems accompanied by the release of a myriad of mediators. These include eicosanoids, cytokines, chemokines, adhesion molecules, reactive free radicals, platelet-activating factor (PAF), and nitric oxide (NO). At the present time, it is impossible to evaluate a hierarchy of cellular and humoral components because of their intensive interactions and potential synergisms. However, vasoactive substances such as histamine and serotonin as well as activated complement are among the initial mediators. The presence of LPS in the circulation rapidly activates complement via both the classical and alternate pathways leading to the formation of the anaphylatoxins, C5a and C3a, which results in the release of inflammatory mediators from perivascular mast cells, endothelial cells and platelets as well as the priming of leukocytes and macrophages for enhanced reactive oxygen generation. Moreover, endotoxins and their mediators are potent inducers of a prothrombotic state by eliciting the release of von Willebrand factor, reducing thrombomodulin, and increasing tissue factor production [9,10] leading to disseminated intravascular coagulation [11]. Further details of the many biological activities of LPS are contained in several reviews [12–16].

The consequences for the host of all of these events are dependent on the extent of LPS exposure and the status of the host defense mechanisms. They may be beneficial under physiological conditions or they can be deleterious when sufficient amounts of LPS are involved triggering an overwhelming systemic inflammatory response that no longer can be controlled. The decisive role of these events is played by the functional state of the macrophages, the major cellular target of endotoxic activities. The phenomena of endotoxin tolerance or increased nonspecific resistance to microbial infection, radiation, or tumor growth is based on the induction of a hyporeactive state of the macrophages to injurious noxious substances by a number of agents, such as minute amounts of endotoxins or cytokines. In addition to macrophages, endothelial cells and leukocytes also are primary targets of endotoxic effects. The initial manifestation of injury elicited by LPS and the various mediators mentioned above is a basic microvascular inflammatory response which is characterized by activation of the endothelium with increased adhesiveness for leukocytes and platelets. Such intercellular interactions involve regulated production and interactions of endothelial cell and leukocyte adhesion molecules (selectins and integrins) triggered by a variety of stimuli and resulting in leukocyte–endothelial adhesion, activation, and subsequent release of superoxide anions (O_2^-) generated from xanthine oxidase which induce oxidative stress and lipid peroxidation, further exacerbating the injury, vascular permeability, and finally transmigration of leukocytes [17–22]. Thus, the initial events when the organism is exposed to sufficient amounts of LPS are severe alterations in the microvascular system; the effects of endotoxemia on lymphatic microvessels is largely unknown.

The purpose of this paper is to briefly review the LPS-induced microvascular changes, the dynamics of which can best be observed by in vivo microscopy, and to discuss some of the mechanisms involved.

3. In vivo microscopic observations of the microcirculation during endotoxemia

In the initial phase of endotoxemia irrespective of its course and outcome, the earliest effects of endotoxin are seen in the microcirculation accompanied by a significant decrease in the numbers of leukocytes and platelets in the peripheral blood.

3.1. Mesentery and cheek pouch

In vivo microscopic observations of the mesenteries of rabbits and guinea pigs as well as the cheek pouch of hamsters and in rabbit ear chambers [23–28] have revealed that within 5–10 min after a bolus injection of endotoxin either intravenously or locally as a bleb, the velocity of blood flow is reduced, particularly in venules. By this time, perivascular mast cells also are degranulated (Fig. 1). Modest arteriolar constriction and venular dilatation tend to briefly occur followed by more sustained arteriolar dilatation, loss of vasomotion, and venular constriction. The mechanisms involved in these early responses are not completely understood but are thought to involve products of mast cells (see below) [24] as well as NO and kinins [28–30]. Within 30 min, the deformability of erythrocytes is reduced and may be accompanied by changes in shape to acanthocytes and spherocytes as well as subsequent rouleaux formation. These changes may be due to increased membrane viscosity associated with increased intracellular Ca^{2+} levels [31–34]. Granulocytes begin to roll and then adhere on the endothelium of venules and to

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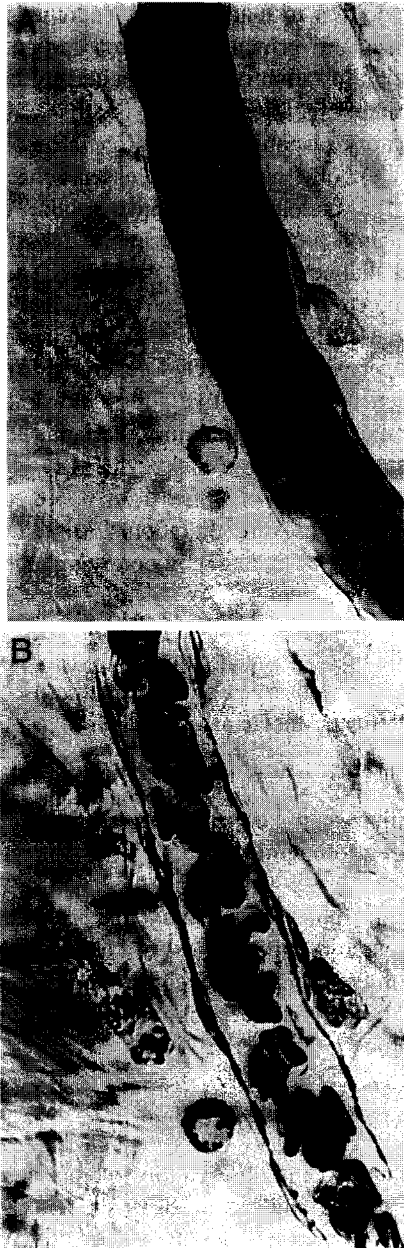

Fig. 1. In vivo microscopy of the microcirculation of the mesentery of a guinea pig. (A) Before endotoxin. (B) 15 min after endotoxin (125 μg/kg i.v.), slowing down of the blood flow, beginning degranulation of a mast cell.

a lesser extent in capillaries. By 1 hour numerous granulocytes are firmly adherent to the endothelium and pave the venular endothelial lining as well as occlude some capillaries. Erythrocytes and platelets also may adhere to the granulocytes to form mixed thrombi; platelet aggregates and microthrombi also are seen at this time. Frequently, thrombi that have developed will fragment and be discharged into the microcirculation to form microemboli at distant sites. Concomitantly, there is swelling of endothelial cells and associated perivascular cells which together with the adhering leukocytes narrow the lumina of venules and capillaries, thereby impairing nutritive blood flow. Whether endothelial swelling is a passive osmotic response or is due to active contraction of the endothelial cells remains to be determined. Associated with this is an increase in capillary and venular permeability [24,28] with the formation of tissue edema which is neutrophil-dependent [21,35–37] and can be reproduced by the injection of tumor necrosis factor alpha (TNFα) or interleukin-1 beta

Fig. 2. In vivo microscopy of the microcirculation of a rabbit after endotoxin (100 μg/kg i.v.) [23]. (A) 35 min after endotoxin. Swelling of the endothelial lining of a venule, massive aggregations of platelets and adhesion of leukocytes in a precapillary showing prestasis. (B) 40 min after endotoxin. Rouleaux formation of erythrocytes, an acanthocyte, and an adhering leukocyte. (C) 55 min after endotoxin. Extravasation of leukocytes and erythrocytes, swelling of endothelial cells, wall-adhering leukocytes, plasma skimming.

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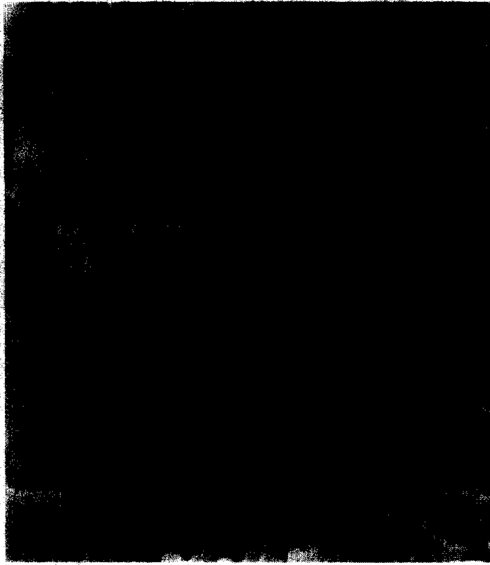

Fig. 3. Scanning electron microscopy of the microcirculation of the hamster cheek pouch. 50 min after endotoxin (300 μg i.v.). Platelet thrombi, a few single platelets and fibrin adhere to the surface of deformed erythrocytes [23].

(IL-1β) [37]. Granulocytes may release some of their granules and begin to undergo diapedesis through the injured walls of these blood vessels. The leukocytes may be followed by extravasation of erythrocytes and the appearance of massive micro-hemorrhages. Some of the microcirculatory changes are depicted in Fig. 2.

Detailed examination of these responses with scanning and transmission electron microscopy confirms the above (Figs. 3 and 4) [38,39]. In addition, it reveals the extensive nature of the intravascular coagulation involving cellular and fibrin components as well as severe damage to the endothelial cells as evidenced by their fragmentation and vacuolization resulting in discontinuity of the endothelial lining and exposure of the basal lamina to the blood. The mitochondria of remaining endothelial cells and pericytes are swollen and lack cristae. Gaps in the endothelial lining permit the extravasation of blood elements into the surrounding tissue as well as enhance edema formation. Perivascular macrophages appear to be activated and contain numerous lysosomes.

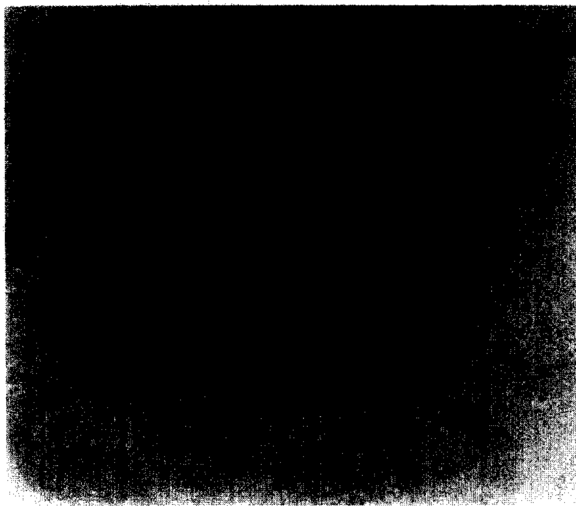

Fig. 4. Electron microscopic study of the microcirculation in a translucent rabbit ear chamber 55 min after LPS administration (50 μg/kg i.v.). Platelet attached to the basement membrane having empty secretion granules [39].

All of the above responses produced by endotoxin can be reproduced by histamine and serotonin, the only difference being the immediate initiation of these responses with these biogenic amines [24] and suggesting that the degranulation of perivascular mast cells described above plays a major role in the responses of the mesentery and cheek pouch to endotoxin. Other studies demonstrate that this degranulation and the resulting microvascular response is mediated by the activated complement components, C3a and C5a, and is not elicited by direct action of endotoxin on the mast cells [24]. Further studies with the interactions of these biogenic amines and endotoxin revealed these substances to be synergistic with each other so that very low, subthreshold doses in combination with each other produce exaggerated microvascular inflammatory responses [24,40]. Based on the fact of synergistic activities of LPS with other mediators and complement-dependent release reactions, an additional vicious circle perpetuating the pathophysiological events occurs that is caused by the non-enzymatic activation of C5 by oxygen radicals [41]. More recently, it has been shown that most of the microcirculatory reactions described can be mimicked by injecting platelet-activating factor (PAF) [42] and is inhibited by PAF antagonists [43,44]. They also can be reproduced by the injection of either TNFα or IL-1β [37] as previously mentioned. The specific roles and cellular mechanisms involving each these various mediators, however, remains unclear.

3.2. *Liver and lung*

Similar microvascular inflammatory responses to endotoxemia are evidenced early and exacerbated in the liver and lung which contain significant numbers of fixed macrophages. In fact these two organs constitute an hepatic–pulmonary macrophage axis. Activation of hepatic Kupffer cells and alveolar macrophages by blood- or airborne endotoxin leads to the release of substances that mediate and/or contribute to the microvascular inflammatory responses in the lung and liver as well as other organs. Spillover or release of toxic substances or inflammatory mediators from the injured or failing liver are transported directly to the lung where they may elicit an acute respiratory distress syndrome (ARDS) [45–47]. These substances include cytokines, eicosanoids, reactive free radicals, nitric oxide and proteolytic enzymes.

There have been very limited in vivo microscopic studies of these responses in the lung, although numerous static

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microscopic studies have been reported [48–51]. In contrast, the hepatic microvascular responses during endotoxemia associated with LPS injection, with sepsis, and/or with ethanol ingestion have been studied extensively by in vivo microscopy.

In response to stimulation by endotoxin, hepatic Kupffer cells release a variety of toxic, beneficial, and vasoactive substances which have been implicated as mediators of hepatic inflammation, injury and subsequent liver disease or organ failure [4,18,19,52–70]. Some of the principal substances released that have been implicated in liver injury include: (a) cytokines, especially TNFα, IL-1β, interleukin-6 (IL-6), and interleukin-8 (IL-8); (b) eicosanoids, especially prostaglandin E_2, thromboxane B_2, and leukotriene B_4; (c) reactive free radicals or their precursors, especially O_2^- and nitric oxide (NO) which through its rapid interaction with O_2^- forms hepatotoxic peroxynitrite anions ($OONO^-$). All of these substances may directly and/or indirectly affect surrounding parenchymal and sinusoidal lining cells as well as circulating blood elements resulting in an inflammatory response and tissue injury. As above, the initial manifestation of injury is the basic microvascular inflammatory response characterized by activation of the endothelium with increased adhesiveness for leukocytes and platelets. Tissue damage subsequently results, not only from hypoxia from reduced perfusion of the exchange vessels (sinusoids), but also from oxidative stress and lipid peroxidation due to injurious substances released from activated, sequestered leukocytes as well as the activated macrophages (Kupffer cells) [17–20,71]. Additional interest has been focused on the role of nitric oxide which is produced in the liver by Kupffer cells [72], hepatocytes [73] and sinusoidal endothelial cells [68,74]. NO is produced from L-arginine by the action of nitric oxide synthase which exists in both constitutive (cNOS, endothelial) and inducible (iNOS, macrophage and hepatocyte) forms [75,76]. Beneficial functions attributed to cNOS include inhibition of vascular smooth muscle contraction, leukocyte adherence, platelet aggregation and adherence, and maintenance of vascular integrity [75–78]. However, cytotoxic effects including inhibition of hepatic protein synthesis [72] and mitochondrial electron transport [69] have been attributed to iNOS to form very high amounts of NO which can react with O_2^- to form toxic $OONO^-$ which oxidize tissue sulfhydryls [79]. The pathophysiological importance of all of these mechanisms in the liver have received considerable attention in recent years in relation to ischemia/reperfusion injury and shock which also may involve bacterial translocation and endotoxemia [56,57,71,80–89].

Some of the dynamics of the hepatic microvascular inflammatory response have been characterized directly using in vivo microscopic imaging of the liver during endotoxemia and/or sepsis alone or in combination with the ingestion of ethanol [4,60–66,84,90,91]. The basic response includes an increase in the number of leukocytes and platelets adhering to the sinusoidal wall plugging these vessels, swelling of sinusoidal endothelial cells, decreases in the number of sinusoids with blood flow as well as flow velocity, and reduced phagocytic function of Kupffer cells. During severe intoxication, microthrombi of platelets, fibrin and leukocytes not only plug the sinusoids but also central venules. The sequelae of these responses are summarized in Table 1.The responses of the hepatic microvasculature during endotoxemia can be correlated with the state of activation and numbers and distribution of Kupffer cells within the hepatic lobule [84,92]. In animals having a highly activated reticuloendothelial system, the responses are exaggerated and amounts of endotoxin which normally are innocuous produce lethal responses and profound microcirculatory disturbances [93,94]. In contrast, in animals having a paucity of functional Kupffer cells (e.g., endo-

Table 1
Sequelae of the hepatic microvascular responses to endotoxin.

Time	Sequelae
1–15 min	Increased phagocytosis by Kupffer cells Transient adhesion of granulocytes to endothelium in sinusoids and central venules Aggregation and adhesion of platelets, particularly to Kupffer cells Transient plugging of sinusoids by adhesion of granulocytes and platelets which alters blood flow rates and patterns Decreased phagocytosis by Kupffer cells
15–60 min	Swelling of Kupffer and endothelial cells Circulating crenated erythrocytes Influx of monocytes which adhere to endothelium of sinusoids and central venules Influx of lymphocytes which adhere to Kupffer cells Further increased plugging of sinusoids and reduced blood flow Intermittent flow in portal and central venules
1–2 h	Efflux of Kupffer cells to central venules Stasis in many sinusoids and coagulation
24 h (survivors)	Increased blood flow Kupffer cell activation and increased phagocytosis

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toxin-resistant C_3H/HeJ mice) massive doses of endotoxin are not lethal and minimal microvascular dysfunction is observed [93,95]. However, activation of the Kupffer cells in such animals restores endotoxin lethality and microvascular dysfunction [93]. In animals having functional Kupffer cells, pretreatment with minute doses of endotoxin prior to a challenge with a normally lethal dose of endotoxin or the induction of sepsis induces tolerance by affecting Kupffer cell function [93,96,97]. Microcirculatory disturbances under the latter conditions are minimal. Temporary destruction of Kupffer cells with gadolinium chloride minimizes the hepatic injury induced by endotoxin or chronic ethanol ingestion [98,99]. Finally, periportal Kupffer cells are more numerous and sensitive to endotoxin than those in the centrilobular region, resulting in a regional distribution of adhering leukocytes within the lobule which is dose-dependent [84,92,97].

All of the above observed responses support the central role of Kupffer cells and gut-derived endotoxin in modulating Kupffer cell function and participating in microcirculatory disturbances. Further support is provided by studies of animals subjected to end-side portacaval anastomoses which have a paucity of functional Kupffer cells; this is thought to be due, in part, to the diversion of gut-derived endotoxin into the systemic circulation [100]. In addition, selective injury and subsequent destruction of Kupffer cells by Frog Virus 3 infection results in severe microcirculatory disturbances such as seen during endotoxemia [101].

TNFα elicits hepatic microvascular responses very similar to those produced by endotoxin including leukocyte adhesion to the sinusoidal endothelium and swelling of endothelial cells [62,102]. This is consistent with the suggested role of TNFα as a primary mediator of endotoxin events [103]. However, under in vivo conditions the effects of TNFα in the microvasculature may be mediated by other substances induced by endotoxin since the alterations were not immediate and the responses required almost 1 hour to develop [62,102]. In contrast, the earliest microvascular responses to endotoxin are visualized within 15–30 minutes after its injection [96,97,104]. This suggests that TNFα is not the initial or sole mediator of endotoxic events in the hepatic microvasculature since the response is not as rapid as elicited with biogenic amines released from LPS-stimulated mast cells [105–109].

Acute ethanol ingestion also elicits a microvascular inflammatory response similar to that seen following administration of endotoxin or TNFα (Fig. 5). A dose-dependent increase in leukocyte adhesion and endothelial cell swelling in hepatic sinusoids occurs during the first few hours following ethanol ingestion [61]. Activation of Kupffer cells is initially elicited at low doses while depression occurs at high doses [61], with daily ingestion for several days, and with chronic exposure [62,63]. The responses are exacerbated in the presence of endotoxemia or sepsis and are not seen in endotoxin-resistant animals, implicating a

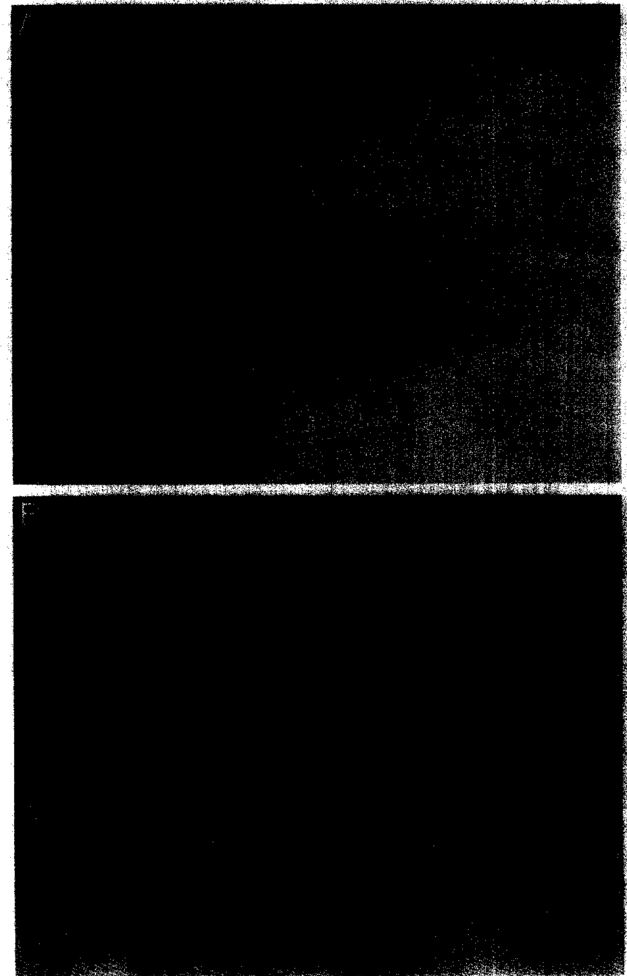

Fig. 5. In vivo microscopy of adhesion of leukocytes (L) and plugging of sinusoids (S) during hepatic microvascular inflammatory response to endotoxemia following ingestion of ethanol. H = hepatocyte; CV = central venule; BC = bil canaliculus at end of arrow.

role for endotoxin in the ethanol-induced inflammatory response [4,62,65]. In addition, the responses are abolished with anti-TNFα, suggesting that TNFα is a primary mediator of these events [4,60]. NO initially appears to play an important role in these events by stabilizing the TNFα-mediated hepatic microvascular inflammatory response to endotoxin or acute ethanol ingestion, thereby helping to protect the liver from ischemia and leukocyte-induced oxidative injury [4,66].

While these results are consistent with reports by others that chronic ethanol ingestion enhances the production of endotoxin-stimulated TNFα production and hepatic injury [53,54,110], they appear at odds with the reported inhibition of LPS-induced TNFα, O_2^-, and NOS following acute ethanol infusion [56,68]. The latter studies, however, were evaluating the response to endotoxin after the rats had been infused for several hours with ethanol, by which time their Kupffer cells may have been rendered tolerant to the challenge injection of endotoxin by the effects of ethanol-induced absorption of gut-derived endotoxin as has been

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suggested recently [111]. In this regard, increased levels of endotoxin are detected in the portal blood within 30 minutes after gastric gavage with ethanol and minute doses of endotoxin render Kupffer cells [4], the hepatic microvasculature, and animals tolerant to subsequent challenge doses for a period of time [93,96,104,112–115].

All of these results are consistent with significant interactive roles for endotoxin, cytokines, reactive free radicals, NO, sinusoidal lining cells, leukocytes and platelets in the pathophysiology of liver injury resulting from endotoxemia following infection and/or ethanol ingestion. It should be noted, however, that products of intrahepatic mast cells (e.g., histamine, serotonin, PAF, etc.) also play a role in modifying the hepatic microvascular and parenchymal function during endotoxemia [105–109]. As in the mesentery and cheek pouch, serotonin and histamine administration elicit responses that mimic many of those seen with endotoxin.

4. Modification of cardiac function and microvascular reactivity

In addition to the microvascular inflammatory responses discussed above, endotoxemia results in a transient hyperdynamic cardiovascular state followed by depressed cardiac function, lowered peripheral resistance, and hypotension in both patients and experimental animals. This latter hypodynamic state alters the distribution of blood to exchange vessels in tissues and organs throughout the body including the coronary microcirculation of the heart. Coronary hypoperfusion and ischemia *per se*, however, are not thought to be the sole cause of depressed myocardial contractility [116,117]. Increased levels of circulating cytokines elicited by endotoxin stimulate iNOS in cardiac myocytes resulting in the production of NO and coronary arteriolar vasodilation accompanied by high flow rates and reduced oxygen extraction [117,118]. In addition, NO has been suggested by some to adversely affect cardiac myocyte contractility [119,120], while other studies suggest that NO alone is not responsible for cardiac contractile dysfunction during endotoxemia [121]. In the periphery, the massive release of NO from iNOS in vascular smooth muscle is thought to be largely responsible for the lowered peripheral resistance that accompanies endotoxemia [122] which, together with reduced cardiac output, results in hypoperfusion and potential circulatory collapse.

Endotoxemia also modifies the reactivity of the microvasculature to a variety of agonists. The vasoactive sensitivity to catecholamines and sympathetic stimulation is greatly reduced [123–125]. Isolated pulmonary and coronary arteriolar relaxation responses to serotonin were reduced and converted to contractile responses while the dilatory response to adenosine diphosphate was unaffected [126]. Endotoxin has been suggested to impair β_2-adrenoreceptor-mediated relaxation in this system [127]. In contrast, arteriolar constrictor sensitivity to arginine vasopressin is increased in the rat cremaster muscle [128]. This response required the presence of endothelin but also was dependent on the presence of NO [129]. Other studies revealed that NO release from these arterioles is unaffected by endotoxemia [130]. Recent studies in the hamster cheek pouch, however, revealed that LPS stimulates iNOS to produce NO that is responsible for the sustained arteriolar vasodilation and loss of vasomotion and that induction of cNOS is protective [28]. In contrast, *E. coli* bacteremia caused rapid constriction of arterioles in the small intestine which was reversed by treatment with L-arginine, suggesting that cNOS was inhibited by endotoxin [30]. These latter responses in the microvasculature are consistent with studies in isolated large vessels (aorta) and in cultured aortic endothelial cells which indicate that endotoxemia inhibits the formation of cNOS in endothelial cells and therefore inhibits vascular responses to endothelial-dependent agonists [131,132]. However, differences in microvascular responses to endotoxin probably occur between various tissues and organs.

5. Protective mechanisms and endotoxin tolerance

The protective effects of heat shock protein (HSP) have been studied during the past few years in context with their major function of preserving essential cellular proteins and functions. The productions of heat shock proteins is enhanced by stress stimuli such as heat exposure, toxic oxygen radicals, ischemia reperfusion injury, and inflammation. Recently, it was demonstrated for the first time that endotoxins stimulate HSP production in vivo [133]. It has been suggested that HSP participate in host defense against invasive pathogens by inducing the production of proinflammatory cytokines [134], which are able to activate antimicrobial functions of phagocytes [135]. Thus, cells and organisms that produce HSPs develop tolerance to additional stress as well as to endotoxin or TNFα [134,136] and have improved myocardial function after ischemia and reperfusion [137]. The mechanisms by which such protections occur include prevention of reactive oxygen species-induced DNA strand breaks and lipid peroxidation, as well as protection from mitochondrial structure and function [138].

The phenomenon of endotoxin tolerance was described decades ago when it was observed that sublethal doses of LPS induce refractoriness to subsequent LPS challenges [139]. The complexity of endotoxin tolerance involving the central role of macrophages and their mediators have been reviewed [140,141]. All the pathophysiological events in the microvascular bed described above can be prevented or mitigated when mice were pretreated with endotoxin or detoxified endotoxin to induce tolerance 24 hours prior to the injection of endotoxin or burn trauma [93,96,97,142–144].

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The fact that endotoxin tolerance to the lethal effects of LPS and enhanced nonspecific host defense is associated with downregulation of cytokine production, particularly TNFα and IL-1β, was first described in in-vivo experiments in mice [145] when a marked alteration in macrophage response to subsequent endotoxin stimulation was observed. These findings have been confirmed and extended [146,147]. The failure of macrophages to produce soluble factors including eicosanoids responsible for endotoxicity had been described after in vitro LPS stimulation of macrophages from tolerant animals [148]. The LPS-induced transient state of hyporeactivity also can be mimicked by cytokine administration [149,150].

Besides the downregulation of cytokine production in endotoxin- or cytokine-induced tolerance, a reduction in a variety LPS-induced activities has been described that contribute to the understanding of the lack of endotoxic microcirculatory disturbances observed in tolerant animals by in vivo microscopy. These include reduced procoagulant activity and leukotriene synthesis [151], increased NO synthesis during the induction of tolerance [152] followed by reduced induction of iNOS to subsequent LPS challenge [153], and a decrease in myocardial ischemia reperfusion injury [154].

Endogenous antioxidants such as manganous superoxide dismutase that was shown to be induced in response to endotoxins, and their mediators, IL-1 and TNF, may contribute to the protective effect of endotoxin and cytokines against oxygen-radical-mediated injury [155,156], thus improving microvascular perfusion.

Many attempts have been made to modify the LPS structure to eliminate their toxicity while retaining their beneficial activity. A detoxified, potassium-methylated endotoxin preparation induced tolerance within 24 hours against the lethal effects of endotoxins from various gram-negative bacteria [157]. The effect of this detoxified LPS on minimizing microcirculatory disturbances following endotoxin injection or burn trauma was confirmed by in vivo microscopy in guinea pigs, rabbits and hamsters [142,143]. Similar protective effects were seen in the hepatic microcirculation as described above [62,90,93,97].

These observations suggest a possibility to prophylactically influence in a non-specific manner excessive inflammatory responses for patients at risk to developing septicemia or, e.g., in elective abdominal surgery, by administering detoxified LPS preparations, precursor Lipid A, cytokines, or other immunomodulators. The difficulties that may be involved are that the condition under which endotoxic preparations or cytokines exert beneficial or detrimental effects are complex because they are dependent on the dose, timing and, most importantly, on the status of the macrophages. Endotoxin tolerance or enhanced nonspecific resistance to microbial infections is inducible within 24 hours to 7 days, peaking at 3 to 4 days before the challenge. On the other hand, such pretreatment has the potential of being harmful. Extreme hyperreactivity to endotoxin results when the pretreatment is at < 24 hours before the challenge, or when the macrophages are in a prolonged activated stage that has been shown to occur, for instance, following injection with Bacille Calmette-Guérin when an explosion of cytokine release is triggered by minute doses of endotoxin [158]. Thus, it is difficult to predict whether endotoxin will exert its beneficial or toxic effects.

In addition, a variety of therapies have been developed and tested in experimental animal models directed at reducing the release or blocking the actions of cytokines, minimizing oxidative stress, blocking complement activation, or neutralizing bacterial toxins with antibodies. A number of these regimens now are in various phases of clinical trials. Preliminary results suggest various degrees of efficacy probably due to the complex nature of the endotoxic response.

6. Conclusions

The response of the microvascular system to endotoxemia is an inflammatory response characterized by activation of the endothelium stimulating these cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for leukocytes and platelets. Tissue damage subsequently results not only from reduced perfusion of the exchange vessels but also from injurious substances released from activated, sequestered leukocytes as well as activated macrophages and platelets. Alterations in the responsiveness of the vessels to vasoactive stimulants also occurs, which additionally affects the microcirculation. Multiple mediators are able to reproduce most if not all of these responses, suggesting that there is redundancy and that no one mediator is responsible for the microvascular inflammatory response. Future investigation hopefully will determine more specifically which mediator system(s) are operative under what conditions and during what periods of time. While our understanding of the complex mechanisms involved in the consequences of gram-negative bacteremia or endotoxemia that are reflected in multiple microcirculatory alterations has increased during recent years, to date, no totally effective treatment of patients has yet been developed.

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