Epithelial Barrier Dysfunction: A Unifying Theme to Explain the Pathogenesis of Multiple Organ Dysfunction at the Cellular Level

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The multiple organ dysfunction syndrome (MODS) is the most common cause of death among patients requiring care in an ICU. There is widespread agreement that MODS is the clinical manifestation of a dysregulated inflammatory response. Indeed, most of the published research regarding the pathogenesis of MODS has focused on the various signaling pathways that lead to the activation of the innate immune system and the elaboration of cytokines, oxidants, tissue-destructive enzymes, and other proinflammatory mediators (Fig. 1, steps 1–3). Interestingly, however, the biochemical and cell biologic basis for organ dysfunction per se (Fig. 1, step 4) remains very poorly understood. As is emphasized later, the histopathology of MODS in humans is remarkably bland; massive cell death, whether caused by necrosis or apoptosis, is almost certainly not the cause of MODS. Rather, the final step in the development of MODS is probably the widespread dysfunction of parenchymal cells in multiple organs as a result of the deleterious effects of a poorly controlled systemic inflammatory response. A hugely underexplored area of research can be summarized by this question: How does the inflammatory response lead to cellular dysfunction that translates into dysfunction of those organs (ie, the lungs, liver, kidneys, and intestine) that are...
Is cytopathic hypoxia the underlying mechanism responsible for multiple organ dysfunction syndrome?

Previously, the authors' laboratory entertained the possibility that cellular dysfunction in sepsis and MODS is caused by an acquired intrinsic derangement in mitochondrial function leading to inadequate production of ATP, a pathologic state called cytopathic hypoxia [1,2]. Although subsequent clinical studies have confirmed that cellular respiration is deranged in patients with sepsis [3,4], recent data from the authors' laboratory suggest that mitochondrial dysfunction is more likely to be an epiphenomenon rather than a fundamental factor contributing to organ dysfunction in MODS. Specifically, Berg et al [5] performed a study using corticotropin-stimulated IEC-6 (nontransformed rat) enterocyte-like cells in a reductionist in vitro model of sepsis, and showed that the rate of ATP turnover actually increases in immunostimulated as compared with control monolayers. The increase in ATP use (and production) is maintained by a marked increase in the rate of glycolysis. In related studies, the authors showed that incubating Caco-2 or IEC-6 cells with corticotropin increases DNA binding by hypoxia-inducible factor-1 [6,7], the key transcription factor responsible for acute cellular adaptation to hypoxic conditions. Moreover, they showed that incubating IEC-6 cells with corticotropin leads to the transcriptional activation of two key hypoxia-inducible factor-1 responsive genes, aldolase A and enolase-1, which encode proteins important in the glycolytic pathway [6]. Along with results from studies from other laboratories [8,9], their data support the view that sepsis is associated with a shift toward anaerobic ATP production and away from oxidative phosphorylation. Along with some key observations from other laboratories [10,11], however, their results suggest that inadequate ATP production is not a plausible explanation to account for organ dysfunction caused by sepsis (at least in the absence of profound vascular collapse).

Increased cellular apoptosis is another mechanism that might underlie the development of organ dysfunction in patients with sepsis or other causes of multiple organ dysfunction syndrome.

Certainly, massive apoptosis among lymphoid cells is a prominent feature of sepsis in both human patients and mice [12-14]. Moreover, using pharmacologic or genetic approaches to limit lymphoid cell apoptosis improves survival in mice with bacterial peritonitis [12,14], a finding that supports the view that increased programmed cell death of lymphocytes is significant in the pathogenesis of sepsis. Intestinal epithelial apoptosis also occurs in both patients and animals with sepsis [13,15], although most cells in the epithelial sheet are not affected. Despite these observations, neither apoptosis nor necrosis are prominent features in other organs, notably the lungs, liver, or kidneys, which are commonly involved in cases of MODS [13]. It is highly improbable that loss of cell mass per se can account for the development of lung, liver, gut, or kidney dysfunction in patients with MODS.

Tight junctions maintain epithelial polarity and barrier function

The normal functioning of the lungs, liver, kidneys, and intestine, among other organs, depends on the establishment and maintenance of compositionally distinct compartments that are lined by sheets of epithelial cells. An essential element in this process is the formation of TJs between adjacent cells making up the epithelial sheet. The TJ serves as a fence that differentiates the cytosolic membrane into apical and basolateral domains. This fence function preserves cellular polarity and, in combination with transcellular vectorial transport processes, generates distinct internal environments in the opposing compartments that are formed by the epithelial sheet. In addition, the TJ acts as a regulated semipermeable barrier that limits the passive diffusion of solutes across the
paracellular pathway between adjacent cells. The barrier function of the TJ is necessary to prevent dissipation of the concentration gradients that exist between the two compartments defined by the epithelium. In some organs, notably the gut and the lung, this barrier function is also important to prevent systemic contamination by microbes and toxins that are present in the external environment [16].

**Multiple proteins are necessary for the assembly and functioning of tight junctions**

The formation of TJs involves the assembly of at least nine different peripheral membrane proteins and at least three different integral membrane proteins [17]. Among the peripheral membrane proteins associated with TJs are the membrane-associated guanylate kinase-like proteins, ZO-1, ZO-2, and ZO-3. The integral membrane proteins involved in TJ formation include, but are not limited to, occludin and members of a large class of proteins called claudins. Both occludin and the claudins contain four transmembrane domains and are thought to be the actual points of cell-cell contact within the TJ [18]. ZO-1 has been shown to interact with the cytoplasmic tails of occludin and the claudins [19]. In addition, ZO-1 interacts with ZO-2 and ZO-3, which then interact with various actin-binding proteins, such as pp120<sup>CA<sub>5</sub></sup> [20,21], thereby linking the TJ with the cytoskeleton. Studies of mouse embryos indicate that ZO-1 localizes in plasma membrane plaques well before occludin is incorporated [22,23], suggesting that ZO-1 probably plays a central role in the assembly of mature TJs.

**Organ dysfunction caused by systemic inflammation may be the aggregate macroscopic manifestation of derangements in cellular physiology that are not necessarily sufficient to cause cell death**

Because many of the organs commonly affected in MODS (e.g., the lungs, liver, kidneys, and gut) depend on the proper functioning of an epithelial component, it is reasonable to hypothesize that epithelial cell dysfunction is important in this syndrome. Of course, the proper functioning of epithelia depends not only on the formation of TJs, but also on the appropriate expression, localization, and activity of many other cellular constituents (e.g., membrane pumps, cytoskeletal proteins, and cell-surface receptors). Epithelial polarization (and vectorial transport) and barrier function, however, depend on the formation of TJs. Although it is unlikely that any single mechanism accounts for all forms of organ failure in MODS, it is plausible that some key molecular events (e.g., alterations in the expression of Na<sup>+</sup> -K<sup>+</sup> -ATPase or impaired targeting of TJ proteins) are common factors contributing to cellular dysfunction in multiple tissues.

**What are the expected physiologic and clinical manifestations of tight junction dysfunction in epithelial cells?**

The proper functioning of the lungs, kidneys, liver, and gut depends on the generation and maintenance of compositionally distinct compartments. Table 1 summarizes some predicted physiologic and clinical consequences of epithelial TJ dysfunction. In the lungs, failure to maintain normal TJ formation is expected to promote alveolar flooding, and hence pulmonary edema, on the basis of backleakage of salt and water that is pumped from the apical side of the alveolar epithelium to the basolateral side. Of course, TJ dysfunction is not the only potential cause of impaired alveolar fluid clearance. Another cause might be decreased expression of Na<sup>+</sup> -K<sup>+</sup> -ATPase, the pump responsible for the vectorial transport of sodium ions [24,25], or the epithelial sodium channel [25].

Cholestatic jaundice is a fairly common clinical manifestation of MODS and is associated with an increased risk of mortality [26,27]. Efforts to understand the pathophysiologic mechanisms responsible for cholestatic jaundice caused by sepsis have largely focused on alterations in the function and expression of various bile acid transporters [28–31]. Nevertheless, another factor that could contribute to the development of intrahepatic cholestasis is backleakage of bile from the canalicular spaces into the sinusoids [32,33].

Alterations in the barrier function of the intestinal epithelium could permit the leakage of bacteria or microbial products, such as lipopolysaccharide (LPS) or bacterial (CpG rich) DNA or flagellin, from the lumen of the gut into the systemic compartment, leading to the initiation or amplification of a deleterious inflammatory response. The notion that this process actually occurs in patients with MODS is supported by results from a number of clinical studies, which have documented increases in intestinal epithelial permeability in a variety of acute conditions that are associated with systemic inflammation [34–37]. Moreover, in several recent studies, increased intestinal permeability in critically ill patients has

**Table 1**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Compartments</th>
<th>Physiologic consequence(s) of TJ dysfunction</th>
<th>Expected clinical manifestation(s) of TJ dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Blood/air</td>
<td>Alveolar flooding</td>
<td>Pulmonary edema, impaired gas exchange, arterial hypoxemia</td>
</tr>
<tr>
<td>Kidney</td>
<td>Renal interstitium/ tubular fluid</td>
<td>Backleakage of tubular fluid</td>
<td>Azotemia</td>
</tr>
<tr>
<td>Liver</td>
<td>Blood/bile</td>
<td>Backleakage of bile</td>
<td>Intrahepatic cholestatic jaundice, Endotoxemia, persistent activation of systemic inflammation</td>
</tr>
<tr>
<td>Intestine</td>
<td>Lumen/interstitium of the lamina propria</td>
<td>Paracellular leakage of microbial products (e.g., CpG DNA, flagellin, LPS)</td>
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*Abbreviations: LPS, lipopolysaccharide; TJ, tight junction.*
been shown to be associated with an increased risk of complications, MODS, or even mortality [36–40].

Acute renal failure in patients with sepsis or MODS is manifested by the development of azotemia and often also oliguria. The mechanisms underlying development of acute renal failure caused by sepsis are likely multiple and remain poorly understood. One important factor is thought to be excessive renal vasoconstriction [41], and another might be glomerular thrombin deposition [42]. Without minimizing the importance of these and other mechanisms, it is reasonable to postulate that another factor might be backleakage of tubular fluid as a result of TJ dysfunction in the tubular epithelium.

Nitric oxide or peroxynitrite are involved in the regulation of tight junction protein expression and function

The authors [43–45] and others [46–48] have shown that the permeability of cultured epithelial monolayers increases when the cells are incubated with various proinflammatory cytokines. The mechanisms responsible for cytokine-induced epithelial hyperpermeability are incompletely understood. It is known, however, that compounds that spontaneously release nitric oxide (NO·) increase the permeability of cultured intestinal epithelial cell monolayers [49,50]. This observation is pertinent, because incubating Caco-2 human enterocyte-like cells with the proinflammatory cytokine, interferon (IFN)-γ, or a mixture of the proinflammatory cytokines, IFN-γ plus tumor necrosis factor and interleukin-1β, leads to increased expression of inducible NO· synthase (iNOS) and increased production of NO· [44,51,52]. Moreover, compounds that inhibit iNOS have been shown to ameliorate the development of hyperpermeability induced by exposing Caco-2 cells to IFN-γ [44] or “cytomix” (IFN-γ plus tumor necrosis factor plus interleukin-1β) [52]. Similarly, 1-[(6-tosylamido)-2-naphthyl]methyl-3-isonicotinamide (1-TMA), an iNOS inhibitor, blocks the development of hyperpermeability when Calu-3 human alveolar epithelial) monolayers are incubated with cytomix [53]. IFN-γ or cytomix seems to increase epithelial permeability, at least in part by increasing the production of NO· by epithelial cells.

NO· reacts rapidly with superoxide (O₂⁻) to form the potent oxidizing and nitrating species, peroxynitrite (ONOO⁻) [54,55]. Several lines of evidence support the view that ONOO⁻ (or some related species) rather than NO· per se is responsible for the deleterious effects of NO· on intestinal epithelial barrier function. When Caco-2 monolayers are incubated with the NO· donor, S-nitroso-N-acetylpenicillamine (SNAP), permeability is significantly increased, but the magnitude of the effect is small [50]. Furthermore, the permeability of Caco-2 monolayers is not affected when the cells are incubated with pyrogallol, a compound that spontaneously generates O₂⁻ in aqueous solutions [50]. If Caco-2 cells are incubated with both SNAP and pyrogallol, however, then epithelial permeability is dramatically increased [50]. SNAP-induced hyperpermeability is also markedly enhanced by coincubating the cells with diethylthiocarbamate, a compound that is known to inactivate Cu-Zn superoxide dismutase and is expected to increase the concentration of endogenously generated O₂⁻ [50]. Taken together, these findings support the view that NO·-induced hyperpermeability is enhanced by the simultaneous availability of O₂⁻ (ie, conditions favoring the formation of ONOO⁻). Because ONOO⁻ is a weak acid (pKa approximately 6.8) and many of the effects of ONOO⁻ are thought to be mediated by an unstable form of the protonated species, studies from the authors’ group showing that NO·-induced hyperpermeability is enhanced under mildly acidic conditions further support the notion that ONOO⁻-ONOOH is the responsible moiety [43,56].

The mechanisms responsible for NO·-, or ONOO⁻-mediated intestinal epithelial hyperpermeability remain to be elucidated. The authors’ laboratory reported, however, that NO· generated endogenously as the result of iNOS expression induced by incubating Caco-2 cells with cytomix or exogenously from the NO· donor DETA-NONOate [(Z)-1-[2(aminooethyl)-N-(2-aminoethyl) amino]diazene-1-ium-1,2-diolate] decreases the expression and impaired proper localization of the TJ proteins, ZO-1, ZO-3, and occludin [45]. The authors also showed that incubating Caco-2 cells with either DETA-NONOate or cytomix increases the expression of another key TJ protein, claudin-1, and promotes the accumulation of this protein in what seem to be vesicles within the cells. These findings support the view that NO· (or a related reactive species) increases epithelial permeability by causing derangements in the expression or localization of several key TJ proteins.

Nitric oxide-dependent changes in Na⁺, K⁺-ATPase activity can affect tight junction assembly and function

Sugi et al [57] proposed that one way that NO· might alter the expression or localization of various TJ proteins is by modulating the activity of the membrane pump, Na⁺, K⁺-ATPase. In a series of studies using monolayers of T84 enterocyte-like cells, these investigators reported that intracellular sodium concentration and cell volume increase following exposure to the proinflammatory cytokine, IFN-γ. Additionally, Sugi et al [57] showed that incubating T84 cells with either NO· or IFN-γ decreases the expression and activity of Na⁺, K⁺-ATPase. Remarkably, growing the monolayers in medium with low sodium concentration inhibits the development of hyperpermeability following exposure to IFN-γ and also prevents IFN-γ-induced alterations in occludin expression. These findings suggest a pathway that involves the following steps: IFN-γ (or other proinflammatory cytokines) → iNOS induction → NO· production → inhibition of Na⁺, K⁺-ATPase expression and function → cell swelling → altered expression or targeting of TJ proteins (eg, occludin) → hyperpermeability.
Quyyum et al. [58] reported that treatment of rat brain membranes with ONOO• in vitro decreases the activity of the Na⁺,K⁺-ATPase, and hypothesized that this effect may be caused by nitration of the Na⁺,K⁺-ATPase. These authors, however, did not demonstrate that ONOO• actually modifies the Na⁺,K⁺-ATPase. These authors also implicated lipid peroxidation in the inactivation of the Na⁺,K⁺-ATPase in a previous report [59], but only showed an association between lipid peroxidation and altered Na⁺,K⁺-ATPase activity in their studies. These authors convincingly showed that ROS decrease the affinity of the Na⁺,K⁺-ATPase for Na⁺ and K⁺, however, inhibiting transport of these ions [58,59]. Taken together, these results support the notion that oxidative or nitrosative posttranslational modifications of the Na⁺,K⁺-ATPase can lead to decreased epithelial barrier function.

**Functional inducible nitric oxide synthase expression is essential for lipopolysaccharide-induced alterations in intestinal permeability in mice**

When Han et al. [63] injected C57BL-6J mice with a small sublethal (2 mg/kg) dose of *Escherichia coli* LPS, intestinal mucosal permeability to the permeability probe, fluorescein isothiocyanate-labeled dextran (FD4) molecular mass 4 kDa, increased significantly (Fig. 2A). Treatment of endotoxemic mice with L-NIL, an isoform-selective iNOS inhibitor [60], ameliorated LPS-induced ileal mucosal hyperpermeability (Fig. 2B). Basal ileal mucosal permeability in control (phosphate buffered saline [PBS]-treated) iNOS knockout (iNOS−/−) mice on a C57BL-6J background was greater than that measured in control (wild-type) iNOS+/+ mice (Fig. 2C), a finding that is consistent with reports that basal levels of NO• are required for normal gut homeostasis [61,62]. Despite a basal defect in intestinal barrier function in iNOS−/− mice, permeability to FD4 failed to increase further when these mice were challenged with LPS (see Fig. 2C).

**Functional inducible nitric oxide synthase expression is essential for lipopolysaccharide-induced bacterial translocation in mice**

Bacterial translocation from the gut lumen to mesenteric lymph nodes is another measure of in vivo mucosal barrier function. In a recent study from the authors’ group, endotoxemia increased the number of bacteria that were recovered from mesenteric lymph nodes from wild-type (iNOS+/+) mice [63]. Treatment of endotoxemic iNOS+/+ mice with L-NIL to block iNOS-dependent NO• production decreased LPS-induced bacterial translocation. Similarly, LPS failed to induce bacterial translocation in iNOS−/− mice. These findings are consistent with data reported by other investigators [64].

**Lipopolysaccharide decreases the expression of several tight junction proteins in mice**

Han et al. [63] used a portion of ileal tissue to prepare total and NP-40 (detergent)-insoluble protein extracts, the latter being enriched for TJ-associated and other cytoskeletal proteins. Total protein extracts were subjected to immunoblotting. NP-40-insoluble proteins were first solubilized with detergent-containing buffer and concentrated by immunoprecipitation before immunoblotting. The expression of occludin in NP-40-insoluble extracts was decreased in samples obtained 6 hours after injecting mice with LPS [65]. Occludin expression in NP-40-insoluble extracts decreased still further at 12 hours, but was starting to return toward normal 18 hours after LPS challenge. In total protein extracts, changes in occludin levels were less dramatic, and the maximal decrease was observed at 12 hours. ZO-1 expression decreased slightly in total protein extracts from ileal mucosa of mice exposed to LPS. There was a large decrease, however,
in ZO-1 levels in the NP-40-insoluble fraction. This finding suggests that the ZO-1 that is present in the cells of endotoxemic animals is unable to assemble into TJ's. Consistent with the authors' observations obtained using the Caco-2 system [45], claudin-1 expression increased in total protein extracts prepared from ileal mucosa. Immunoblotting total protein extracts for actin revealed equivalent loading of the samples in these gels. As expected, iNOS protein expression increased in total protein extracts from ileal mucosa of LPS-treated mice.

**Endotoxia is associated with derangements in ileal mucosal tight junction protein localization**

Immunohistochemical studies of ileal tissue from endotoxemic mice were performed using samples harvested 12 hours after injection of LPS. ZO-1 formed a continuous staining pattern around the enterocyte layer near the apical region of the lateral membrane of crypt and villous cells of the epithelial and endothelium of the lamina propria from normal mice (Fig. 3). Following injection of mice with LPS, ZO-1 staining was maintained in the crypts, but staining progressively decreased at the tips of the villi (see Fig. 3). In sections from endotoxemic mice, the staining patterns for ZO-1 were disrupted only in focal regions of the ileum; approximately 60% of the villi in a given section stained normally. If the endotoxemic mice were treated with L-NIL to block pharmacologically iNOS-dependent NO production, then the correct targeting of ZO-1 in the ileal mucosa was preserved (see Fig. 3). Similar findings were obtained when staining was performed for occludin instead of ZO-1 (data not shown).

![Fig. 3. Indirect immunofluorescence images of ileum from control mice, mice exposed to LPS for 12 hours, and endotoxemic mice that were treated with LPS plus L-NIL. In ileum from control mice, ZO-1 staining was continuous along the villous epithelium (TRITC-conjugated goat anti-rabbit secondary antibody, red fluorescence). LPS caused marked down-regulation of ZO-1 expression in the epithelium. L-NIL treatment ameliorated the deleterious effects of LPS on the localization of ZO-1. The insets are confocal images. The original magnification for the LPS plus L-NIL panel was ×400. The bar represents 50 μm. L-NIL, 1-NaO).](image)

Parallel experiments were performed using iNOS−/− mice. The levels of occludin and ZO-1 in ileal mucosa from control iNOS−/− mice (i.e., those not challenged with LPS) were reproducibly lower than the levels of these proteins in control iNOS+/− mice. To some extent, these basal differences in occludin and ZO-1 expression confounded interpretation of the results obtained in LPS-challenged animals. Nevertheless, it was apparent that injecting iNOS−/− mice

![Fig. 4. Effect of LPS on circulating concentrations of conjugated bilirubin (A) and bile acids (B) in mice. Total bile acids were measured in plasma samples from iNOS−/− and iNOS+/− mice that were challenged with phosphate buffered saline (PBS) (Con) or LPS (L) 12 hours earlier. In a separate experiment, groups of animals were treated with phosphate buffered saline (Con) or LPS. Some of the LPS-challenged animals also were treated with two 5 mg/kg doses of L-NIL, administered by intraperitoneal injection, 2 and 8 hours after the injection of LPS. Plasma samples were assayed for total bile acids 12 hours after the animals were injected with PBS or LPS. Plasma concentrations of conjugated bilirubin were assayed in plasma samples from mice treated with PBS (Con) or LPS 12 hours earlier. Some of the LPS-challenged animals also received L-NIL. In a separate experiment, plasma samples of conjugated bilirubin were measured in groups (N = 5) of iNOS−/− and iNOS+/− mice injected 12 hours earlier with LPS. Results are means ± SEM (N = 5, 7 for each treatment group). * Indicates P < .05. Con, control; iNOS, inducible nitric oxide synthase; NH₃, N(O)(-iminoethyl)lysine; LPS, lipopolysaccharide. (Adapted from Han X, Fink MP, Yang R, et al. Increased iNOS activity is essential for the development of hepatic epithelial tight junction dysfunction in endotoxemic mice. Am J Physiol Gastrointest Liver Physiol 2004;286:G126-G133, with permission.)](image)
with LPS failed to cause a further decrease in the expression of ZO-1 or occludin in ileal mucosa. The localization of ZO-1 and occludin was preserved in ileal sections prepared from LPS-treated iNOS 
−/− mice, being essentially unchanged from what was observed in sections from iNOS +/− animals injected with vehicle (data not shown).

Lipopolysaccharide impairs hepatobiliary barrier function by an inducible nitric oxide synthase–dependent mechanism

Lora et al [66] reported that hepatic TJ function can be assessed by measuring serum concentrations of bile acids and conjugated bilirubin. In a recent study from the authors' laboratory, Han et al [67] showed that circulating levels of both of these bile components were increased in mice injected 12 hours earlier with LPS (Fig. 4). When endotoxemic mice were treated with L-NIL, however, serum levels of bile acids and conjugated bilirubin were not different from normal. Although basal serum conjugated bilirubin levels were somewhat higher in vehicle-treated iNOS +/− as compared with iNOS −/− mice, LPS-induced changes in circulating bile acid and conjugated bilirubin levels were prevented by genetic ablation of iNOS function. Collectively, these data support the view that systemic inflammation in mice (induced by injecting LPS) is associated with hepatobiliary epithelial barrier dysfunction by an iNOS-dependent mechanism.

To further confirm their findings, the authors used another approach for assessing hepatobiliary tight junctional integrity. They cycled the common bile duct of mice, and assayed bile for the appearance of fluorescein isothiocyanate–labeled dextran ([FD40], molecular mass 40 kD) following intravenous injection of the tracer. In control mice, biliary FD40 concentration increased only very slowly following injection of the tracer (Fig. 5) [67]. In mice injected with LPS (2 mg/kg) 12 hours earlier, however, the concentration of FD40 in bile increased rapidly after intravenous injection of the tracer. The LPS–induced increase in biliary FD40 concentration was prevented if the endotoxemic animals were treated with L-NIL. The rate of bile flow was about 50% lower in endotoxemic mice as compared with control mice, a finding that is consistent with other studies [30]. Although decreased bile flow rate tends to increase the measured concentration in bile of a marker like FD40, the approximately 50% decrease in bile flow rate observed in the endotoxemic mice is insufficient to account for the approximately 10-fold increase in FD40 concentration in bile that was detected in LPS-challenged as compared with control animals. Accordingly, the marked increase in biliary FD40 concentration was evidence of deranged hepatobiliary TJ function.

Lipopolysaccharide induces hepatic inducible nitric oxide synthase expression and alterations in hepatic tight junction protein expression

In the studies performed by Han et al [67] of the effects of endotoxemia on hepatobiliary epithelial barrier function in mice, immunoreactive iNOS was not detectable by Western blotting of hepatic protein extracts from control mice. Within 6 hours after the injection of LPS, however, hepatic iNOS expression was clearly evident. Levels of iNOS protein in liver increased still further 12 and 18 hours after the injection of LPS. Following the induction of endotoxemia, occludin and ZO-1 expression decreased in NP-40 insoluble (cytoskeletal fraction with associated TJ proteins) extracts of hepatic tissue. Decreased expression of these TJ proteins was also observed in total protein extracts, but the change in occludin expression occurred more gradually. ZO-2 and ZO-3 levels also decreased in total protein extracts. Claudin-1 expression did not change reproducibly in total protein extracts.

Endotoxemia is associated with derangements in hepatic tight junction protein localization

Han et al [67] found minimal evidence of hepatic inflammation or necrosis when they examined hematoxylin and eosin stained thin sections of liver tissue from mice injected 12 hours earlier with LPS, irrespective of whether or not
the animals were treated with L-NIL. In control specimens, occludin and ZO-1 were largely detected as parallel strands of staining representing the outlines of canalicular (Fig. 6). Consistent with previously reported data [68], staining of occludin and ZO-1 in normal liver tissue was predominantly limited to focal regions of hepatocyte-hepatocyte contact and endothelial cell cell junctions. Hepatic tissue from endotoxemic mice showed a widespread decrease in occludin staining (see Fig. 6). The remaining areas of occludin staining were tortuous and discontinuous. Similarly, ZO-1 staining was greatly reduced following injection of LPS, and the residual ZO-1 staining was distorted. In contrast to the dramatic decrease of immunostaining for ZO-1 and occludin in hepatocytes of the LPS group, there was obvious preservation of occludin and ZO-1 staining along the outlines of canalicular in endotoxemic mice treated with L-NIL. Similar protection against LPS-induced alterations in ZO-1 and occludin staining was observed when hepatic sections from LPS-challenged iNOS−/− and iNOS−/− mice were compared (data not shown).

A proinflammatory milieu decreases pulmonary epithelial barrier function

Prompted by the findings noted previously supporting the notion that systemic inflammation induced by injecting LPS causes alterations in epithelial TJ formation in two organs (liver and intestine), Han et al. [53] from the authors' laboratory sought to extend these observations by examining the effect of LPS (2 mg/kg) on the leakage of FD4 from plasma into the alveolar space in C57Bl/6J mice. At various time points after injection of LPS (or PBS), the authors injected mice intravenously with FD4 in saline. Within 6 hours after the induction of endotoxemia, the bronchoalveolar lavage fluid serum FD4 ratio increased significantly (Fig. 7). At 12 hours, the bronchoalveolar lavage fluid serum FD4 ratio increased still further. By 18 hours, however, the bronchoalveolar lavage fluid–serum FD4 ratio normalized. Delayed treatment with L-NIL significantly ameliorated the increase in lung permeability caused by LPS. These findings indicate that injecting mice with LPS transiently impairs bronchoalveolar epithelial barrier function and supports the view that LPS-induced bronchoalveolar barrier dysfunction is mediated, at least in part, by iNOS-dependent NO− synthesis.
Lipopolysaccharide induces inducible nitric oxide synthase expression and increases nitric oxide synthase production in mice

Injecting mice with LPS significantly increased the concentration of the NO⋅-breakdown products, NO2− and NO3−, in bronchoalveolar lavage fluid and serum (data not shown). Treatment with L-NIL only partially inhibited the accumulation of NO2− and NO3− in serum, whereas treatment with the iNOS inhibitor almost completely blocked accumulation of these NO⋅-metabolites in bronchoalveolar lavage fluid.

Endotoxemia is associated with alterations in the expression of tight junction proteins in the lung

NP-40 insoluble occludin and ZO-1 levels decreased within 6 hours after injecting mice with a sublethal dose of LPS, and were maximally decreased at 12 hours. By 18 hours, NP-40 insoluble occludin and ZO-1 levels were starting to normalize. In lung tissue specimens from normal mice, ZO-1 was localized as a continuous line along the boundaries between bronchial and alveolar epithelial cells. The intensity of this staining was markedly reduced in lung tissue harvested from mice that were challenged with LPS 12 hours earlier.

Summary

Collectively, the results from more than a decade of work by scientists in the authors’ laboratory and many other laboratories around the world support the view that an inflammatory milieu leads to marked alterations in the structure and function of TJs in multiple epithelia. These data have been obtained using both reductionist in vitro models, such as Caco-2 enterocyte-like cells growing as monolayers in diffusion chambers, and in vivo models, such as mice injected with the proinflammatory bacterial product, LPS. Although the mechanisms responsible for TJ dysfunction associated with acute inflammation are likely to be complex, and in any case remain poorly delineated, findings from the authors’ group suggest that induction of iNOS and increased production of NO⋅ is almost certainly important. Determining how NO⋅ production leads to alterations in the expression and targeting of TJ proteins currently is the focus of a major research program in the authors’ laboratory. In addition to determining how NO⋅ and proinflammatory cytokines (and possibly other mediators) cause epithelial barrier dysfunction, another important goal of the authors’ research program is to move beyond studies using just cultured cells or rodent models of sepsis and begin obtaining data to support (or refute) the notion that the expression and targeting of TJ proteins is impaired in the pulmonary, hepatic, renal, or intestinal epithelia of patients with sepsis and MODS. Finally, it is important to seek to find therapeutic strategies that can prevent or even reverse TJ dysfunction in patients with MODS.

References
