Intestinal epithelial hyperpermeability: update on the pathogenesis of gut mucosal barrier dysfunction in critical illness

Mitchell P. Fink, MD

Purpose of review

Tight junctions between adjacent epithelial cells are essential for the maintenance of compositionally distinct fluid compartments in various organs, such as the liver, lungs, kidneys, and intestine. These epithelial organs are commonly affected in the condition known as multiple organ dysfunction syndrome, which can complicate the clinical course of patients with sepsis or other conditions associated with poorly controlled systemic inflammation. The gut serves as a useful model for this problem, and studies using reductionist *in vitro* models and experiments carried out using laboratory animals are starting to clarify the cellular and biochemical mechanisms that are responsible for intestinal epithelial hyperpermeability secondary to critical illness.

Recent findings

One key factor that has been identified is excessive production of nitric oxide and related species, although other factors, such as increased expression of the cytokine interleukin 6, appear to be important as well. A newly described, cytokine-like molecule, high-mobility group B1, increases permeability of cultured epithelial monolayers *in vitro* and murine ileal mucosa *in vivo*.

Summary

Epithelial dysfunction may be a common final pathway contributing to organ dysfunction in sepsis and other forms of critical illness.

Keywords

high-mobility group B1, nitric oxide, reactive oxygen species, tight junctions

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Departments of Critical Care Medicine and Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.

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Correspondence to Mitchell P. Fink, MD, Department of Critical Care Medicine, Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15260, USA; e-mail: finkmp@ccm.upmc.edu

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Abbreviations

HMGB1	high-mobility group B1
MODS	multiple organ dysfunction syndrome
NOS	nitric oxide synthase
PKC	protein kinase C
TNF	tumor necrosis factor

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A common consequence of sepsis and other conditions associated with systemic inflammation is multiple organ dysfunction syndrome (MODS) [1,2]. Although the clinical manifestations of MODS can be varied, the organs involved typically include the lungs, kidneys, liver, and gut. A common feature of these organs is that their normal functioning depends on the establishment and maintenance of compositionally distinct, fluid-containing compartments that are lined by sheets of epithelial cells. For example, in the lung, the alveolar epithelium is essential to define the distinct air- and blood-containing spaces. In the kidney, the tubular epithelium separates the compartment containing tubular fluid from the interstitial space. In the gut, the lumen is separated from the lamina propria by the intestinal epithelium. An essential element in this process is the formation of tight junctions between adjacent cells making up the epithelial sheet.

The tight junction serves as a fence that divides the plasma membrane into apical and basolateral domains [3]. The tight junction also serves as a regulated, semipermeable barrier that limits the passive diffusion of solutes across the paracellular pathway between adjacent cells [4,5]. These properties of tight junctions, in combination with transcellular vectorial transport processes, generate distinct internal environments in the opposing compartments. Thus, the barrier function of the tight junction is necessary to prevent dissipation of the concentration gradients that exist between adjacent compartments defined by the epithelium and that are essential for normal organ function. In some organs, notably the gut and lung, this barrier function is also important to prevent systemic contamination by microbes and toxins that are present in the external environment.

Proper functioning of tight junctions is essential to maintain normal physiologic processes in all organs containing epithelia. The intestine is essentially a simple tube lined by epithelium. Furthermore, immortalized cell lines, such as Caco-2 and T84, recapitulate many of the morphologic and functional attributes of the normal intestinal epithelium. These cell lines have been invaluable for studying intestinal epithelial tight junction function using reductionist *in vitro* models. For these reasons, alterations in intestinal epithelial function owing to sepsis or proinflammatory cytokines may serve as a useful para-

digm for the more general problem of epithelial dysfunction in critical illness.

The tight junction is a narrow band that consists of several strands wrapping around the circumference of the apical portion of epithelial cells. The number of the strands correlates with the tightness of the junction [6,7]. In the intestine, the tight junctions of the villus epithelium have more strands and are more restrictive than those in the epithelium of the crypts, which have fewer strands. The formation of tight junctions involves the assembly of at least nine different peripheral membrane proteins and at least three different integral membrane proteins [8]. Among the peripheral membrane proteins associated with tight junctions are the membrane-associated, guanylate kinase–like proteins ZO-1, ZO-2, and ZO-3. The integral membrane proteins involved in tight junction 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 points of cell–cell contact within the tight junction [9]. ZO-1 has been shown to interact with the cytoplasmic tails of occludin and the claudins [10]. In addition, ZO-1 interacts with ZO-2 and ZO-3, which then interact with various actin-binding proteins, such as $pp120^{CAS}$ [11,12], thereby linking the tight junction with the cytoskeleton.

Measurement of intestinal epithelial permeability

Several means are available to investigators studying the mechanisms responsible for the development of intestinal epithelial hyperpermeability under normal or pathologic conditions. The models used in this field can be classified as *in vivo*, *ex vivo*, and *in vitro*. Two main approaches are used to measure intestinal epithelial permeability *in vivo*. In the first, a hydrophilic permeability probe is administered enterally, and the subsequent appearance of the molecule in urine [13] or plasma [14,15] is monitored. In the second approach, the experimental animal is infused intravenously with an appropriate hydrophilic probe while the lumen of an isolated segment of intestine is perfused with a buffer solution. In this method, permeability is assessed by monitoring the plasma-to-lumen clearance of the marker substance [16,17].

There are also two main ways of assessing intestinal epithelial permeability *ex vivo*. First, small segments of intestine can be harvested from an experimental animal (usually a rodent), everted, and ligated at both ends to form a gut sac with the mucosal surface facing out [18,19,20•]. To assess permeability, the everted gut sac is incubated in a bath of oxygenated nutrient solution containing an appropriate hydrophilic permeability probe. Permeability is quantitated by determining the flux of the probe from the bath into the serosa-lined lumen of the sac. The second way to assess permeability *ex vivo* is to harvest segments of intestine, strip away the seromuscular coat, and mount the remaining mucosal layer in a diffusion (Ussing) chamber [21]. In this approach, paracellular conductance can be assessed by both measuring transepithelial electrical resistance and determining the rate of permeation from the serosal to the mucosal chamber of appropriate hydrophilic probe substances.

Purely *in vitro* methods also are available for studying the regulation of epithelial permeability. These approaches typically use various immortalized cell lines that form polarized epithelial monolayers when grown on permeable supports in bicameral diffusion chambers. Two human intestinal epithelial cell lines, T84 and Caco-2, are commonly used for studies of this sort. Both of these cell lines form tight junctions *in vitro*. Permeability of the monolayer is measured by loading the upper (apical or luminal) chamber with a fluorescent or radioactive probe and, after an appropriate period of incubation, determining the amount of the probe in the lower (basolateral) chamber. Permeability commonly is expressed as the flux of the probe per unit area of membrane divided by the concentration of the probe in the apical compartment, a term with the units of a clearance ($nL/h/cm^2$) [22].

When intestinal permeability is estimated by measuring the urinary excretion of an enterally administered probe, care is taken to use a marker substance that is thought to be transported across the intestinal epithelium only by passive diffusion through the paracellular pathway [23,24]. In addition, the probe must not be metabolized or degraded *in vivo* and must be cleared only by urinary excretion. In addition to alterations in the intrinsic permeability of the intestinal epithelium, various pre- and postmucosal factors might influence the urinary excretion of an enterally administered probe. For example, changes in the rate of intestinal transit might alter absorption of the probe on kinetic grounds by changing the amount of time that the probe is in contact with the epithelium of the gut and hence available for diffusion. Furthermore, changes in the glomerular filtration rate might alter the rate of urinary excretion of the probe.

In an effort to circumvent these problems, clinical investigators interested in assessing mucosal permeability have measured the ratio of the urinary excretion of two probes. Typically the two probes have been nonmetabolizable saccharides, such as lactulose and rhamnose [25,26], lactulose and mannitol [27,28], and cellobiose and mannitol [29]. This approach is based on a number of critical assumptions. The first assumption is that the larger sugar (lactulose or cellobiose) can traverse the intestine barrier only via the paracellular pathway, whereas the smaller molecule (rhamnose or mannitol) can diffuse

through cell membranes and pass between enterocytes. The second assumption is that the rate of absorption of the smaller probe is more or less constant, being relatively unaffected by changes in intestinal permeability. The third assumption is that the only pertinent process within the intestine is passive diffusion from the lumen of the gut into the subepithelial compartment. The fourth assumption is that potentially confounding pre- or postintestinal factors, such as alterations in gut motility or renal function, affect both probes to the same extent.

Recently, some of the assumptions underlying the differential sugar absorption test have been called into question. For example, Tomita *et al.* [30] showed that a variety of compounds, including both lactulose and mannitol, are actively transported by the ileal mucosa of rats in a polarized fashion from the serosal to the mucosal compartments. Thus, a change in the net rate of absorption of one of these permeability probes could represent the effect of a change in the rate of passive diffusion from the lumen or a change in the rate of active transport into the lumen or both. Additionally, the assumption that alterations in renal function affect both probes to the same extent is almost certainly wrong. Hallemeesch *et al.* [31] reported that increasing the rate of urine flow increases the rate of excretion of an intravenously administered lactulose load without affecting the rate of excretion of an intravenously administered load of rhamnose. Similar findings were reported recently by Oudemans-van Straaten *et al.* [32••], who showed that the urinary recovery of an enterally administered load of cellobiose, mannitol, or sucrose is highly correlated with urine output in critically ill patients. Additionally, the urinary recovery of cellobiose was strongly correlated with creatinine clearance, whereas the urinary recovery of mannitol was not. Thus, the clearance ratio for these two sugars was confounded by alterations in renal function.

Active, polarized secretion of some tracers, as described by Tomita *et al.* [30], might also confound interpretation of studies using Ussing chambers or sheets of Caco-2 or T84 cells growing on filters in diffusion chambers. Clearly, further investigation along these lines is warranted.

Oxidant stress

Because partially reduced derivatives of molecular oxygen, including superoxide radical anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$), have been implicated as important mediators of inflammation [33], it is noteworthy that oxidant stress increases the permeability of intestinal epithelial monolayers [34–40]. Oxidant stress causes lipid peroxidation in epithelial cells and disturbs cellular homeostasis by inhibiting the proper functioning of important cellular processes, such as sodium–hydrogen ion exchange [41,42]. Oxidant stress also perturbs cellular mitochondrial function and depletes intracellular levels of ATP [43–45]. A particularly critical pathway leading to oxidant-induced epithelial barrier dysfunction, however, may be activation of various isoforms of the enzyme protein kinase C (PKC). Intestinal epithelial cells express at least six different PKC isoforms: PKC-α, PKC-β1, PKC-β2, PKC-δ, PKC-ϵ, and PKC-ζ [34,46,47]. In recent studies, activation of PKC-δ has been implicated as being essential for the development of hyperpermeability in Caco-2 monolayers because cells expressing a dominant negative form of the enzyme are completely protected from barrier disruption induced by incubation with H_2O_2 [35]. Interestingly, epidermal growth factor, which can protect epithelial monolayers from oxidant-induced hyperpermeability, appears to provide protection by activating other PKC isoforms, including PKC-α, PKC-β1, and PKC-ζ [34,47,48]. In addition to being important in oxidant-mediated damage to the intestinal epithelial barrier, signaling via the PKC pathway also has been implicated as being an important factor contributing to intestinal epithelial hyperpermeability induced by other factors, such as *Clostridium difficile* toxin A [49]. The various mechanisms whereby activation of PKC modulates intestinal epithelial permeability remain to be elucidated, but one pathway likely involves phosphorylation of the enzyme myosin light chain kinase [50].

Hypoxia

In hemorrhagic shock, intestinal blood flow is reduced [51], and the intestinal villi are damaged [52,53]. Both mucosal hyperpermeability to macromolecules and bacterial translocation to extraintestinal sites are observed in animals subjected to hemorrhage [15,19,20•,54–56,57•]. However, providing an extravascular source of oxygen to enterocytes (by perfusing the lumen of the gut with oxygenated crystalloid or perfluorocarbon solutions) ameliorates mucosal injury induced by ischemia and maintains mucosal barrier function [58–60]. *In vitro*, exposing Caco-2_{BBe} intestinal epithelial monolayers to hypoxic conditions for prolonged periods depletes cellular ATP levels and increases paracellular permeability to macromolecules [61]. Collectively, these data support the view that cellular hypoxia is a key factor in the pathogenesis of the mucosal hyperpermeability.

The effects of cellular hypoxia on cellular functions are complex. Of course aerobic metabolism is impaired under hypoxic conditions, and ATP levels can be depleted on this basis. However, other factors are probably also important. For example, reducing equivalents (*eg*, ubiquisemiquinone) accumulate in hypoxic cells. These moieties are then available to donate electrons to molecular oxygen, promoting the formation of partially reduced species, such as $O_2^{-}\cdot$ and other reactive oxygen metabolites [62]. The reductive stress caused by hypoxia has been shown to be more deleterious to cells than complete anoxia [63]. Hypoxia also is associated with

high intracellular concentrations of the reduced forms of nicotinamide adenine dinucleotides (reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate). These entities can serve as reducing equivalents that are necessary for enzyme-mediated release of soluble iron from intracellular iron stores (ferritin) [64]. Free iron can participate with $O_2^{-}\cdot$ and H_2O_2 in reactions, producing the highly reactive species OH· [65,66]. Free iron also can promote lipid peroxidation by reactive oxygen metabolites [67]. Thus, by promoting iron delocalization, hypoxia fosters formation of oxidants and lipid peroxidation within cells [67].

Pharmacologic agents that block mitochondrial or glycolytic metabolism have been used extensively to deplete cells of ATP, a technique referred to as *chemical hypoxia*. Although clearly only a laboratory model system with limited relevance to the clinical setting, the induction of chemical hypoxia has provided much useful information about the effects of energy deprivation on cellular physiology. Acute and profound ATP depletion to approximately 10% of normal levels has been shown to impair the structure and function of tight junctions in cultured epithelial monolayers [68,69]. Even relatively minor reductions in ATP content to approximately 70% of normal levels, if maintained for a prolonged period (12–72 hours), are sufficient to increase the permeability of cultured intestinal epithelial monolayers [61].

Derangements in barrier function–induced metabolic inhibition have been linked to alterations in the cytoskeleton and particularly to changes in the stability of microfilaments [70,71]. Indeed, in many systems, ATP depletion has been associated with cytoskeletal destabilization [61,72,73]. This observation is not surprising because the normal cycling between polymerized filamentous (F) actin and monomeric (G) actin is known to be an ATP-dependent phenomenon [74].

Impaired synthesis of ATP is not the only way that hypoxia can affect intestinal epithelial permeability. Under hypoxic conditions, enterocytes stimulated with the proinflammatory cytokine interferon (IFN) γ secrete increased amounts of another proinflammatory cytokine, tumor necrosis factor (TNF) [75]. The combined effects of the two cytokines lead to a greater increase in permeability than that which is observed after incubation of the cells with IFN-γ alone [75]. Perhaps as part of a counterregulatory adaptive response, hypoxic enterocytes upregulate the expression of intestinal trefoil factor, a small protease-resistant peptide that promotes intestinal barrier function [76].

Nitric oxide

Derived from the amino acid L-arginine, NO· is a pluripotent signaling and effector molecule [77]. NO· is synthesized by a wide variety of cell types in a reaction catalyzed by the enzyme nitric oxide synthase (NOS). Three isoforms of NOS have been identified: neuronal NOS (NOS-I), endothelial NOS (NOS-III), and inducible NOS (NOS-II) [78]. Whereas neuronal NOS and endothelial NOS tend to be expressed constitutively, inducible NOS expression generally requires induction under the influence of various cytokines and/or lipopolysaccharide [77,78].

The effects of NO· on intestinal permeability are complex. Physiologic levels of NO· are essential to maintain mucosal integrity under normal conditions [79]. Furthermore, low concentrations of NO· can be salutary under pathologic conditions as well. Evidence in support of this view comes from studies showing that using an isoform-nonselective NOS inhibitor to block all or most endogenous NO· synthesis tends to exacerbate gastrointestinal mucosal injury caused by mesenteric ischemia and reperfusion [80], endotoxemia [81], or systemic injection of platelet-activating factor [82] or endothelin 1 [83]. By the same token, supplementing endogenous NO· production by administering a NO· donor ameliorates gut mucosal injury in a number of similar models [84,85]. Proposed mechanisms of the beneficial effects of NO· include prevention of leukocyte adhesion to vascular endothelial cells [86] and inhibition of the release of chemical mediators (*eg*, histamine and platelet-activating factor) from mast cells [87].

However, NO· also can be toxic to the intestinal epithelium and interfere with normal barrier function. Various NO· donors, such as sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine, decrease the viability of freshly harvested rat colonic epithelial cells [88] or gastric mucosal cells [89]. Furthermore, exposure to NO· donors or authentic NO· gas decreases cellular ATP levels, promotes derangements in the actin-based cytoskeleton, and increases the permeability of Caco-2 human intestinal epithelial monolayers [22,90].

The mechanisms responsible for the cytopathic effects of NO· on the gastrointestinal epithelium are incompletely understood. It is likely, however, that NO· *per se* is not the toxic moiety. Rather, it is more likely that peroxynitrite anion ($ONOO^{-}$) or its protonated form, peroxynitrous acid (ONOOH), is the principle species responsible for causing ATP depletion, cytoskeletal derangements, and hyperpermeability. $ONOO^{-}$, a weak acid, is formed when NO· reacts with superoxide radical anion $O_2^{-}\cdot$ [91,92]. Many of the toxic effects of NO· are thought be mediated by $ONOO^{-}$ [92–97]. In many respects, the chemistry of the protonated form ONOOH is similar to that of hydroxyl radical (OH·) [92]. Although theoretically ONOOH could spontaneously decompose to form $NO_2\cdot$ plus OH·, it is more likely that a vibrationally activated intermediate, sometimes represented

as ONOOH*, is responsible for the hydroxyl radical–like characteristics of ONOOH [92].

Several observations support the notion that $ONOO^-$/ONOOH is the critical species responsible for the toxic effects of NO·. First, the deleterious effects of various NO· donors on intestinal epithelial monolayers are exacerbated by lowering the pH of the intra- or extracellular environment, conditions that favor protonation of $ONOO^-$ to form ONOOH [98,99]. Second, scavengers of $ONOO^-$ (deferoxamine) or ONOOH (ascorbate) ameliorate the deleterious effects of NO· donors on epithelial permeability [99]. Third, tiron, an O_2^-· scavenger, ameliorates the deleterious effects of NO· donors on epithelial permeability [100]. In contrast, diethyldithiocarbamate, which inhibits of superoxide dismutase and thereby increases the availability of O_2^-·, promotes the development of hyperpermeability when Caco-2 monolayers are exposed to an NO· donor [100].

Recently, Sugi *et al.* [101••] carried out an elegant series of experiments that may explain why NO·/$ONOO^-$/ONOOH increases intestinal epithelial permeability. These authors showed that incubation of T84 cells with an NO· donor results in rapid downregulation of sodium–potassium–adenosine triphosphatase activity. Furthermore, they showed that inhibiting sodium–potassium–adenosine triphosphatase activity leads to cell swelling and hyperpermeability of monolayers. However, if cell swelling is prevented (by incubating the cells in a buffer containing a low concentration of sodium ion), then the development of hyperpermeability is prevented. These data support the notion that inhibition of the sodium–potassium–adenosine triphosphatase may be a final common pathway leading to altered barrier function caused by a variety of proinflammatory stimuli.

The notion that NO· is a mediator of gut mucosal hyperpermeability secondary to inflammation is also supported by data obtained from *in vivo* studies. Unno *et al.* [102] showed that intestinal hyperpermeability and bacterial translocation are both ameliorated when endotoxemic rats are treated with either aminoguanidine or methylthioureasulfate, two chemically dissimilar isoform-selective inducible NOS inhibitors. Similar findings were obtained by other investigators [103,104]. Subsequent studies using inducible NOS knockout mice have confirmed that increased inducible NOS expression is necessary for the development of gut mucosal barrier dysfunction after injection of lipopolysaccharide [105].

Cytokines

The lamina propria and the mucosa itself contain large numbers of immune cells, including T and B lymphocytes, plasma cells, macrophages, mast cell eosinophils, and neutrophils. In inflammatory states, these cells can release a wide array of cytokines, including interleukin (IL) 1, IL-4, IL-6, IL-10, IFN-γ, and TNF [106]. Several cytokines, including IL-4, IL-13, IFN-γ, and TNF, have been reported to increase the permeability of T84 [107–111] or Caco-2 [112] intestinal epithelial monolayers. In contrast, the antiinflammatory cytokine IL-10 has been shown to ameliorate IFN-γ– or TNF-induced gut epithelial hyperpermeability [109]. Secretion of IFN-γ by intraepithelial lymphocytes was recently shown to be a major factor underlying the loss of intestinal epithelial barrier function in a murine model of total parenteral nutrition [113].

The mechanisms responsible for cytokine-induced epithelial hyperpermeability are yet to be completely elucidated. However, it is known that IFN-γ induces expression of inducible NOS mRNA in Caco-2 cells [112,114], particularly when it is combined with IL-1β and TNF, a cytokine cocktail commonly referred to as *cytomix* [114]. Thus, it is plausible to hypothesize that cytokine-induced intestinal epithelial hyperpermeability is mediated, at least in part, through the induction of inducible NOS mRNA, which leads to increased NO· production and perturbed barrier function on that basis. Indeed, IFN-γ–induced hyperpermeability in Caco-2 human intestinal epithelial monolayers is ameliorated by NOS inhibitors, such as N^G-nitro-L-arginine-methyl ester and N^G-monomethyl-L-arginine [112]. Moreover, IFN-γ–induced epithelial hyperpermeability is potentiated under acidic conditions [115] and is ameliorated by scavengers of either NO· or ONOOH [114]. These results suggest that NO· produced by epithelial cells after stimulation by IFN-γ or cytomix may react with O_2^-· to form $ONOO^-$, ultimately leading to cytopathic effects, including barrier dysfunction, as a result of ONOOH-mediated toxicity. Upregulation of inducible NOS expression and increased NO· or $ONOO^-$ production also have been implicated as factors contributing to increased permeability of Caco-2 monolayers after incubation with H_2O_2 [36].

The mechanisms responsible for the increase in epithelial permeability that is induced by a relatively high concentration of cytomix and/or NO· or $ONOO^-$ are not completely understood. As noted previously, however, the formation of tight junctions depends on proper expression and localization of key proteins, such as ZO-1, occludin, and various members of the claudin family. It is noteworthy, therefore, that Han *et al.* [90] recently reported that exposure of Caco-2 monolayers to either cytomix or an NO· donor leads to decreased expression of both ZO-1 and occludin. In these studies, expression of claudin 1 was shown to increase, but proper targeting of this protein to tight junctions was found to be impaired [90]. Complementary findings have been reported by other investigators. For example, Youakim and Ahdieh [116] reported that incubating T84 cells with IFN-γ resulted in dramatically decreased expression of ZO-1,

whereas expression of ZO-2 and occludin was only minimally decreased. These authors also showed that ZO-1 mRNA levels were decreased after incubation of T84 cells with IFN-γ. In another study, Cuzzocrea *et al.* [117] exposed Madin-Darby canine kidney epithelial cells to zymosan, a proinflammatory microbial product, to induce inducible NOS expression and showed that NO· production was associated with decreased expression of occludin.

In addition to IFN-γ, IL-1β, and TNF, two other cytokines warrant discussion as potential mediators of intestinal epithelial barrier dysfunction in critical illness. These cytokines are IL-6 and high-mobility group B1 (HMGB1). IL-6 is a 26-kd pluripotent cytokine that is produced by many different cell types, including activated monocytes and macrophages [118], endothelial cells [119], adipocytes [120], T cells [121], and enterocytes [122]. The biologic effects of IL-6 are quite diverse and range from stimulation of hepatocyte proliferation to suppression of the pituitary–thyroid axis [123]. With regard to the innate immune response, IL-6 has been shown to have both proinflammatory [124–127] and antiinflammatory effects [128,129], depending at least in part on whether the cytokine is acting in a paracrine or endocrine manner [130].

Although there is no evidence that IL-6 is directly toxic to enterocytes, several studies suggest that IL-6 is a mediator of intestinal mucosal injury in various conditions associated with transient mesenteric hypoperfusion and subsequent inflammation. For example, Cuzzocrea *et al.* [131] reported that gut mucosal inflammation and histologic damage was markedly attenuated in IL-$6^{-/-}$ compared with IL-$6^{+/+}$ mice subjected to splanchnic artery occlusion and reperfusion. In another study, the same laboratory showed that intestinal inflammation induced by intraperitoneal administration of zymosan is decreased in IL-$6^{-/-}$ compared with IL-$6^{+/+}$ mice [132]. More recently, Wang *et al.* [133•] compared changes in intestinal permeability to FD4 in wild-type and IL-6 knockout mice 16 hours after the induction of sepsis by cecal ligation and perforation. Whereas sepsis was associated with a marked increase in mucosal permeability in IL-$6^{+/+}$ mice, intestinal permeability was essentially unchanged after the induction of sepsis in IL-$6^{-/-}$ mice. In another study, blocking the IL-6–dependent signaling pathway by administering a neutralizing antibody against the IL-6 receptor decreased mucosal inflammation in several murine models of inflammatory bowel disease [134]. Recently, our laboratory showed that IL-6 is essential for the development of gut barrier dysfunction after hemorrhagic shock and resuscitation in mice (Yang *et al.*, Unpublished data, 2003).

High-mobility group B1 was first described as a nonhistone nuclear protein with high electrophoretic mobility [135]. A characteristic feature of the protein is the presence of two folded DNA-binding motifs that are termed the A domain and the B domain [136]. Both domains contain a characteristic grouping of aromatic and basic amino acids within a block of 75 residues termed the HMG-1 box [137]. HMGB1 has several functions within the nucleus, including facilitating DNA repair [138] and supporting the transcriptional regulation of genes [139]. Recently, extracellular HMGB1 was identified as a late-acting, cytokine-like mediator of lipopolysaccharide-induced lethality [140]. High circulating concentrations of HMGB1 can be detected in mice 16 to 32 hours after the onset of endotoxemia [140], and delayed passive immunization of mice with antibodies against HMGB1 confers significant protection against lipopolysaccharide-induced mortality [140]. Administration of highly purified recombinant HMGB1 to mice is lethal [140], and direct application of HMGB1 into the airways of mice initiates an acute inflammatory response and lung injury [141]. Exposure of macrophage cultures to low concentrations of HMGB1 induces the release of TNF and other proinflammatory cytokines [142].

Recently, Sappington *et al.* [143••] reported that both HMGB1 and the B box portion of the molecule increase the permeability of Caco-2 monolayers. HMGB1 B box also was shown to promote gut mucosal hyperpermeability and bacterial translocation *in vivo*. Evidence was presented in support of the view that all of these effects are dependent on the induction of inducible NOS expression and increased production of NO·.

Conclusions

Alterations in intestinal mucosal barrier function are likely an important component of MODS. In addition, studies of the way in which inflammation promotes intestinal epithelial hyperpermeability may serve as a useful paradigm for gaining insights into the more general problem of epithelial dysfunction in multiple organs resulting from sepsis or related conditions. Proinflammatory cytokines, oxidants, and reactive nitrogen–centered moieties seem to be important mediators of gut barrier dysfunction in critical illness.

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