Determinants of intestinal barrier failure in critical illness

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The gut serves not only as a physiological portal for the entry of water and nutrients into the body, but also as a barrier limiting the systemic absorption of intraluminal microbes, microbial products, or both. Evidence exists to support the view that trauma, sepsis or other critical illnesses lead to derangements in the barrier function of the gut. One potential consequence of deranged barrier function is bloodstream invasion by gut-derived pathogens, leading to “primary” bacteraemias or fungaemias, or even metastatic infections.

Another consequence of gut barrier dysfunction might be a several step process leading to a poorly controlled systemic inflammatory response and organ system failure. In the first step of this postulated pathophysiological process, microbes or toxins passively diffuse or are actively transported out of the lumen of the gut into submucosal tissues. In step two, these microbes or toxins activate immune cells in tissues or organs, which are “downstream” from the mucosa (e.g. Kupffer cells in the liver; macrophages in the lamina propria of the gut, Peyer’s patches or mesenteric lymph nodes). In the third step, the activated immune cells release various inflammatory mediators (cytokines, nitric oxide, eicosanoids, platelet activating factor), which have been implicated as being important in the pathogenesis of multiple organ dysfunction. The hypothesis that the gut is an endogenous source of pro-inflammatory agents is attractive because it might explain why signs and symptoms of sepsis, such as fever, leucocytosis, hypermetabolism and organ system dysfunction, frequently are present in critically ill patients, even in the absence of a well-defined focus of infection [42, 91, 92].

Components of the intestinal barrier

Intestinal barrier function depends upon a stratified system of defences. These defences include the normal microbiological flora of the gut, enteric secretions, peristalsis, gut-associated immune cells and the epithelium itself.

Under normal conditions, anaerobic organisms, growing as a carpet on the mucosal surfaces of the gut, limit colonization and overgrowth of other potentially invasive microbes [148]. However, in critically ill patients, possibly because of the administration of broad-spectrum antibiotics, the gut commonly becomes colonized by potentially pathogenic facultative Gram-negative bacilli, Gram-positive cocci and fungi [92, 93]. These organisms are believed to express specialized virulence factors enabling mucosal adherence and penetration [136].

Mucins, secreted by epithelial goblet cells, create a protective viscous gel that hampers bacterial penetration [148]. In addition, active Cl− transport by epithelial cells promotes intraluminal fluid flux that washes away harmful agents [94]. Uprogulation of mucosal Cl− secretion occurs rapidly after ischaemic [95] or toxic [94] insults, or both, through a host of autocrine, paracrine and, perhaps, hormonal mechanisms. Additionally, mucosal secretions are rich in IgA antibodies that bind and aggregate bacteria [3, 4, 136] and thereby aid in preventing adherence to the mucosa and subsequent trans-epithelial invasion.

As the largest immunological organ of the body, the intestine contains numerous immunoreactive cells, including various subtypes of B- and T-lymphocytes, plasma cells, macrophages, neutrophils, Paneth cells and specialized “M cells” [8, 65]. Together these cells are thought to help prevent systemic infection by lumenerally derived invasive microbes. The gut associated lymphoid tissue (GALT) consists of three distinct populations: Peyer’s patches, immunocytes within the lamina propria, and interepithelial lymphocytes (IEL).

Found throughout the length of the small intestine, Peyer’s patches are mucosal and submucosal lymphoid follicles that are the site of B-cell differentiation and immunoglobulin (IgA) class commitment [8, 65]. A proportion of these B-lymphocytes eventually migrate to the lamina propria, where they are critical in producing IgA that is secreted transcellularly by the epithelial cells. Antigen priming in intestinal lymphoid follicles produces selective humoral responses by stimulating clonal B-cell growth and migration to local as well as systemic sites. This function is facilitated by specialized epithelial cells, termed “M cells” [144], which selectively expose underlying lymphoid aggregates to luminal antigens thereby allowing continuous priming of lymphocytic defences.

IEL and lamina propria lymphocytes are predominately T-cells [8, 21]. These two populations
differ in phenotype and purpose. IEL predominantly express the CD8 cell surface marker and display cytolytic activity. As the first T-cells exposed to luminal antigens, their defensive role against microbial invasion is an active one. Exposure to infiltrating pathogens triggers both direct cytolytic activity as well as the release of cytokines that stimulate antigen-specific immune responses (IL-5) and epithelial growth (TGF-β). In contrast with IELs, lamina propria T-cells primarily express CD4 antigen and act as facilitators of inflammation and epithelial defences [8]. Activated by specific antigenic recognition, these cells turn on a cascade of protective reactions via cytokines.

The most critical barrier against systemic absorption of intraluminal microbes and microbial products is the epithelium per se. Structurally, the intestine is comprised of a single layered columnar epithelium arranged into villus and crypt components. Specialized cell–cell junctional complexes allow for selective paracellular permeability (tight junctions) [83], maintain intercellular adhesion (intermediate junctions and desmosomes) [131] and permit intercellular communication (gap junctions) [166]. The zonula occludens is a circumferential band of apical tight junctions that limits paracellular passage of ions and fluid. Under normal circumstances, tight junctions exclude passive movement of hydrophilic non-charged compounds with a molecular radius >11.5 Å [83]. Substances that are therefore prevented from paracellular transepithelial movement include lipopolysaccharide (LPS) [149] and a variety of other bacterial-derived proinflammatory substances, such as formyl-methionyl-leucyl-phenylalanine (FMLP) [22] and peptidoglycan-polysaccharides [77], which are present in high concentration within the lumen of the distal small intestine and colon.

Assessment of intestinal mucosal barrier function

In clinical and experimental studies, the integrity of the gut mucosal barrier has been assessed using two very different approaches. One method estimates the degree of transmucosal movement of bacterial or fungal pathogens from the intestinal lumen into the vascular or lymphatic systems, a process referred to as microbial translocation. The second method quantitates the permeability of the gut to various water-soluble probes.

Microbial translocation does not necessarily imply the loss of epithelial integrity. Under some conditions, translocation in experimental animals subjected to a major stress (e.g. haemorrhage or endotoxaemia) appears to occur via tiny breaks in the continuity of the mucosal epithelial sheet [28, 31]. However, for the most part, translocation is a transcellular rather than a paracellular process [2, 155, 158, 159]. Moreover, in many experimental studies, the extent of translocation is quantitated by enumerating viable colony-forming units (CFU) in mesenteric lymph nodes (MLN) or other organs. However, data using radioactively labelled luminal bacteria indicate that this approach vastly under-estimates the extent of translocation, because most microbes breaches the epithelial barrier are killed [40]. Increases in "translocation" evidenced by increases in CFU in MLN or other organs are predominantly the result of decreased killing rather than increased transepithelial penetration.

Normally, the healthy intestinal mucosa manifests a very low basal level of permeability to large and middle-sized hydrophilic molecules. Systemic uptake of luminally placed water-soluble non-charged compounds such as mono- and disaccharides (e.g. rhamnose and lactulose), radiolabelled organic acid-transition metal complexes (e.g. chromium ethylenediaminetetra-acetate [51Cr-EDTA]), synthetic polymers (polyethylene glycol) or radio-labelled proteins (albumin) can be used to quantitate the rate of transepithelial flux. In such studies, two probes are commonly employed. One probe (e.g. mannitol) typically consists of a relatively small molecule which permeates moderately well through even normal mucosa. A second probe (e.g. lactulose) consists of a larger molecule, which permeates the normal mucosa only minimally. Probes which are non-metabolisable are chosen, and absorption of the probes is determined by measuring concentrations in plasma or urine. By measuring the differential concentrations of two probes, it is possible to remove confounding effects related to changes in intestinal motility (or renal function). Because the zonula occludens restricts intercellular passage of these probes in the luminal as well as vascular direction, permeability can also be assessed in animal models by measuring luminal concentrations of these probes after intravascular administration [35, 59].

Evidence for and against intestinal barrier dysfunction in critically ill humans

Convincing evidence of microbial translocation has been documented in humans under certain conditions. Deitch showed that viable enteric organisms frequently can be isolated from cultures of mesenteric lymph nodes obtained at laparotomy from patients with small bowel obstruction [26]. Similar results were obtained by Ambrose and colleagues in a study of patients undergoing abdominal operations for Crohn’s disease [5]. van Goor and colleagues isolated bacteria in mesenteric lymph nodes from 11 of 21 brain-dead organ donors with anatomically intact gastrointestinal tracts [150]. Sedman and colleagues found evidence of translocation to mesenteric lymph nodes in 10.3% of 267 patients undergoing general surgical procedures [132]. Braithwaite and colleagues used immunofluorescent staining of Escherichia coli-derived β-galactosidase (rather than standard microbiological methods) to document the presence of bacteria in macrophages in mesenteric lymph node specimens from every one of 20 trauma patients [13]. Unfortunately, in this study, samples from normal controls were not obtained, and, therefore, it is unclear whether this represents a normal or a pathological occurrence. Reed and colleagues used both standard microbiological methods and electron microscopy to demonstrate bacteria in mesenteric lymph nodes
from trauma victims [120]. As in the study by Braithwaite and colleagues already cited, samples from normal individuals were not studied. Certainly the most dramatic evidence that translocation can occur in humans comes in the form of a report by Krause, Matheis and Wulf, who documented that oral ingestion of a suspension of viable C. albicans resulted in transient fungaemia and funguria in a normal volunteer [70]. None of these studies, however, has demonstrated a correlation between the presence of viable (or non-viable) organisms in mesenteric nodes (or blood) and the development of multiple organ dysfunction syndrome (MODS).

Rush and colleagues showed a high incidence of bacteraemia and endotoxaemia in blood samples obtained from patients with haemorrhagic shock [127]. Since the samples were obtained within minutes after presentation to the emergency department, the development of bacteraemia in these patients presumably was not due to hospital-acquired infection. Interestingly, most of the isolates in this study were Gram-positive, rather than the enteric Gram-negative bacilli, which are most commonly isolated in studies of bacterial translocation using animal models of haemorrhagic shock. Thus, the implications of the findings obtained by Rush’s group remain uncertain.

Winchurch, Thupari and Munster have shown that endotoxin is detectable in plasma samples obtained within 24 h after the time of injury from victims of thermal trauma [165]. Moreover, these workers have shown that the degree of endotoxaemia correlates with the magnitude of the injury. In a recent report from this group, however, it was shown that prophylactic treatment with an endotoxin-neutralizing antibiotic (polymyxin B) could lower the concentrations of circulating endotoxin detected in burns patients, but that this treatment effect was not associated with a lessening in mortality or with the concentrations of a cytokine (IL-6) in plasma [104]. Thus, Winchurch, Thupari and Munster concluded that the presence of endotoxaemia in burns patients may be an epiphenomenon rather than a primary factor leading to organ dysfunction.

Two other recent studies of trauma victims failed to validate the notion that bacterial translocation is increased in one important subpopulation of critically ill patients, namely those with multiple trauma. Moore and colleagues [102] were unable to detect elevated endotoxin concentrations in portal venous or systemic blood samples obtained over the first 5 days of hospitalization from 20 victims of major abdominal trauma, even though 60 % of the patients were in shock at the time of presentation and six of the subjects developed MODS. Although nine (2 %) of 424 systemic and portal venous blood cultures were positive over the 5-day period of observation, many of the isolates were probable contaminants. Cultures of ileocolic lymph nodes were positive in four of 12 cases, although in one case the isolated organism (S. epidermidis) was probably a contaminant.

Peitzman and coworkers [115] obtained biopsies of mesenteric lymph nodes at the time of coeliotomy from 25 trauma patients. Although 40 % of the trauma patients had one or more major complications, including adult respiratory distress syndrome (ARDS), serious pulmonary infections, and Gram-negative bacteraemia, all of the lymph node cultures were sterile. As a control for technique, the authors of this study also cultured lymph nodes obtained from patients with primary gastrointestinal tract diseases, and in three of four cases, were able to isolate enteric organisms. Thus, these two studies must be viewed as casting doubt on the idea that translocation of gut-derived bacteria or endotoxin plays a crucial role in the development of serious complications, at least in victims of major trauma.

During the past several years, a number of studies have documented the presence of gut mucosal hyperpermeability in human volunteers challenged with tiny doses of endotoxin [109], or patients with a variety of critical illnesses [27, 51, 110, 124, 125, 128, 170]. However, just as is the case for translocation, data are lacking to show that the presence of hyperpermeability is associated with or predicts the development of MODS.

Despite the “negative” studies just cited, the notion that the gut is somehow a pivotal organ in critical illness remains viable because of other circumstantial evidence from clinical studies. Certainly, it is well established that septic complications are diminished by providing early enteral nutrition to trauma patients [74, 101]. In addition, colonization of the proximal gastrointestinal tract with certain pathogens, particularly Pseudomonas aeruginosa and coagulase-negative Staphylococcus, is associated with subsequent development of intensive care unit-acquired infections caused by these same organisms [92, 93]. Thus, the role of the gut as a “motor of multiple organ failure” remains a controversial but tenable hypothesis.

**Pathophysiological mechanisms responsible for gut barrier failure**

The pathophysiological mechanisms responsible for gut barrier dysfunction in critically ill humans remain to be elucidated. Given the complex nature of the gut barrier, however, it seems probable that no single mechanism can explain all aspects of barrier dysfunction or all cases of barrier dysfunction precipitated by different causes.

**MUCOSAL HYPOXIA**

In general, the rate of oxygen consumption ($V_{O_2}$) by tissues is determined by the metabolic demand and not by oxygen delivery ($D_{O_2}$); this is a statement of Pflüger’s law. Certain pathophysiological processes can, however, reduce $D_{O_2}$ below a critical value ($D_{O_2\text{crit}}$) such that $V_{O_2}$ becomes supply-dependent [57]. Supply-dependency of $V_{O_2}$ can occur as a result of tissue hypoperfusion, arterial hypoxaemia or anaemia. Hypermetabolism, secondary to sepsis and other critical illnesses, necessitates higher $V_{O_2}$ and thereby a higher $D_{O_2\text{crit}}$ [52]. Alone or in combination, these factors may limit intracellular $D_{O_2}$ to levels inadequate to support normal mitochondrial
respiration. There are two important effects of supply-dependent mitochondrial respiration. First, glycolytic metabolic rate is increased in an effort to preserve adequate intracellular concentrations of adenosine triphosphate (ATP) by substrate level phosphorylation of adenosine diphosphate (ADP). In contrast with oxidative phosphorylation, substrate level phosphorylation is associated with the production of protons; i.e. enhanced glycolytic rate leads to intracellular acidosis [100]. Second, if mitochondrial respiration is sufficiently limited, then intracellular concentrations of ATP are depleted. As will be discussed below, both intracellular acidosis and ATP depletion can lead to increases in intestinal epithelial permeability.

In the clinical setting, the most likely cause of intestinal mucosal hypoxia is mesenteric hypoperfusion. The architecture of the microcirculation of the intestinal villi may act to increase the susceptibility of the epithelium to hypoxic injury because of inadequate perfusion. Each villus is supplied by a single central arteriole, which is surrounded by an arbor of draining venules. Probably as a result of both counter current arteriovenous diffusion and shunting of oxygen as well as consumption of oxygen by cells along the length of the villus, the tissue PO₂ at the tips of villi is low even under normal conditions. During low flow states, these conditions are exacerbated, further increasing the likelihood of supply-dependency of oxygen uptake [81, 82, 133].

Another factor which may predispose the villous epithelium to ischaemic injury during low-flow states is “plasma skimming” [67]. This phenomenon occurs because the nutrient arterioles supplying each villus originate at right angles to the parent artery. Since erythrocytes tend to cluster away from the walls of arteries, this architecture leads to relative haemodilution (and reduced oxygen carrying capacity) in the blood perfusing villous arterioles.

Two common conditions, which are likely to induce marked mesenteric ischaemia in critically ill patients, are haemorrhage and cardiogenic shock [17, 97, 98, 122]. Studies using animal models have documented that global decreases in cardiac output because of haemorrhage or cardiac tamponade lead to activation of the renin-angiotensin axis, which, in turn, triggers a dramatic increase in mesenteric arteriolar resistance [17, 97, 122]. Splanchnic hypoperfusion also has been reported in animal models after thermal injury [61, 103].

Sepsis is another common condition, which may be associated with mesenteric hypoperfusion. In contradistinction to haemorrhage or cardiogenic shock, however, the effects of sepsis on mesenteric (or mucosal) perfusion and oxygenation are poorly understood. Total hepatosplanchnic perfusion is elevated in humans with compensated sepsis [25, 48, 163]. Data are lacking regarding the effects of septic shock in humans on mesenteric and, particularly, intestinal mucosal perfusion.

We [36, 37, 130] and others [6, 119, 138, 161, 167] have documented that mesenteric perfusion is compromised in experimental models of sepsis and septic shock. Interestingly, however, we have never been able to document a decrease in transmesenteric VO₂ in endotoxaemic pigs [36, 37, 130]. Other investigators have reported similar findings [7, 119, 151]. In other words, transmesenteric oxygen extraction seems to be increased sufficiently in sepsis to maintain normal (or even elevated) levels of VO₂. Nevertheless, ileal mucosal acidosis is a characteristic feature of sepsis in experimental animals [36, 37, 119, 130]. Gastric mucosal acidosis occurs commonly in septic patients [49, 88, 135].

The development of gastrointestinal mucosal acidosis in sepsis, whether in animals models or patients, has been interpreted as evidence of gut epithelial hypoxia. However, normalization of superior mesenteric arterial blood flow ameliorates but does not completely normalize endotoxin-induced mucosal acidosis in pigs [36], a finding which suggests that mechanisms other than, or in addition to, mucosal ischaemia might contribute to tissue acidosis in sepsis. To further investigate this issue, we recently conducted an experimental study using a resuscitated, normodynamic pig model of endotoxaemic shock [152]. Mucosal oxygenation was measured directly using an array of eight Clark-type tissue Po₂ microelectrodes. Mucosal perfusion and acidosis were measured by laser Doppler flowmetry and intraluminal tonometry, respectively. After infusion of endotoxin, pigs developed ileal mucosal acidosis despite the absence of any significant change in mucosal blood flow and a significant increase in mucosal oxygen tension (PmO₂). These observations are consistent with previous findings obtained by Hurtado and colleagues, who showed that lactic acidosis develops in skeletal muscle of septic rabbits despite normal tissue oxygenation [62]. Although an unexpected finding, the increase in PmO₂ induced by endotoxin can be explained on the basis of the Bohr effect (i.e. an increased unloading of oxygen from haemoglobin as a result of acidosis) [75].

Oxidant stress

Oxidants have been implicated in a variety of causes of intestinal barrier dysfunction. In experimental animals, intestinal ischaemia followed by reperfusion leads to mucosal hyperpermeability and biochemical evidence of oxidant stress [44, 45]. During the reperfusion phase, spin trapping techniques have been used to demonstrate the formation of free radicals [108]. There are two main sources of reactive oxygen metabolites (ROM) in intestinal ischaemia/reperfusion: (1) the reaction catalysed by xanthine oxidase and (2) the reaction catalysed by NADPH oxidase in neutrophils sequestered in the intestinal microvasculature. Agents which scavenge ROM or inhibit xanthine oxidase have been shown to ameliorate derangements in intestinal barrier function after intestinal ischaemia/reperfusion [112]. These strategies also have been shown to have salutary effects on barrier function in animals subjected to haemorrhage [28, 29] or systemic inflammation [30, 32, 85].

The mechanisms whereby oxidants impair barrier function are not well understood. In various in vitro models, however, oxidants, such as hydrogen per-
oxide ($\text{H}_2\text{O}_2$) or superoxide radical ($\text{O}_2^-\cdot$) have been shown to increase the permeability of epithelial [160] or endothelial [55] monolayers, and also disrupt the cytoskeleton (by promoting excessive actin polymerization) [55, 56]. Although most research related to alterations in epithelial (or endothelial) permeability and the actin-based cytoskeleton have focused on the paracellular pathway, it is plausible that cytoskeletal derangements also promote increased endo- and transcytosis. Sundqvist and Liu reported that endocytosis of FITC-dextran (70 000 Da) is promoted when cultured endothelial monolayers are incubated with $\text{H}_2\text{O}_2$, an oxidant known to cause cytoskeletal destabilization [141]. Bhalla and Crocker reported that exposure of tracheal epithelial cells to ozone, another potent oxidant, promotes vesicular transcytosis of a macromolecule (horseradish peroxidase) [9]. Thus oxidant-induced epithelial damage might promote barrier dysfunction via transcellular as well as paracellular pathways.

ROM can lead to ATP depletion [53, 54, 164]. As will be discussed below, ATP depletion is another potential factor contributing to epithelial barrier dysfunction. The mechanisms responsible for oxidant-mediated ATP depletion probably include inhibition of glyceraldehyde-3-phosphate dehydrogenase (in the glycolytic pathway) and inhibition of mitochondrial phosphorylation of ADP [64].

MUCOSAL ACIDOSIS

In an effort to maintain adequate energy levels, hypoxic cells increase their rate of anaerobic metabolism (i.e. glycolysis). In contradistinction to oxidative phosphorylation, substrate level phosphorylation of ADP during glycolysis leads to a net release of protons. Under anaerobic conditions, there is net formation of two moles of $\text{H}^+$ per mole of glucose metabolized (at physiological pH) [100]. Since anaerobic metabolism is less efficient than oxidative metabolism for generating high-energy compounds, hypoxia results in ATP depletion. In sepsis, tissue acidosis can occur even in the absence of hypoperfusion or hypoxia [60, 152]. Sepsis down-regulates the activity of pyruvate dehydrogenase (PDH), the enzyme complex catalysing the first irreversible step in the mitochondrial oxidative pathway [153]. The shift of PDH to its inactive form in sepsis fosters increased flux through the glycolytic pathway [38]. In cultured MDCK (renal epithelial) monolayers, exposure to A23187 increases permeability [116]. The effects of increased $[\text{Ca}^{2+}]$, on intestinal epithelial permeability have not been investigated.

ATP DEPLETION

As noted above, ATP depletion is a consequence of impaired mitochondrial function, the result of intracellular hypoxia. ATP depletion can also be caused by oxidant stress, leading to inhibition of glyceraldehyde-3-phosphate dehydrogenase (in the glycolytic pathway) and inhibition of mitochondrial phosphorylation of ADP [64]. Acidosis, in a form of positive feedback, can also promote ATP depletion.
we found that relatively minor reductions (permeability of Caco-2BBe monolayers [146]. In both scenarios, we found that relatively minor reductions (<30%) in ATP content were sufficient to increase the permeability of Caco-2BBe monolayers. The mechanism appears to involve derangements in the actin-based cytoskeleton, since laser confocal micrographs using rhodamine-phalloidin to stain polymerized (F) actin demonstrated perijunctional condensation of actin filaments after prolonged 2-DOG exposure.

Although it is well established that ATP depletion increases epithelial [71, 76, 87, 146] and endothelial permeability [157], the precise mechanism(s) underlying this phenomenon are not established. It is known that ATP depletion disrupts actin microfilaments [56, 71] by fostering the conversion of monomeric (G) actin to F-actin [56, 99]. As noted above, proper functioning of the actin-based cytoskeleton is essential for the maintenance of normal epithelial permeability characteristics. The tendency for actin to polymerize under conditions of ATP depletion [27, 28, 34] is paradoxical, since in vitro, the polymerization of G-actin to F-actin is an energy-requiring event, which depends upon the irreversible hydrolysis of ATP [35]. Only tiny quantities of G-actin are required for filament growth in vitro, thus, at equilibrium, the majority of actin in vitro is present in its polymerized form (36). In vivo, however, approximately half of the actin in cells is present in its monomeric form (36). No adequate explanation has been advanced to explain this paradox, but we hypothesize that ATP depletion may foster cytoskeletal derangements by interfering with normal intracellular calcium homeostasis.

NITRIC OXIDE

Produced by mammalian cells from the amino acid, l-arginine, the small molecule, nitric oxide (NO·), has been identified as an important regulator and/or effector of many phenomena in the cardiovascular, nervous, and immune systems [106]. Increasingly, it is apparent that NO· also plays a versatile role in the physiology and pathophysiology of the gastrointestinal tract [137]. The reaction responsible for the formation of NO· is catalysed by a family of enzymes called nitric oxide synthase (NOS) [68, 90]. There are three isoforms of this enzyme, including: neuronal or type I NOS found primarily in neuronal tissues and skeletal muscle; endothelial or type III NOS (eNOS), found primarily in endothelial cells; and inducible or type II NOS found in a wide variety of cell types, including macrophages and hepatocytes. Both nNOS and eNOS are constitutively expressed, and the production of NO· by these enzymes is dependent on the presence of calmodulin and intracellular concentrations of Ca2+. In contrast, iNOS contains calmodulin as a tightly bound subunit even under nominally Ca2+-free conditions, and the enzyme is expressed only after induction by various cytokines or LPS.

A large variety of cell types in the gut are potential sources of NO·. These cell types include myenteric neurons [16, 78, 107, 156], vascular endothelial cells [107, 117] and interstitial cells of Cajal [118]. Inflammatory cells in the submucosa, including mast cells [58], macrophages [43, 139], and polymorphonuclear leucocytes [47, 85], are capable of producing NO·. Luminal bacteria represent another potential source of NO· in the gastrointestinal tract [41]. Enterocytes also appear to be capable of producing NO·, at least under certain conditions. Boughton-Smith and colleagues reported that iNOS activity is elevated in biopsy specimens of inflamed colonic mucosa from patients with ulcerative colitis [10]. These investigators have also documented that administration of LPS to rats leads to the induction of iNOS activity in homogenates of jejunal or colonic tissue [11]. These results suggest that various inflammatory states (e.g. ulcerative colitis and acute endotoxicosis) are associated with induction of iNOS activity in gut epithelial cells, although the observed data also could be explained by iNOS induction in resident or infiltrating inflammatory cells in the mucosa or lamina propria. Tepperman and colleagues, however, have documented upregulation of iNOS activity in isolated enterocytes harvested from the colons of rats injected 4 h earlier with LPS [142]. Furthermore, these workers have shown that prior administration of an anti-neutrophil antiserum prevents LPS-induced leukosequestration in the colonic mucosa, but has no effect on measured concentrations of iNOS activity, supporting the view that infiltrating neutrophils are not the source of the iNOS activity in enterocytic preparations from endotoxic rats.

The idea that enterocytes can be induced to produce NO· is further supported by preliminary findings obtained by several laboratories. For example, Grisham has demonstrated that exposure of cultured IEC-18 (rat intestinal epithelial) cells to LPS, IL-1, or TNF-α plus IFN-γ, increases production of nitrite plus nitrate (markers of NO· production) [46]. Dignass, Podolsky and Rachmilewitz showed that incubation of cultures of another rat enterocytic cell line (IEC-6) with a number of different cytokines (e.g. IL-2, TNF-α) results in modest increases in the production of nitrogen oxides [133]. Recently, our group has shown that incubation of Caco-2BBe cells with the pro-inflammatory cytokine, IFN-γ, increases the release of markers of increased NO· production (nitrite and nitrate anions) into the media [147]. Even more recently, we have shown that incubation of Caco-2BBe cells with INF-γ upregulates expression of iNOS mRNA [unpublished observations]. NO· appears to be capable of modulating intestinal epithelial permeability. Interestingly, depending upon the experimental system employed, NO· can either diminish or increase intestinal permeability to various water-soluble molecules. The idea that NO· plays a beneficial role to preserve normal
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Recent studies performed using cultured T84 enterocytic cells suggest that certain proinflammatory cytokines, namely IFN-γ and IL-4, are capable of increasing the permeability of model epithelia [1, 24, 84]. The mechanism(s) whereby cytokines, such as IFN-γ, induce intestinal epithelial hyperpermeability have not been determined. It is known, however, that IFN-γ, acting either alone or in combination with other cytokines, is capable of triggering increased production of NO by a number of different cell types [69, 89, 154, 162]. Thus, it seems plausible that cytokine-induced intestinal epithelial hyperpermeability is mediated through induction of iNOS leading excess NO production. In support of this view are recent data published by our group, showing that: (1) incubation of Caco-2 cells (enterocytic) cells with IFN-γ increases the release of NO oxidation products (nitrite and nitrate) into culture supernatants; (2) Caco-2 monolayers can be rendered hyperpermeable by incubation in media containing IFN-γ; and (3) coinubation of Caco-2 monolayers with IFN-γ and various NO inhibitors significantly blunts the development of hyperpermeability [147].

Conclusions

Although attractive on theoretical grounds, the notion that gut barrier dysfunction contributes to morbidity, including MODS, in critically ill patients remains unproved. Despite the presence of negative data, however, this concept remains a fruitful line of investigation both at the bedside and in the laboratory. At the very least, learning about the mechanisms underlying gut barrier failure in critical illness will provide insights into the mechanisms of cellular dysfunction of all kinds associated with sepsis or other causes of diffuse inflammation. At best, this avenue of investigation will lead to improvements in management and a reduction in morbidity and mortality in victims of trauma, sepsis or other acute life-threatening illnesses.

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