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Review Article

THE EPITHELIUM AS A TARGET IN SEPSIS

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ABSTRACT—Organ dysfunction induced by sepsis has been consistently associated with worse outcome and death. Regardless of the organ compromised, epithelial dysfunction is present throughout the body, affecting those organs that contain epithelia like the skin, lungs, liver, gut, and kidneys. Despite their obvious differences, sepsis seems to alter common features of all epithelia, such as barrier function and vectorial ion transport. Such alterations in the lung, the gut, and the kidney have direct implications that may explain the profound organ functional impairments in the absence of overt cell death. Epithelial injury in this context is not only an explanatory real pathophysiologic event, but also represents a source of biomarkers that have been explored to identify organ compromise earlier, predict outcome, and even to test novel therapeutic interventions such as blood purification. However, this remains largely experimental, and despite promising results, work is still required to better understand the response of the epithelial cells to sepsis, to define their role in adaptation to insults, to comprehend the interorgan cross-talk that occurs in these circumstances, and to exploit these aspects in pursuit of targeted therapies like blood purification, which may improve outcome for these patients in the future.

KEYWORDS—Barrier function, epithelium, inflammation, sepsis, vectorial transport

INTRODUCTION

In organs, such as the lung, intestine, or kidney, epithelial layers generate and maintain compositionally distinct compartments. For example, in the lung, the alveolar epithelium separates the blood-containing capillary space from the air-containing alveolar space. In order to provide these functions, epithelial cells are polarized (i.e., they have a ''top'' and ''bottom''), they are capable of vectorial transport (moving things in one direction from top to bottom or bottom to top), and they are capable of regulating trans- and paracellular permability (to solutes, water, and even microbes). When patients develop sepsis, the function of epithelia in various organs is often compromised, even if the source of infection is remote. Epithelial dysfunction due to sepsis can contribute to organ dysfunction and/or the development of secondary infections. Therapies and interventions that can ameliorate epithelial dysfunction in sepsis may improve outcomes.

As a part of the International Acute Disease Quality Initiative (ADQI) XIV Sepsis Consensus Conference, we assessed the diagnostic and therapeutic targets of septic epithelia. We sought

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Dr. Mitch P. Fink died on November 17, 2015. The remaining authors would like to dedicate this paper in his memory. Dr Fink was an inspiring figure in medical science and in critical care in particular. He touched all of us in many ways and his ideas and passion for medicine will endure for many years to come.

LSC, MF, SLG, HG, SO all contributed to the pre-conference and post-conference email discussions on this review. In addition, all contributed to the group breakout sessions during the ADQI XIV conference. LSC drafted the first manuscript, and MF, SLG, HG, SO, AG, PM and JAK helped develop subsequent drafts. All authors (Appendix 1) contributed to group discussion and consensus.

LSC and MF denote workgroup facilitators.

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to determine if diagnostics and therapeutics across epithelia and within certain organ epithelia could be utilized to improve outcomes in patients with sepsis.

METHODS

Complete methods are available in the companion article to this series. Briefly, we assembled a group of international experts with distinct clinical and scientific backgrounds; this group included physicians, specialists in critical care, anesthesiology, nephrology, surgery and emergency medicine, and basic scientists with expertise in biology and physiology, who were recruited based on their expertise in sepsis and organ dysfunction. The group consisted of 23 international experts from 5 continents. A set of questions was generated through mutual agreement and we sought evidence to answer each question by searching the Cochrane Controlled Trials Register, the Cochrane Library, MEDLINE, and EMBASE from 1966 to present. Search terms for question regarding epithelial dysfunction are provided in Appendix 2. Finally, we reviewed the evidence with the group and used the Delphi method to achieve consensus.

RESULTS

Based on literature review and consensus among the workgroup members, the following key questions were considered:

(1) How can we assess organ epithelia in health and disease?
(2) What tests/biomarkers can be utilized to make these assessments?
(3) Do these assessments differ between the dynamic and static forms of disease?
(4) Can these measures inform the clinician about prognosis, therapeutic targets, or response to therapy?
(5) Is there a role for provocative/functional testing?
(6) Are these biomarkers targets for blood purification (defined as an extracorporal therapy used in the setting of sepsis for removal of inflammatory, bacterial toxins of both, as a means to modulate the inflammatory response) or other therapies?
(7) What are the similarities and differences between the epithelial responses of the various organs systems?
(8) Is the response of the epithelia adaptive or maladaptive? (similar or different across organs and is there organ crosstalk?)
(9) How do our current therapies affect the, epithelial structure/ function, microbiome, and GU tract?
(10) What therapies can improve these functions?

In our assessment, we recognized that each of the major organs, skin, lung, gut, and kidney, have highly specialized epithelia cell linings and thus that their responses to sepsis are equally specialized. Therefore, for each key inquiry, we have provided assessments for each organ (Fig. 1).

Assessment of epithelial health, function(s), and organ injury, biomarkers and association to outcome

Skin/mucosa—The skin as an epithelial site for clinical research has a decided advantage over other epithelial membranes, as the epidermis and some portions of the mucosa are readily available for direct inspection (1–4). Skin lesions related to various types of infections have been used for centuries to diagnose and evaluate sepsis (e.g., purpura fulminans, Janeway lesions, petechial rashes, etc.). The mucosa of oropharynx, the stomach, and the colon have been utilized with tonometry and the oropharynx with capillary microscopy to assess level of resuscitation, and further evaluate the microcirculation (5–8).

Lung—Clinical evaluation of pulmonary function during sepsis is usually focused on the global assessment of lung/chest wall mechanics, radiologic changes, gas exchange, and occasionally, the cytologic and microbiologic analysis of bronchoalveolar lavage fluid. However, a growing body of evidence suggests that the alveolar epithelium (9) is key in maintaining normal conditions in the alveolar space in health, and during the response to injury, and that the loss or alteration of this function, not only can explain some of the clinical and pathophysiologic findings characteristic of acute respiratory distress syndrome (ARDS), but can also serve as indicators of disease and potential targets for future therapies.

One of the key functions of the epithelial barrier is regulating permeability, which is directly related to the configuration of the epithelial monolayer, and the interaction between adjacent epithelial cells by means of highly specialized (but common to all epithelia) points of contact or tight junctions. Injury to type I and type II alveolar epithelial cells with subsequent liberation of specific mediators also provides an opportunity to assess epithelial damage in plasma or pulmonary edema fluid. Thus, the biomarkers that relate to tight junction integrity and the health of alveolar epithelial cells appear to be the most promising in assessing the pulmonary epithelium in sepsis.

Disruption of the epithelial barrier by inflammatory mediators, neutrophil induced damage, and hypoxia are key mechanisms for the development of ARDS during sepsis, which alters permeability of the barrier, and allows passage of fluid and proteins into the alveolar space (10). Injury to type I and type II alveolar epithelial cells with subsequent liberation of specific mediators also provides an opportunity to assess epithelial damage in plasma or pulmonary edema fluid. A group of promising epithelial biomarkers have shown potential in recent clinical studies: Krebs von den Lungen-6 (KL-6), receptor for advanced glycation endproducts (RAGE), surfactant proteins, Club Cell protein 16 (CC16), and soluble Fas/FasL (10–13) (Table 1). These biomarkers can be measured in the bronchoalveolar lavage (BAL) fluid, and some in plasma, and can be used to assess lung epithelial integrity, injury, and inflammation (Table 1, pulmonary epithelial biomarkers).

Gut—Several clinically relevant assays are available for assessing intestinal epithelial mass and function. These assays can be classified as: dual-sugar absorption studies for assessment of mucosal permeability; other tests of gut mucosal permeability; circulating biomarkers to assess intestinal epithelial mass or damage. Although one key role of the gut is to permit the movement of water, small ions (e.g., Na^+ and Cl^-) and nutrients (e.g., glucose and amino acids) from the lumen into the systemic compartment, another crucial function of the intestine is to serve as a barrier, preventing or at least limiting absorption of luminal microbes or microbe-derived pro-inflammatory substances (e.g., lipopolysaccharide, bacterial DNA) (14). In critical illness, another function of the gut mucosal

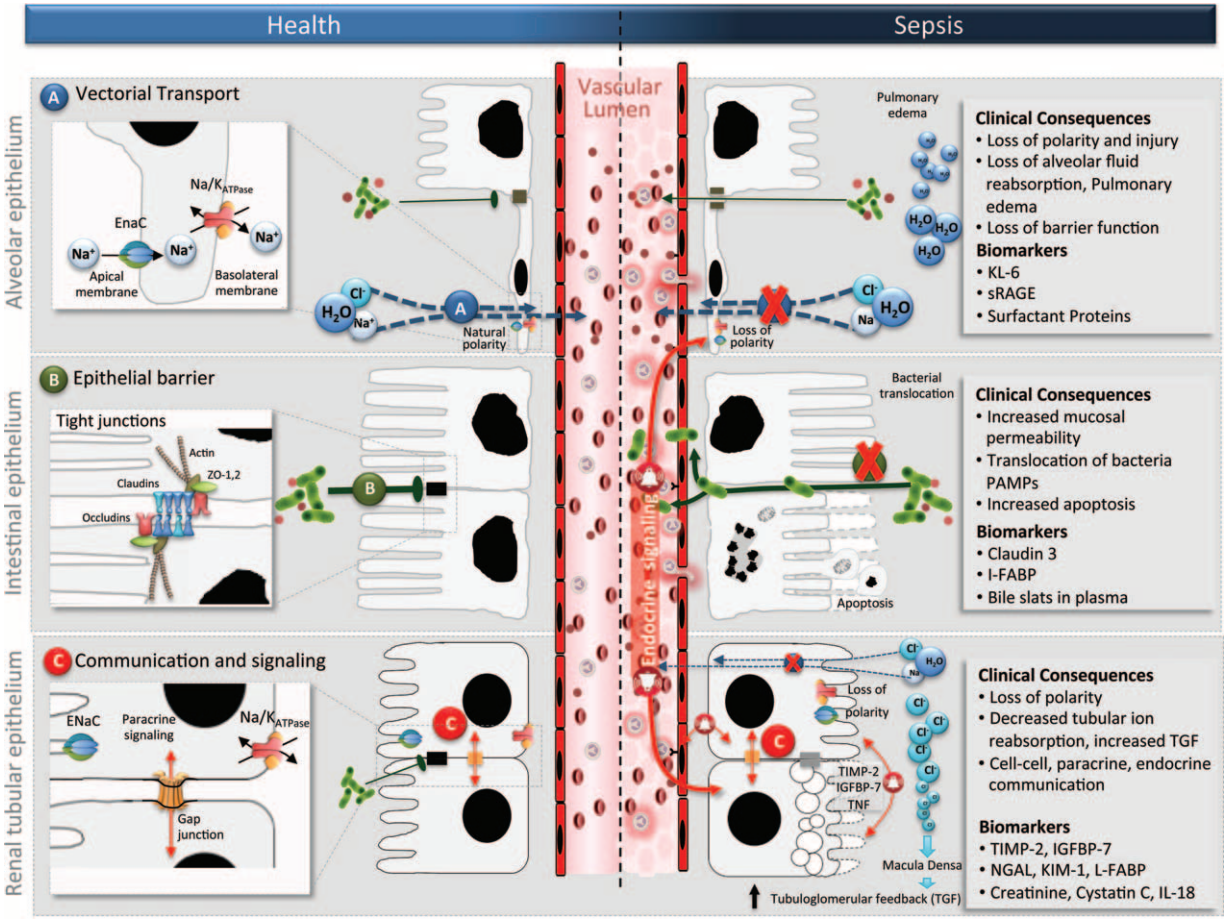

FIG. 1. **Despite obvious differences in epithelial function across different organs, sepsis alters common features of all epithelia, such as vectorial ion transport, barrier function and communication.** These alterations in the lung, intestine, and kidney can explain at least, in part, the profound functional alterations at the organ level. The figure shows a schematic and simplified representation of these common epithelial functions in the lung (A), intestine (B), and kidney (C) during normal conditions on the left-hand side, and their alterations during sepsis on the right-hand side. Importantly, despite these common epithelial functions are characteristic of the epithelia of the lung, intestine, and kidney, the figure emphasizes only one in each panel in the interest of clarity (A) vectorial transport; (B) epithelial barrier function; (C) communication and signaling.) Sepsis induces alterations in vectorial transport in the alveolar epithelium (A) due to loss of polarity, and endocytosis of the Na/K ATPase pump, resulting in impaired alveolar fluid clearance and thus in pulmonary edema. In addition, the alteration in the Na/K ATPase pump has been associated with loss of tight junctions, impairing the important barrier function of the alveolar epithelium. The epithelial barrier function is also severely impaired in the intestinal mucosa as shown in (B). Claudin-3 has been recognized as an important component of the tight junctions, and has been shown to be excreted in urine after intestinal mucosal injury. (C) It shows how sepsis may induce increased cell-to-cell, paracrine, and even endocrine communication. Although it is relatively well known that gap junctions serve as communication ports between adjacent cells, it is still unknown how epithelia communicate to nonadjacent epithelial cells, or with epithelia from remote organs. There is associative evidence of this organ-to-organ cross talk between for example the kidney and the lung. In addition, panel C shows how alterations in vectorial transport in the tubular epithelial cell due to loss of polarity and endocytosis of epithelial ion channels like the Epithelial sodium channel (ENaC) may induce increments in delivery of chloride to the macula densa, triggering increased tubuloglomerular feedback, and resulting in decreased glomerular filtration rate, urine output and increased creatinine. Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission. KL-6 indicates Krebs Von den Lungen-6; sRAGE, soluble Receptor of Advanced glycation end products; PAMP, pathogen associated molecular patterns; I-FABP, intestinal-fatty acid binding protein; TGF, transforming growth factor; TIMP-2, tissue inhibitor of metalloproteinase-2; IGFBP-7, insulin-like growth factor binding protein-7; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; IL-18, interleukin 18; ZO, zonula occludens; Na, sodium; K, potassium; Cl, chloride; H_2O, water.

barrier might be to limit absorption of pancreatic digestive enzymes or cytotoxic fatty acids generated within the lumen (15–17). Regardless of the mechanisms responsible for the deleterious effects of intestinal barrier dysfunction, numerous studies support the view that gut mucosal permeability is increased in patients with sepsis (18), acute respiratory distress syndrome (19), and other forms of critical illness (20–22). Moreover, some data support the view that gut mucosal hyperpermeability in critically ill patients is associated with an increased likelihood of mortality or multiple organ dysfunction syndrome (19–22).

The maintenance of normal intestinal mucosal permeability depends on the formation and proper functioning of specialized structures called tight junctions that are situated at the apical poles of enterocytes. Claudin-3 is a key protein involved in the assembly of the tight junctions between adjacent enterocytes. It is notable, therefore, that elevated urinary levels of claudin-3 were detected in a rodent model of hemorrhagic shock and patients with active inflammatory bowel disease, a condition that is known to be associated with gut mucosal hyper-permeability (23). It remains to be determined whether monitoring of urinary claudin-3 concentration can serve as a noninvasive way to assess gut mucosal barrier integrity in patients with sepsis.

A class of small proteins, called fatty acid binding proteins (FABP), are present in the cytosol of enterocytes and certain

Table 1. **Pulmonary epithelial biomarkers**

Biomarker	Characteristic	Biological (Dys)function	Diagnostic/Therapeutic Potential
KL-6	MUC1 type mucin expressed by alveolar type II cells (ATII). Presence in alveolar fluid and plasma	Chemotactic factor. Promotes migration, proliferation of lung fibroblasts	Diagnosis: OR for diagnosis of ARDS in population at risk 6.06 (95% CI 3.04–12.1; 4 studies, n = 137) (13). Prognosis: OR for death in patients with ARDS of 4.29 (95%CI 1.84–9.99) (13). Therapy: Elevated in non-protective mechanical ventilation (15).
sRAGE	Expressed (not exclusive) by alveolar type I cells (ATI). Presence in alveolar fluid and plasma	Induces gene/protein expression. Involved in recognition of inflammatory ligands.	Diagnosis ALI/ARDS in patients at risk: OR of 3.48 (1.69–7.17; 5 studies, n = 317) (13). Prognosis: Not associated with mortality. Therapy: Decreases with protective ventilator strategies.
SP-D	Hydrophillic surfactant associated apolipoprotein, produced by ATII. Presence in alveolar fluid and plasma	Maintains and modulates innate immune response of the lung	Diagnosis ALI/ARDS in patients at risk: OR of 2.77 (1.17–6.65) (13). Day 2 of admission in patients with sepsis – AUC 0.69 for general population, and 0.72 when evaluating only severe cases (12). Prognosis: Conflicting evidence (9). Therapy: Low tidal volume ventilation limited increment in SP-D levels (19).
CC16	Expressed in Club (Clara Cells). Present in plasma	Suppression of Neutrophil-mediated epithelial damage	Conflicting results in ARDS. (13, 21, 22).
Fas/FasL	The Fas/FasL axis is one of the primary extrinsic pathways of induction of apoptosis. Fas is expressed in alveolar epithelial cells, Club cells, macrophages. FasL is expressed in neutrophils and lymphocytes. Present in alveolar fluid	Induction of apoptosis in different cell lines	Conflicting results in ARDS.

KL-6 indicates syalilated carbohydrate antigen; sRAGE, soluble receptor of advanced glycation end products; SP-D, surfactant protein D; CC16, Clara/Club Cell protein; Fas/FasL, Fas-cell surface Fas receptor (or Apo-1); FasL, transmembrane protein from he tumor necrosis factor family also known as CD95L that interacts with Fas.

other cell types. *Intestinal* fatty acid binding protein (I-FABP) is found almost exclusively in mature enterocytes and this protein appears in the circulation soon after damage to the gut mucosa as a result of ischemia, ischemia/reperfusion injury, or intestinal inflammation. I-FABP is rapidly cleared from the circulation by the kidneys and the protein appears in the urine. Among surgical ICU patients with postoperative sepsis, there was a strong correlation between high gastric mucosal pCO_2 levels, a marker for hypo-perfusion of the stomach, and high circulating concentrations of I-FABP (24). In this study, a high circulating I-FABP level at the time of admission to the ICU was associated with an increased risk of mortality.

Liver—Historically, hepatocellular (liver epithelial) injury or dysfunction has been assessed by measuring serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and (conjugated, unconjugated, and total) bilirubin. Elevated circulating concentrations of the liver enzymes (ALT, AST, and LDH) reflect damage or death of hepatocytes, but measurements of these markers fail to provide information about hepatocellular function. Circulating levels of bilirubin are often increased in patients with sepsis for a variety of reasons (25) and the presence of cholestasis is associated with an increased likelihood of mortality (26). In a retrospective study of 251 patients with sepsis or septic shock, Patel et al. reported that mortality was 12% for those with an admission serum bilirubin concentration <1 mg/dL, whereas mortality was 42% for those with an admission serum bilirubin level >2 mg/dL (27).

Hepatocytes maintain the barrier between the canalicular (bile-containing) space and the (blood-containing) sinusoids by forming tight junctions between adjacent cells. Lora et al. (28) reported that the functional integrity of hepatic tight junctions can be assessed by measuring serum concentrations of bile acids. Han et al. (29) reported that hepatocellular tight junctions were disrupted and circulating levels of bile acids increased when mice were challenged with a sublethal dose of LPS to induce a sepsis-like state. Recently, high circulating bile acid levels were shown to be associated with mortality in patients with community-acquired pneumonia and sepsis (30). In this study, serum levels of several metabolites were measured in 15 patients with sepsis who died and compared with levels measured in 15 carefully matched patients who survived. Plasma levels of sulfated bile acids (taurochenolate sulfate and glycochenolate sulfate) and a primary bile acid (taurocholate) were significantly higher in nonsurvivors as compared with survivors. These findings support the view that bile acids may be a useful marker of hepatocellular barrier dysfunction due to sepsis. Given the potential for bile acids to induce inflammation by acting as damage-associated molecular patterns (DAMPs) (31), it is possible that blood purification, or other therapies, could be used to target these molecules.

Kidney—The standard assessment of kidney function most often refers to measurement of solute clearance capacity, which itself is dominated by glomerular filtration. While renal tubular epithelia contribute to functional clearance via secretion, their impact on standard clearance measurements such as serum creatinine levels per se, is minimal. However, the renal tubule does more that transport ions and small molecules back and forth. The normal kidney(s) filter over 150 L of plasma water each day, and the proximal renal tubule is well positioned to sample the plasma water filtrate of the glomerulus. Elegant studies demonstrate that the proximal tubule is an immune ''sensor'' by routinely assessing the glomerular filtrate (32). This concept explains the presence of pattern recognition receptors (e.g., toll-like receptors) on renal tubular epithelia cells (RTEC). A network of monocytes and dendritic cells, which are well positioned to respond to pattern recognition receptor activation, surrounds RTEC. Thus, RTECs play an important role in regularly sampling the plasma water for various host threats. In addition, the renal epithelium works in concert with the renal endothelium; this interaction has been termed the renal epithelial–endothelial axis.

In sepsis, the previous belief that renal dysfunction was solely due to acute tubular necrosis (ATN) appears to be erroneous. Recent studies demonstrate that in sepsis patients with AKI, minimal ATN (less than 10% of the area sampled) and apoptosis were observed in rapid renal autopsy specimens (33). Thus, a reassessment of the renal epithelial biology is essential for any advancement in AKI diagnosis and treatment to be realized. Fortunately, urine provides a biological fluid that is readily available for ''biopsy'' to assess the integrity and functional capacity of renal epithelial cells.

Classic tests of proximal tubular function assess the resorptive capacity of various solutes normalized to resorption of creatinine, usually expressed as the fractional excretion of that solute. However, recent studies in human sepsis evaluating the fractional excretion of sodium and urea nitrogen have cast doubt on their ability to predict transient, sustained and/or worsening AKI (34, 35).

Serum creatinine, which has been the standard biomarker of renal function for over 50 years, has been repeatedly shown to be a late functional readout for kidney damage, so extensive research has been expended to discover and validate novel renal tubular AKI markers (36). A complete discussion of novel AKI biomarkers is beyond the scope of this section, but a table of the purported biology and potential therapeutic targets for each biomarker is provided (36) (Table 2).

Another approach to functional testing of the thick ascending limb of the Loop of Henle is the recently described ''furosemide stress test'' (FST) (37). The FST employs a standardized dose of furosemide with a following timed 2-h urine output as the clinical readout. Lack of response (defined as less than 200 mL of urine), was highly predictive of AKI progression. Functional testing of the renal tubular epithelium can be integrated with acute kidney biomarkers to improve diagnostic performance (38).

Are these biomarkers targets for blood purification or other therapies?

The therapeutic approaches available for the treatment of sepsis have changed as our vision of the pathophysiology of sepsis has evolved. The recognition that a disregulated immune response is a key trait, and that it is the result not only of the exposure to pathogens and bacterial toxins (i.e., pathogen associated molecular patterns (PAMPs)), but also, to endogenous mediators such as cytokines, chemokines, and released products from damaged tissues (DAMPs) has fueled the interest in blood purification techniques as potential therapeutic strategies for septic patients (39). Within the realm of possibilities, blood purification techniques have been portrayed to provide advantages that range from the simple concept of just removing the excess of DAMPs and PAMPs, to the removal of chemokines from the blood stream in order to re-establish key chemokine gradients that determine immune cell trafficking to the site of infection (40). However, one of the limitations of these techniques strives from the lack of biomarker availability to guide therapy. Accordingly, we will focus on summarizing and revising the available evidence on potential epithelial biomarkers that could serve the purpose of directing these and other therapeutic interventions.

Table 2. **Renal epithelial biomarkers**

Biomarker	Characteristic	Biological (Dys)function	Therapeutic Potential
NGAL	25 kD increased in proximal and distal tubule after renal tubular ATP depletion	Binds iron-siderophore complexes/iron trafficking	Holo-NGAL administration prevents apoptosis associated with IRI
IL-18	22 kD pro-inflammatory cytokine increased in proximal tubule after IRI	Promotes intra-renal infiltration of macrophages and neutrophils	Development of an anti-IL-18 antibody strategy to prevent AKI development
KIM-1	39 kD transmembrane protein localized to de-differentiated PT cells after injury	Promotes phagocytosis of apoptotic bodies and necrotic debris	Prevention of KIM-1 shedding could prevent AKI; KIM-1 upregulation after AKI development could enhance renal recovery
L-FABP	14 kD protein localized to the proximal tubule	Anti-oxidant properties exhibited through HIF-1α	Increased L-FABP production could prevent AKI in patients at-risk for hypoxia induced AKI
TIMP-2/IGFBP-7	Expressed ubiquitously	G_1 cell cycle arrest to prevent tubular apoptosis	Dose targeting for other potential reno-protective molecules

L-FABP indicates fatty acid binding protein; TIMP-2, tissue inhibitor of metalloproteinase-2; IGFBP-7, insulin-like growth factor binding protein-7, NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; IL-18, interleukin 18.

Lung—Within the available evidence, there are two interventions that have been shown to improve outcome in sepsis-associated lung injury (i.e., ARDS) which are low tidal volume ventilation and a fluid conservative resuscitation strategy. Three randomized trials have shown the protective effects of low tidal volume and low airway pressure ventilation in patients with ARDS (41–43). High tidal volume ventilation results in alveolo-capillary barrier damage, increased permeability to water and solutes and pulmonary edema, a condition termed ventilator induced lung injury (VILI) (44). The second intervention is conservative fluid management. The ARDSNet fluid and catheter trial demonstrated that treating patients with ARDS with a fluid conservative strategy is beneficial (reduced duration of mechanical ventilation free days but no difference in mortality) (45). Although there is no evidence suggesting that fluids cause direct injury on the alveolar epithelial cell, it is intuitive that lower hydrostatic pressure could help reduce pulmonary edema in the setting of a permeable, dysfunctional alveolo-capillary barrier.

However, none of the mechanisms that we identified appear to be good targets for removal to improve outcomes in ARDS. One potential target to assist with ARDS management is CO_2 removal. When patients have severe lung injury, very low tidal volume and ''still-lung'' ventilation strategies may be utilized, but these protective ventilation strategies often result in severe hypercapnia. Extracorporeal carbon dioxide removal ($ECCO_2R$) offers a potential solution to this problem because carbon dioxide can be ''dialysed'' out of the blood, while the lungs are ventilated in a maximally protective manner (46, 47). Variations of these techniques have existed for decades, but there has been a renewed interest given the trend toward protective ventilation in critical illness (46). The devices that perform $ECCO_2R$ have become easier to use and are effective at CO2 removal. However, evidence for a reduction in mortality and other important clinical outcomes is still lacking.

Gut—At present, the only widely accepted therapy to support intestinal epithelial function in critical illness is the administration of enteral nutrition. Extensive studies using animal models support the view that the administration of pharmacological doses of the amino acid, L-glutamine, can prevent gut mucosal damage and preserve intestinal epithelial barrier function in animal models of intestinal ischemia and reperfusion injury, endotoxemia, trauma, and graft-versus-host disease after bone marrow transplantation (48–51). Although results from some small clinical trials support the view that enteral or parenteral administration of pharmacological doses of L-glutamine can ameliorate gut barrier dysfunction in critically ill patients (52–54), contrary results have been obtained in other randomized clinical studies (55, 56). Heyland et al. (57) reported the results of a large, multicentric, randomized clinical trial that evaluated the effects of combined enteral and parenteral L-glutamine supplementation in critically ill patients. Mortality at 28 days was significantly higher for patients randomized to treatment with L-glutamine as compared with controls. Accordingly, administration of pharmacological doses of L-glutamine to critically ill patients is not recommended. Results from a single-center single-armed ''before and after'' trial support the view that continuous veno-venous hemofiltration can ameliorate gut barrier dysfunction in critically ill patients with multiple organ dysfunction syndrome (58).

At present, the only potential therapy identified as a target for extracorporeal therapy linked to gut epithelial injury/dysfunction is endotoxin removal. When gut integrity is compromised, significant bacterial translocation can ensue (47, 48). Polymyxin B (PMX) is a cationic cyclic polypeptide antibiotic which binds with high affinity to endotoxin, but this antibiotic has significant nephrotoxic and neurotoxic effects, and these toxicities limit their systemic use (58). This subsequently led to the development of an adsorptive cartridge in which PMX is covalently bound to polystyrene fibers (59). The device has been approved for use in Japan and Europe in the 1990s. More than 70,000 patients have been treated with PMX-F in Japan and Italy over the last 15 years (60). Systematic reviews demonstrate improvement in outcomes, and the overall results for this therapy have been positive (61). This device is currently being investigated in Phase III clinical trials (Safety and Efficacy of Polymyxin B Hemoperfusion (PMX) for Septic Shock (EUPHRATES- NCT01046669).

For liver dysfunction, particularly injury linked to retention of bilirubin and bile salts, albumin dialysis may have a role to help remove these molecules that are typically eliminated by the liver. Albumin dialysis-based therapies, such as the molecular adsorbent recirculating system (MARS), and single pass albumin dialysis (SPAD), or the Prometheus system have previously been investigated predominantly in acute and acute-on-chronic liver failure, in which the removal of both albumin-bound and small water-soluble molecules (i.e., ammonia, creatinine, cytokines, urea) has been characterized (62). Several case studies and series have demonstrated that the application of albumin dialysis can effectively remove albumin-bound molecules (i.e., bilirubin, bile acids, middle- and short-chain fatty acids) that accumulate from liver injury (63–67). This removal has been shown to improve hepatic encephalopathy, but whether this intervention can improve outcomes in septic shock has not been studied (68, 69).

Some preclinical sepsis models document that blood purification with cytokine removal may improve liver epithelial function. For example Peng et al. (69) found that in experimental sepsis using CLP in the rat, treatment with sorbent-based blood purification (CytoSorb) resulted in reduced hepatic injury, as measured by ALT and histology. Similar results were seen in a study by Namas using Cytosorb treatment in *E. coli* impregnated fibrin-clot-induced peritonitis (68). The precise mechanisms whereby these observed effects occur are unknown but in addition to removal of DAMPs and PAMPs, restoration of the appropriate chemokine gradients from the systemic circulation to the compartment of actual infection may be a critical factor (40).

Kidney—Currently, maintenance of renal perfusion, prevention of secondary insults, and supportive dialytic therapy represent the mainstay of AKI therapy. Early evidence suggests that some of the novel biomarkers listed above may herald the onset of renal recovery (70, 71) to potentially guide withdrawal of renal supportive therapy, yet none have been identified as targets for blood purification. Although the clinical role of

extra-corporeal therapy for renal epithelial dysfunction remains supportive and primarily focused on the removal and uremic toxins, volume control, and acid–base/electrolyte management, experimental data have suggested that blood purification techniques may be beneficial for the tubular epithelial cell. Cantaluppi et al. studied the effect of plasma obtained from septic patients on tubular epithelial cell cultures. Plasma from septic patients caused injury by enhancing granulocyte adhesion, inducing apoptosis and altering tubular epithelial cell polarity and function. Importantly, these effects were significantly attenuated when septic plasma was pretreated with Aberchrom resin (72). Although this study focused on non-selective removal of cytokines, blood purification has proven to be beneficial even in the absence of significant cytokine removal in animal models of sepsis (73). Peng et al. (74) showed that septic rats treated with extracorporeal hemoadsorption using CytoSorb polymer beads had better survival than those treated with the same circuit without sorbent, even though TNF, IL-1B, IL-6, and IL-10 did not change, suggesting that simple cytokine removal may not explain the beneficial effects. Moreover, blood purification resulted in significant liver and kidney protection at 72 h compared with control (68). In subsequent studies the association of hemoadsorption with the re-establishment of the chemokine gradients that usually drives granulocyte migration to areas of infection, led the authors to postulate that these extracorporeal techniques may exert beneficial effects through optimization of the immune response by redirecting neutrophil trafficking (40).

Role of the microbiome

The microbiome of the skin, gut, and lung all interact with their respective epithelium and this interaction is bidirectional. Thus, the microbiome can affect the pathophysiology of injury and organ dysfunction when the various epithelia are altered in sepsis. The metagenome (total complement of unique genomes determined by molecular methods) of the entire bacterial population along epithelial membranes can now be accurately estimated by characterizing the net product of amplified sequences from the 16S rRNA complex (1, 2).

The recent completion of the first phase of the human microbiome project consortium has provided some useful insights into the nature of the host microbiome in health and disease (1). A richly diverse community of microorganisms is distributed along gastrointestinal surfaces, the urogenital tract, the skin, the nasal mucosa, lower airways, and the oropharynx (1, 28, 29, 74–77). These communities are quite distinct from each other and remain remarkably stable in composition for prolonged periods of time after infancy and throughout most of the adult life. Some areas of the body such as the skin are highly variable, whereas other sites such as the oropharynx are remarkably similar between individuals (1).

Using traditional culture-based microbiologic investigations, it was widely believed that the lower airways and the upper genitourinary tract of healthy humans were sterile. Using non-culture-based methods to detect the entire microbial community existing in these anatomic sites, it is now clear that there is a definable and fairly stable microbiome existing even in the lower airways and uroepithelium of healthy humans (74, 78, 79). There are multiple clinical examples wherein changes in the microbiome along an epithelial membrane can be associated with clinical illness and might even be of etiologic significance.

The microbiome of the gut has been the best-characterized microbiome among the various organ systems. Fungi, bacteria, viruses, and Archaea species comprise the microbial community that colonizes that gastrointestinal tract in vertebrates (80). In humans, the intestinal lumen contains about 10^{14} bacterial cells and many thousands of individual species or strains (81). Remarkably, the intestinal microbiome contains more than 100 times as many genes and 10 times as many cells as the host (80). The intestinal microbiome is now recognized as playing a crucial role in human health and disease (2). Commensal gut microbes play important roles in metabolism, nutrition, immunity, and host defenses against pathogens.

Under normal conditions, the composition of the gut microbiome is temporally stable in adult humans (82). In critical illness, however, the composition of the intestinal microbiota can be dramatically altered as a result of changes in nutritional intake, administration of antibiotics, and the effects of myriad pharmaceutical agents in addition to antimicrobial agents. For example, opioids (whether administered as a drug or produced endogenously during critical illness) promote expression of a virulent phenotype by *Pseudomonas aeruginosa* within the gut lumen (83). Importantly, the presence of virulent *P. aeruginosa* in the intestine is associated with epithelial disruption and decreased mucus production (83). There is evidence that changes in the composition of intestinal microbiome can have a major impact on the course and outcome of critical illness (84).

Critical illness itself can be associated with processes that cause factors released by the epithelium of the gut to trigger expression of virulence factors by microbes colonizing the gut lumen. In a landmark paper, Wu et al. (85) showed that the pro-inflammatory cytokine, interferon-γ, binds a specific receptor on *P. aeruginosa* (OprF), leading to increased expression of the virulence factors, *P. aeruginosa* lectin and pyocyanin. These virulence factors are capable of disrupting enterocyte function.

There is increasing interest in using active measures to modify the gut microbiome to improve outcomes for critically ill patients. One potentially attractive approach is the administration of prebiotic or probiotic agents (86). Probiotic agents contain viable bacteria that can be administered enterally in an effort to restore a more normal intestinal microbial ecology. Prebiotic agents are non-digestible compounds, such as galactooligosaccharides, that selectively promote the growth of specific bacterial species. A number of prospective clinical trials have evaluated the effects of administration of probiotics on outcomes for critically ill patients. In a recent meta-analysis of 13 such trials, Barraud et al. (87) reported that administration of probiotics did not affect ICU or hospital mortality but did reduce the incidence of ICU-acquired pneumonia and shorten ICU length of stay. A case of fulminant *Clostridium difficile*-induced colitis was successfully treated by transplantation of fecal microbiota from a healthy donor (88).

Differences in the epithelial response to sepsis between organs, organ cross-talk, and the concept of adaptation

Epithelial response—Vectorial Na^+ and water transport is an active, energy-consuming process and one of the main functions of several epithelia important in the alveolar epithelial cell, fundamental to the lung to perform adequate gas exchange. Hyperoxia (89), ischemia (90), hypercapnia (9), and inflammation (91) have been shown to induce down-regulation of Na and Cl transporters, as well as Na/K ATPase pump endocytosis, severely impairing epithelial Na and fluid transport and clearance (92). Similarly, ionic transport at the apical membrane of the proximal tubular epithelial cell represents the largest energy sink of the kidney, and there too, endocytosis of the Na/K ATPase pump and ENaC, as well as loss of ionic transport has been demonstrated in the setting of experimental sepsis (93). In the liver, exposition of hepatocytes to LPS induces a rapid decline in ATP levels, without significant apoptosis, suggesting downregulation of the energetic metabolism (94). Thus, it is possible that different epithelia share common, evolutionarily conserved responses to injury. An important difference in how different epithelia respond to the septic insult though is apoptosis. There is some controversy in the literature regarding the role of apoptosis in the setting of all cause ARDS however. Evidence has suggested that the Fas/FasL axis is active and that there seems to be a fair degree of apoptosis in the alveolar epithelia. However, in sepsis, Hotchkiss et al. (95) and others (96) have consistently demonstrated that apoptosis is rare in most organs (including the lung, liver, and kidney), but present in the gut epithelia, suggesting potentially different response mechanisms or perhaps distinct patterns of exposure to injury (95). Although evidence is still inconclusive, the lack of apoptosis in most epithelia may suggest that at least at the cellular level the epithelial response may be adaptive.

Organ cross-talk—Relevant kidney–lung interactions have been demonstrated by clinical and experimental studies. Ranieri et al. (97) demonstrated an association between increased multiple organ dysfunction and in particular, acute kidney injury with a non-protective ventilator strategy. The ARDSNet trial further supported this finding, showing a decrease in AKI-free days in the patients with lung protective ventilation (41). There is also experimental evidence showing that ventilation with high tidal volumes resulted in increased apoptosis of renal tubular epithelial cells in a model of acid-aspiration induced ARDS in rabbits (98). Although there seem to be enough data suggesting that such cross-talk does occur, it is unclear if the epithelia plays a role in such interactions and what mechanisms may be involved.

DISCUSSION

The various epithelia in the body are all affected by sepsis. Organ epithelial injury/dysfunction, whether the epithelia compromised is skin, lung, liver, gut, or renal epithelia, is associated with worsened outcomes. Multiple biomarkers and functional tests exist which can aid in assessing the degree of dysfunction and injury. Multiple extracorporeal treatments that support the consequences of dysfunctional epithelia have been developed and many are used routinely. However, specific targets for removal by extracorporeal therapies, which could result in the improvement of epithelial function, include various DAMPs and PAMPs. Future work needs to integrate the considerable advances in understanding of basic mechanisms of epithelial cell injury and dysfunction with technologic understanding of what can be removed from the plasma. Available evidence already supports the use of extracorporeal therapies as supportive therapies to help manage the consequences of organ epithelial injury/dysfunction in sepsis. Emerging evidence supports a more direct role in protecting the epithelium in special organs such as the kidney and liver than are directly exposed to plasma filtrates containing DAMPs, PAMPs, and other molecules. Indeed, many of the various pathophysiological mechanisms discussed in this review may yield viable targets for extracorporeal therapy in the future.

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APPENDIX 2. SEARCH TERMS

Epithelium, sepsis, inflammation, barrier function, vectorial transport, sodium, lung, acute respiratory distress syndrome, ARDS, acute lung injury, ALI, claudins, biomarkers, plasma, blood, surfactant, intestine, liver, translocation, bacteria, bacterial translocation, fatty acid binding proteins, FABP, apoptosis, ions, transport, enteral, kidney, renal, tubular epithelial cell, necrosis, creatinine, renal blood flow, skin, microbiota, epidermis, mucosa, capillary, blood purification, hemoadsorption.

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