HHS Public Access

Author manuscript

J Equine Vet Sci. Author manuscript; available in PMC 2019 April 16.

Published in final edited form as:

J Equine Vet Sci. 2017 May ; 52: 10–22. doi:10.1016/j.jevs.2017.02.009.

Alterations in Intestinal Permeability: The Role of the "Leaky Gut" in Health and Disease

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Abstract

All species, including horses, suffer from alterations that increase intestinal permeability. These alterations, also known as "leaky gut," may lead to severe disease as the normal intestinal barrier becomes compromised and can no longer protect against harmful luminal contents including microbial toxins and pathogens. Leaky gut results from a variety of conditions including physical stressors, decreased blood flow to the intestine, inflammatory disease, and pathogenic infections, among others. Several testing methods exist to diagnose these alterations in both a clinical and research setting. To date, most research has focused on regulation of the host immune response due to the wide variety of factors that can potentially influence the intestinal barrier. This article serves to review the normal intestinal barrier, measurement of barrier permeability, pathogenesis and main causes of altered permeability, and highlight potential alternative therapies of leaky gut in horses while relating what has been studied in other species. Conditions resulting in barrier dysfunction and leaky gut can be a major cause of decreased performance and also death in horses. A better understanding of the intestinal barrier in disease and ways to optimize the function of this barrier is vital to the long-term health and maintenance of these animals.

Keywords

Horse; Intestinal permeability; Leaky gut; Barrier function; Decreased performance

1. Introduction

The intestinal tract serves many vital functions that include the selective absorption of essential nutrients, ions, and other compounds while serving as a barrier against harmful, noxious substances. Dysfunction of this barrier and alterations in intestinal permeability, also known as "leaky gut" is an important topic for clinicians and researchers in all species including horses and humans. In humans, changes in intestinal permeability have been

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linked in the pathogenesis of debilitating inflammatory bowel diseases (IBDs) such as Crohn's disease (CD) [1] and in autoimmune diseases such as Celiac disease [2]. Currently conditions resulting in leaky gut are known to occur in equine species, yet few scientific studies have been conducted focusing on this condition. In horses, gastrointestinal issues are reported second to only old age as the leading cause of death [3]. Death or illness can result from the systemic effects of microbial toxins and pathogens that "leak" through the intestinal wall and the subsequent immune response that includes the production of inflammatory mediators. Leaky gut is therefore described in both severe, life-threatening intestinal obstructions as well as in long-term, insidious disorders that result in weight loss and decreased performance (Fig. 1). Thus, a better understanding of the intestinal barrier in disease and how to improve its function remains vital to the long-term health and the maintenance of high-level athletic performance of these animals. This paper serves to review the normal intestinal barrier, measurement of barrier permeability, pathogenesis and main causes of altered permeability, along with highlight potential alternative therapies of leaky gut in horses while relating what is known in other species including poultry, porcine, rodent, and human.

2. Intestinal Barrier and Permeability

Intestinal permeability is determined by the interaction of several components including an unstirred water layer that forms a diffusion barrier in combination with mucus. In addition, mucus protects the villi from physical friction and bacterial adhesion [4,5]. Other barrier components include phospholipids within the mucosal surface, epithelial factors including tight junctions, the intestinal immune system including lymphocytes and the gut microbiota [4,6–8]. The gut microbiota, a complex community of microorganisms that inhabits the intestine, varies with diet, age, and environment, and influences normal physiology and susceptibility to disease through its metabolic activities and host interactions [8]. A full review of the microbiota and its interactions with intestinal barrier function is outside the scope of this article and the interested reader is directed to several recent reviews [8–12].

One of the most important and widely studied intestinal barrier components is the intestinal epithelium. The intestinal epithelium is composed of a single layer of cells and is the largest of the body's mucosal surfaces [13]. These epithelial cells are polarized, contain apical and basolateral membranes, and are responsible for creating a physical barrier, transporting nutrients, and protecting the underlying tissues [14]. The epithelial layer of the large intestine (colon) is folded into invaginated crypts of Lieberkühn that contain undifferentiated stem cells and are supported by the lamina propria (Fig. 2) [15]. The small intestinal epithelium is composed of villi that extend into the lumen and are lined by differentiated, post-mitotic cell types, and the crypts of Lieberkühn that contain Paneth cells and undifferentiated stem cells [16]. The stem cells are responsible for creating new epithelium every 5–7 days [17,18]. Enteroendocrine, goblet and Paneth cells are the specialized, secretory epithelial cells that maintain the digestive or barrier function of the epithelium via hormone, mucin, and antimicrobial peptide secretion, respectively [13]. When healthy, the epithelial barrier is impermeable to toxins, pathogens, and antigens while maintaining a selective permeability for the transport and absorption of nutrients, ions, and water (Fig. 2). Selective permeability occurs via the following two pathways: the paracellular and

transcellular pathway [19]. The transcellular pathway, predominately mediated by transport channels located on the apical membrane, involves the transport of nutrients including sugars, amino acids, and fatty acids across the cell. The paracellular pathway, associated with passage of molecules in the space between adjacent cells, is regulated by an apical junctional complex (AJC) made up of adherens junctions and tight junctions (Fig. 3). Adherens junctions, along with desmosomes, provide strong connective bonds between epithelial cells. Cell to cell contact at the adherens junction is mediated by adhesion molecule complexes made up of protein families including the cadherins and catenins. Tight junctions consist of four unique families of transmembrane proteins including occludin, claudins, junctional adhesion molecules, and tricellulin, and are considered one of the principal determinants of mucosal permeability (Fig. 3) [20,21]. These transmembrane proteins interact with their partners on the opposing plasma membrane and provide a mechanical link between epithelial cells while establishing a diffusion barrier [22]. If disrupted, permeation of potentially noxious molecules and organisms from the intestinal lumen can result in a cascade of events including immune activation and inflammation, eventually triggering the development of intestinal and systemic diseases (Fig. 4).

3. Assessment of Intestinal Permeability

There are a variety of methods to assess intestinal permeability in both human and animal models clinically and experimentally. Clinically, in horses, used methods of assessing intestinal permeability in vivo include urine or serum recovery of enterally delivered radio-labeled markers including ^{51}Cr-ethylene diaminetetra-acetate (^{51}Cr-EDTA) [23], ^{99m}Tc-diethylene triaminopenta-acetate (^{99m}Tc-DPTA) [24], and poorly absorbed sugars such as D-xylose [25,26] and sucrose [27]. After the administration of ^{51}Cr-EDTA via nasogastric tube, the urine of ponies was collected to assess the effects of gastrointestinal motility chemically altered by atropine and bethanechol, as well as the effect of nematode infection on intestinal permeability [23]. There was no difference in recovery after the administration of motility altering medications; however, significant increases in ^{51}Cr-EDTA urine recovery were present when ponies had an experimental cyathostome infection compared with control. ^{51}Cr-ethylene diaminetetra-acetate was well tolerated and was shown to be a useful marker for the assessment of intestinal permeability in horses. Radioactivity of urine and blood technetium (^{99m}Tc) was also used to assess intestinal permeability after the administration of excessive carbohydrates in ponies, and was increased transiently after the administration [28]. This study concluded that excessive carbohydrate administration could directly cause a change in measured ^{99m}Tc, supporting an abnormal intestinal barrier. The measurement of fluorescent probes such as fluorescein isothiocyanate (FITC)-dextrans [29] has also been used to assess intestinal permeability in vivo. To assess abnormal gastric permeability, sucrose has been used in many species including dogs and humans [30,31]. Earlier studies in dogs also quantified the concentrations of several other sugars including rhamnose, 3-*O*-methyl-D-glucose, and lactulose to assess permeability [32]. In horses, significant increases in serum sucrose concentration were recorded following nasogastric intubation of table sugar in horses with moderate to severe gastric ulceration, suggesting the possibility of this test both as a screening tool and diagnostic aid for clinicians [27]. The main advantage of these techniques is that they can be tested in vivo. Other factors must be

taken into consideration when evaluating these testing methods including molecule absorption and metabolism, gastrointestinal motility, concurrent medication administration, as well as blood volume and blood flow to the intestine.

Experimentally, the Ussing chamber provides an ex vivo method for the measurement of electrolyte, nutrient, and drug transport across intestinal epithelial tissues [33]. The use of the chamber has been documented in a variety of species including humans [34], rodents [35], horses [36], swine, and poultry [37]. While tissue is mounted in the Ussing chamber, a variety of measurements can be collected including transepithelial potential difference and transepithelial electrical resistance (TER) indicating tissue viability and barrier integrity, as well as flux of FITC-dextran, FITC-lipopolysaccharide (LPS), or mannitol to measure tight junction damage and leakage across the mucosa [37]. Measurements of TER and permeability in the chamber are calculated based on the serosal surface area, as the surface area of the mucosa varies based on species and the intestinal section under study [38]. Mucosal surface area is amplified by villus projections, which change in shape and height throughout the small intestine. The colon has no villi. When TER data are corrected for differences in surface area, the permeability of the small intestine and colon are similar [38]. The Ussing chamber, therefore, provides opportunities to measure changes in both paracellular and transcellular transport pathways in addition to the ability to manipulate the tissue restitution process through various reagent treatments. In vitro, TER and permeability have been assessed in human colonic epithelial cell (CACO-2) monolayers using a trans-well system [39]. In vitro scratch assays are also used to study cell migration and intestinal “healing” in a variety of cell lines including IPEC-J2 [40,41].

4. Causes of Increased Intestinal Permeability (All Species)

4.1. Introduction

As discussed, intestinal permeability is regulated by the physical barrier created by epithelial cells and junctional complexes (Figs. 3 and 4), other factors such as immune cells and cytokines, and exogenous influences like intestinal pathogens, alterations in intestinal blood flow, and environmental factors such as temperature [21]. With multiple factors contributing to intestinal permeability and barrier function, an understanding of these complex interactions in both health and disease remains critical to the future development of therapeutics aimed at modulating intestinal barrier function.

4.2. Stress-Induced Barrier Dysfunction

A variety of stressors (physiologic, pharmacological, psychologic, and others) affect the intestinal barrier and have been studied using human and animal models [42]. Early models of acute stress in rats first demonstrated that physical restraint was enough to alter normal gastrointestinal function. Using an Ussing chamber, jejunal tissue from rats subjected to several hours of physical restraint was shown to have increased ion transport, increased tissue conductance, and increases in mannitol and Cr-EDTA flux consistent with altered intestinal permeability [43]. In addition, rats had increased plasma corticosterone compared with normal controls, consistent with a stress response. The effect of other physical “stressors,” including exercise, has been studied in a variety of species including humans

and dogs. Humans who participated in moderate exercise had decreases in jejunal absorption of sodium, chloride, potassium, and water [44]. Alaskan sled dogs undergoing sustained strenuous exercise demonstrated altered small intestinal permeability, with an increased lactulose:rhamnose ratio in both serum and urine [45]. The mechanisms of stress-altered permeability are likely related to changes in tissue perfusion and altered gastrointestinal motility; however, the effects seem to be intensity-dependent [46]. Exercise has been shown to reduce splanchnic blood flow [47] in an effort to preferentially provide enough blood to working tissues including the skeletal muscles and skin, leaving the gastrointestinal tract in a state of relative hypoperfusion. Ponies undergoing maximal exertion on a treadmill were found to have significant vasoconstriction of the renal and splanchnic vascular beds and resultant decreases in blood flow to the spleen, pancreas, small intestine, and colon, among other organs [48]. Shunting of the blood from visceral tissues to more active tissues during exercise can therefore result in varying degrees of intestinal damage including alterations in permeability [49,50]. Similar damage is reported in animal models of heat stress [29,42,51,52]. Following periods of extreme hyperthermia, blood is distributed preferentially to the periphery to help maximize heat dissipation, leaving the gastrointestinal tract in a state of hypoperfusion and ultimately leading to alterations in normal permeability. These alterations, secondary to heat damage, lead to increased concentrations of endotoxin and reactive oxygen species (ROS) [52], promoting significant damage to the intestinal mucosa by disrupting cellular membranes and the tight junctions [42]. This damage allows for the continued influx of endotoxins, primarily LPS, into systemic circulation, stimulating the production of proinflammatory cytokines and other immune responses [42]. Local and systemic inflammation can occur due to the increase flood of inflammatory cells. These responses can lead to systemic inflammatory response syndrome (SIRS) and ultimately multiple-organ failure [53,54]. Clinically, veterinary patients who suffer from heat stress have been shown to experience clinical signs that range in severity from mildly decreased feed intake and milk production (reported in dairy cows) to more severe consequences including altered immune responses [55] and death [56,57].

4.3. Gastrointestinal Ischemia-Reperfusion Injury

Interruption of the blood supply results in ischemic injury and the damage of metabolically active tissues including the intestine [58]. Prolonged ischemia alters membrane potential, disrupts normal ion distribution, impairs cytoskeletal organization of cells, and leads to increases in intracellular volume [59]. In addition, energy stores (ATP) become depleted, whereas the expression of proinflammatory cytokines, adhesion molecules, and bioactive agents such as endothelin and thromboxane A_2 become accelerated [59,60]. These agents act as potent vasoconstrictors and may lead to further alterations in blood flow. Restoration of normal blood flow may then further augment tissue injury in excess of that from ischemia alone, termed reperfusion injury. Reperfusion of ischemic tissue results in the formation of toxic ROS. These ROS have high reactivity with other biological molecules, inducing oxidative stress and directly damage cellular membranes via lipid peroxidation [61]. Reactive oxygen species increase leukocyte activation, chemotaxis, and adherence resulting in a vicious cycle of cellular damage as the activated leukocytes continue to release harmful proteases, elastases, and additional ROS [60,61]. Ischemia-reperfusion injury occurs in a variety of conditions including shock, vascular surgery, strangulated bowel, trauma,

intestinal volvulus, and intestinal transplantation, leaving both human and veterinary patients with a decreased chance of survival as the mucosal barrier is lost due to resultant tissue hypoxia, inflammation, and cellular infiltration [60,62]. Ischemia-reperfusion injury of the intestine is also associated with increased bacterial translocation into the systemic circulation, a contributing factor in the development of SIRS [63]. In horses, the survival rate for ischemic/strangulating lesions was lower than that for simple obstructions in a large retrospective study [64]. These lesions resulted in a higher rate of postoperative shock [65], likely due to barrier dysfunction and resultant endotoxemia. In horses, the intestinal damage found in natural obstructions is likely related to the severity and duration of ischemia and the subsequent reperfusion injury [66]. Intestinal ischemia is rarely preventable, and as a result, most research is aimed at targeting early detection and the recovery (postischemic) period, with efforts aimed at minimizing reperfusion injury and hastening epithelial repair.

4.4. Pathogen-Induced Barrier Dysfunction

The effects of pathogenic organisms on host intestinal epithelial cells are complex. These primary pathogen-host interactions may result in disturbances in the normal intestinal barrier, activation of the inflammatory cascade, and alterations of normal fluid and electrolyte secretion [67]. Enteric pathogens can bind to the cell surface and induce changes in the expression of tight junction proteins [21]. In addition, the production of toxins by pathogens can promote cellular damage through disruption of intracellular protein interactions, leading to increased cellular permeability, and ultimately trigger cell death [21]. While some pathogens primarily use one mechanism to alter host physiology, others, including *Salmonella* and *Escherichia coli,* are capable of altering the cellular functions of the intestinal epithelium through multiple mechanisms [67]. Although a detailed review of specific intestinal pathogens affecting both human and veterinary patients is outside the scope of this review article, several previous reports document important pathogens and their role in altering intestinal permeability including *Clostridium, E. coli, Bacteroides, Vibrio, Lawsonia,* and mycotoxin producing fungi such as *Fusarium* sp. [68–73].

Mycotoxin ingestion may be an inciting cause of leaky gut in pasture grazing animals. Pasture grasses, hay, and grain can all support the growth of various types of fungi, depending on the climate and season. Mycotoxins are secondary metabolites produced by fungi that when ingested or inhaled could result in adverse effects including gastrointestinal disease (vomiting and/or diarrhea) and alterations in growth and immune function in both humans and animals [73,74]. Common mycotoxins include aflatoxins, ergot alkaloids, fumonisins, ochratoxin and tricho- thecenes [75]. Deoxynivalenol, a mycotoxin of the trichothecenes family, has been shown to alter intestinal permeability and decrease claudin expression in porcine models [76]. A species sensitivity to mycotoxin exposure is documented, and research in horses is variable [74,77,78]. As horses ingest a variety of feed sources that could become contaminated with mycotoxins, there is a need for research in this field and its potential impact on intestinal health.

4.5. Altered Permeability in Inflammatory Bowel Disease

Inflammatory bowel diseases affect both human and veterinary patients and are associated with gastrointestinal dysfunction due to infiltration of the mucosa, submucosa, or lamina

propria with abnormal populations of immune cells. The two most common forms of chronic IBD in humans are CD and ulcerative colitis (UC) [79]. In dogs, the most frequently detected form of IBD is lymphocytic- plasmacytic enteritis [80]. Horses also suffer from several forms of inflammatory disease including eosinophilic enteritis, granulomatous enteritis, and lymphocytic- plasmacytic enteritis [81]. In advanced equine disease, the large intestine can be involved.

Although the definitive cause is not known, IBD is thought to result from inappropriate and ongoing activation of the mucosal immune system [82]. Genetic factors can contribute to the susceptibility of IBD in humans and studies have shown an increased prevalence of IBD among relatives of patients with CD and UC [83,84]. An association was also found between abnormal small intestinal permeability and a specific mutation in the NOD2 gene, which functions to modulate immune responses to intestinal bacteria, suggesting a genetic pathway in which an abnormal intestinal barrier could lead to chronic intestinal inflammation [85,86]. Other factors proposed in the pathogenesis of IBD include an initial breakdown of the epithelial barrier which can lead to a self-amplifying cycle of immune activation and cytokine release [79], and loss of tolerance to endogenous microflora and dietary antigens [82,87]. Since normal epithelial barrier function is determined in part by the integrity of the AJC, it was initially hypothesized that defects in this structure, made of tight junctions and adherens junctions, led to alterations in intestinal permeability seen in cases of IBD. Initial studies of inflamed epithelium from UC and CD cases, demonstrated reduced expression of the complex proteins, E-cadherin and α-catenin [88]. Additional studies showed the down-regulation of E-cadherin in UC cases, and the upregulation of P-cadherin in both CD and UC cases [89]. Similarly, in dogs with IBD compared with normal controls, the expression of the protein E-cadherin was lower in the villus epithelium, suggesting the role of this protein in the pathogenesis of IBD in dogs [80]. Claudin expression was not significantly different in this canine study. In cases of mild to moderately active ‘CD, alterations in tight junction structure and barrier dysfunction were associated with downregulation of claudins-5 and −8 and an upregulation of claudin-2 [90]. Structurally, both UC and CD cases have abnormal architecture with decreases in the number and complexity of tight junction strands, glandular atrophy, and chronic epithelial damage [79,91]. To the author’s knowledge, no studies have been conducted investigating the expression of AJC proteins and their potential contribution in the pathogenesis of equine IBD.

Immune activation also contributes to altered barrier function in cases of IBD. Early studies demonstrated different cytokine secretion patterns in cases of UC and CD, which may determine the type of inflammatory process present [92]. The proinflammatory cytokines IFN-ϒ and TNF-α are both elevated in the mucosa of IBD patients [93]. These cytokines have been shown to decrease epithelial barrier function in several model epithelial cell lines [91,94,95]. In vitro treatment with IFN-γ and TNF-α led to redistribution of AJC transmembrane proteins, junction adhesion molecule 1, occludin, and claudin-1/4 in an epithelial cell line [93]. Another inflammatory cytokine, IL-13 was shown to be upregulated in patients with UC [96]. Upregulation of IL-13 led to increased epithelial cell apoptosis, conductance, and upregulation of the claudin-2 gene [96]. In dogs with small intestinal enteropathies, there was greater expression of IL-2, IL-5, IL-12, TNF-α, and TGF-β in duodenal mucosa when compared with control dogs [97]. In horses with large intestinal

disease, a significant difference in TNF-α expression was found in diseased mucosa, suggesting a possible role for this cytokine in the pathogenesis of equine IBD [98]. Recent equine research found an involvement of proinflammatory T cells (T helper type 17) in active cases of IBD with greater expression of IL-17 in rectal biopsies [99]. This cytokine was also shown to be increased in human IBD patients [100]. In addition, TNF-α increases myosin light chain kinase (MLCK) phosphorylation [101], which may alter paracellular permeability through its association with actin and myosin (Fig. 3). Myosin light chain kinase expression and enzymatic activity are increased in cases of IBD and correlated with disease activity in one study [102]. It is possible that MLCK plays a role in the induction of intestinal barrier dysfunction in some cases of IBD.

A review of the literature demonstrates a variety of factors influencing the altered intestinal permeability seen in cases of IBD. The etiology is complex and likely involves the interaction between genetic, environmental, and immunological influences. What remains unclear is whether the changes observed within the tight junctions in cases of IBD are causal leading to further barrier dysfunction and abnormal immune responses, or the alterations in the function of tight junctions are related to the inflammation itself [101]. Regardless, further research is indicated in this complicated, multifactorial group of diseases that plagues both humans and veterinary species.

4.6. Microbiota, Diet, and Gastrointestinal Health

The intestinal tract is home to a vast, complex microbial ecosystem in all species. The resident microflora is often thought of as an organ system, in that it provides nourishment, regulates epithelial development, and plays a role in immune responses [103]. The gut microbial species composition is diverse and varies among individuals. Most host-microbiota interactions promote health; however, under certain conditions, such as environmental alterations, normally symbiotic organisms in the gastrointestinal tract have pathogenic potential, resulting in immune system activation and inflammatory disease [104,105]. A wide range of microorganisms have been suggested as causative agents of IBD; however, no single pathogenic agent has been routinely isolated in clinical cases [106]. It is likely that a dysbiosis arises in these conditions in which a decrease of beneficial bacteria and their metabolic byproducts occurs along with an increase in detrimental microbial populations and their toxic metabolites, leading to an altered luminal environment [106]. In work predominantly based on animal models, certain symbiotic microorganisms have been shown to initiate gut inflammation and pathology when colonizing a genetically susceptible host (e.g., *Helicobacter* and segmented filamentous bacteria). In other cases, specific resident microflora can expand following antibiotic use, clearing competing symbiotic organisms, and promoting disease (e.g., *Clostridium difficile)* [104]. In cases of canine dysbiosis, compositional changes in the small intestinal microbiota have been suggested [107].

In horses, the diverse bacterial population of the gastrointestinal tract has been characterized using fecal samples and varies greatly among the different intestinal compartments [108,109]. With such diversity, it is thought that many of the risk factors for gastrointestinal diseases are related to disruption of the intestinal microbiota and function [110]. Increased

risk of colic has been associated with changes in management and nutrition including high amounts of concentrate, forage digestibility, and changes in diet [111–113]. Similarly, alterations in the horse intestinal microbiota have been shown in relation to diet change [114], dietary starch source and concentration [115], systemic antimicrobial usage [116], and administration of excessive carbohydrates [117]. Therefore, it is presumed that alterations in the microbiome may be detrimental to the horse, resulting in abnormal intestinal permeability and subsequent diarrhea, endotoxemia, and laminitis [117,118]. Indeed, disruption of fecal microbiota populations has been associated with incidence of colic [119,120] and colitis [121]. In particular, *Fusobacterium* were shown to be abundant in horses with colitis compared with healthy horses, and this genus has been associated with CD and appendicitis in humans [121].

It is well-recognized that the proportion of complex carbohydrates or fiber (typically found in the diet as long-stem forages) to more simple carbohydrates (such as starches and sugars), as well as abrupt changes in diet, cause fluctuations in the populations of microbes within the hindgut of the horse [109,122–124]. As such, different proportions of fermentative by-products, such as short chain fatty acids (SCFAs, also known as volatile fatty acids [VFAs]), methane, and hydrogen are produced depending on dietary carbohydrate composition. Higher proportions of starch and sugar intake result in higher production of hydrogen, lactate, and propionate, whereas higher intakes of fiber result in increased acetate production [122,125].

Starch intake can be quite high as concentrates are often fed to athletic horses to meet energetic demands. Potter et al [126] showed an upper limit to preileal starch digestion, such that starch intake over 3 g per kg body weight results in a “spillover” of undigested starch to the large intestine. Starch reaching the hindgut increases total VFA production, lactic acid production and results in a drop in pH and acidosis [127]. Acidosis may damage the epithelium and alter permeability, as hyperpermeability has been reported in both cattle and swine mucosa with acidosis [128,129]. Therefore, both microbial composition and dietary composition affect intestinal health. Further research is needed both in healthy and disease states to determine the critical role that the microbiota and diet may play in the progression of disease.

5. Alternative Therapeutic Opportunities in Leaky Gut

Conventional therapies, including the use of systemic antimicrobials and surgical resection of nonviable intestinal tissue remain the cornerstone to successful treatment of several causes of leaky gut. Leaky gut and altered intestinal permeability result from a variety of factors including physical stressors, immune dysfunction, and disruption of normal gut homeostasis. As a result, research regarding manipulation of the intestinal barrier often focuses on therapies aimed at the regulation of host immune and inflammatory responses along with epithelial barrier function. The use of alternative therapies including SCFAs, amino acids, nutrients, and probiotics/prebiotics has been investigated in natural and induced models of altered intestinal permeability in several species. Limited research has been performed with many of these immunomodulatory therapies in the horse, leaving a critical gap in our knowledge as clinicians.

5.1. Short Chain Fatty Acids

The three major SCFAs produced during bacterial carbohydrate fermentation are acetate, propionate, and butyrate. They are readily absorbed and metabolized into energy [130]. Butyrate is the preferred substrate for enterocytes and several studies have shown that SCFAs have anti-inflammatory properties [131]. In an experimental model of 5-fluorouracil-induced mucositis in mice, butyrate treatment improved intestinal permeability and minimized intestinal damage [132]. In a dextran sulfate sodium (DSS)-induced colitis model, mice receiving sodium butyrate in the diet had reduced mucosal inflammation, improvement in diarrhea and an improved inflammatory profile in local lymph nodes [130]. In piglets with acetic acid-induced colitis, butyrate supplementation (in the form of tributyrin) decreased colonic lymphocyte infiltration, decreased expression of cell death marker caspase-3, and increased expression of tight junction protein claudin-1 in the colonic mucosa, suggesting that butyrate may alleviate injury by decreasing cellular apoptosis and improving tight junction formation [133].

The success of butyrate treatment in vitro has yielded variable results. Using an in vitro medium, butyrate and propionate treatment was shown to improve intestinal cell proliferation in human biopsy samples [134]. Sodium butyrate (4 mM) was able to increase wound healing in porcine small intestinal epithelial cells and enhance the expression of tight junction proteins occludin and zonula-occluden protein-1 [40]. A dose-dependent effect of butyrate was demonstrated in vitro using caco-2 cells, in which low concentrations (2 mM) promoted intestinal barrier function, whereas excessive butyrate induced cell apoptosis and increases in inulin permeability [39]. In an equine in vitro model of oxidant-injured right dorsal colon, butyrate treatment (20 mM) did not influence mucosal restitution [135].

Endogenous butyrate production is another potential therapeutic mechanism for improving gut health. In the poultry industry, where antimicrobial growth promoters are now banned, there is significant interest in the role of intestinal butyrate production for pathogen control and for optimizing the intestinal barrier. Increasing butyrate production is a function of both the microbial population (butyric acid producing bacteria) and the diet. The *Ruminococcaceae* and *Lachnospiraceae* are major butyrate producing families, although some clusters of *Clostridium* are also important [136]. Providing these bacteria to the animal directly are difficult because they are strict anaerobes, while prebiotics that stabilize these bacterial families have promise. Prebiotics and probiotics are described further below.

Given the beneficial effects of butyrate and other SCFA noted in a variety of animal models, the use of these therapies warrants consideration in the treatment of equine gastrointestinal disease; however, further research is needed at this time.

5.2. Amino Acids

Amino acids are essential substrates required for protein, nitric oxide, and polyamine synthesis, and serve as a major fuel for the small intestinal mucosa [131,137]. Several studies support the potential therapeutic benefit of amino acids including glutamine, arginine, lysine, threonine, and others in gut-related disease [137].

Arginine, a conditionally essential, versatile amino acid, serves as a precursor for protein synthesis and other important molecules including nitric oxide, urea, and creatine [138]. Arginine has been shown in vitro to improve barrier function in caco-2 cells injured with a commonly used immunomodulatory medication (methotrexate) [139]. In several animal models of intestinal disease, intestinal permeability was maintained by arginine administration [131]. Oral arginine supplementation decreased intestinal mucosal injury following lipopolysaccharide treatment in rats [140]. Recent work demonstrated the safety of arginine supplementation in healthy rats, pigs, and gestating sheep [141] and previous research demonstrated tolerance to long-term supplementation in neonatal and pregnant pigs [142]. Limited studies in horses have demonstrated the successful absorption of arginine in normal mares; however, arginine supplementation altered the absorption of other amino acids [143]. Research is warranted to determine if arginine supplementation may benefit horses with gastrointestinal disease.

Glutamine, a nonessential amino acid with several important functions, accounts for the majority of energy generated in the small intestine along with glutamate and aspartate [131,144]. Glutamine is considered “conditionally essential” in times of stress and disease [145]. Depleted levels of glutamine have been associated with an impaired stress response in human lymphocytes in vitro [146]. Glutamine supplementation was found to decrease jejunal atrophy in weaned pigs and improved growth performance [147]. In weaned piglets, supplementation of glutamine increased the expression of genes necessary for cell growth, and reduced expression of genes that may promote oxidative stress in the small intestine when compared with age-matched controls [148]. Intravenous supplementation of glutamine attenuated the degree of intestinal damage seen in experimental ischemia/reperfusion in rats with reductions in leukocyte infiltration, improved histologic damage scores, and decreased apoptosis [149]. In a DSS-induced colitis model, mice fed glutamine had decreased expression of chemokine receptors and had less colonic T-cell infiltration compared with controls thereby decreasing inflammatory mediators in treated individuals [150].

In horses, glutamine was shown to increase the efficiency of intestinal restitution in an in vitro model of colitis [135]. This is similar to other in vitro experiments demonstrating the role glutamine may play in preventing intestinal cell damage [139,151]. Previous work showed that plasma glutamine concentrations could be increased in the short-term when administered orally to healthy horses [152]. A more recent feed trial in young horses showed no adverse effects of a supplemental product containing L-glutamine [153]. At this time, no research has been performed looking at the effects of glutamine supplementation in horses with gastrointestinal disease, and additional research is necessary to determine an appropriate dose and potential efficacy in diseased gut.

5.3. Other Nutrients

Zinc is a trace element which functions in cellular turnover, regulation, and repair and is a key component of several enzyme systems [1,154]. Several in vivo animal models have demonstrated the potential for zinc to enhance intestinal function in disease. Zinc supplementation helped maintain the stability of the intestinal microflora and diversity of coliforms in treated pigs when compared with control pigs for 2 weeks post weaning [155].

In experimental colitis, zinc supplemented rats had less diarrhea and less weight loss, but no effect on macroscopic inflammation [156]. In a similar model, rats supplemented with zinc had a significant reduction in opened tight junctions when compared with untreated rats with colitis, but no amelioration of clinical disease [157]. A study in broiler chickens supplemented with zinc and challenged with *Salmonella*, demonstrated improved intestinal morphology following infection, lowered plasma endotoxin levels, and enhanced expression of claudin-1 and occludin from ileal mucosa when compared with controls [158]. Following a challenge with enterotoxigenic *E. coli,* piglets supplemented with zinc oxide had reduced expression of inflammatory immune response genes [159]. In humans with quiescent CD, zinc supplementation resulted in improved alterations in intestinal permeability [160]. Serum zinc concentrations following oral supplementation of two different zinc compounds has been investigated in ponies [161], but to date no studies have been performed assessing zinc supplementation in horses with gastrointestinal disease.

Selenium and vitamin E may prove beneficial in improving intestinal permeability. The effects of selenium and vitamin E supplementation were studied on heat-stressed pigs and shown to reduce oxidative stress and improve intestinal permeability when fed in high concentrations [162]. Further data and trials are needed to rationalize the use of vitamin E and selenium for gastrointestinal diseases in horses and other species.

5.4. Probiotics, Prebiotics, and Synbiotics

Probiotics are living microorganisms that when supplemented in certain numbers could offer a beneficial effect to the host. This is in contrast to a prebiotic, which is typically a nondigestible food ingredient that may benefit the host intestinal microflora by stimulating growth and activity of a few selected organisms [163]. Prebiotics often include oligosaccharides that can resist normally produced digestive enzymes, but remain susceptible to fermentation by the colonic microflora [148]. The combination of a prebiotic and probiotic, termed a synbiotic, may offer synergistic therapy [164]. An extensive review of these therapies is outside the scope of this paper, however, the authors direct readers to several published reviews [164–168].

The potential benefits of probiotic use are diverse and may include immune system activation and modulation, enhanced mucosal barrier function, competitive exclusion of pathogens, and decreased risk of infection through production of antimicrobial substances including lactic and acetic acids [131,166]. Probiotics have been used in the treatment and prevention of IBDs, diarrhea, irritable bowel syndrome, and gastroenteritis, among others [169]. Although several organisms have been studied, commonly used species include *Bifidobacterium*, *Lactobacillus*, and *Saccharomyces* [169].

In two murine models of colitis, treatment with *Bifidobacterium bifidum* improved histologic scores and decreased several inflammatory markers [170].

Mice with DSS-induced colitis had improved survival and barrier function following treatment with heat-killed *Lactobacillus brevis* compared with control mice [171]. Using a similar model, *Lactobacillus rhamnosus* was shown to increase the expression of the junctional complex component zonula-occludin-1 and improve intestinal permeability in

treated mice [172]. A recent study demonstrated that *Lactobacillus* fed to broiler chickens subjected to heat stress improved average daily gain [173] when compared with controls.

When *Saccharomyces boulardii* was added as an adjunctive therapy to mesalamine, an anti-inflammatory medication used in the management of CD, the rate of clinical relapse decreased compared with mesalamine alone [174] suggesting that this organism may be useful in improving the effectiveness of commonly used medications for IBD management.

Similar to other species, probiotics commonly used in large animals include the bacterial genera *Lactobacillus*, *Enterococcus, Bifidobacterium,* and *Streptococcus,* and the yeast *Saccharomyces* [110]. In horses, there is limited research regarding the use of probiotics and the results are variable [110,166]. Most studies investigating the use of probiotics in horses have studied the effect of *Saccharomyces cerevisiae* administration to improve hindgut fermentation and diet digestibility in healthy horses. Unfortunately, results are mixed [175–177]. In horses fed a high starch diet, *Lactobacillus acidophilius* alone or in combination with several other species of bacteria including *B. bifidum* and *Enterococcus faecium* had limited effects on nutrient digestibility and did not reduce the risk of acidosis (determined from fecal pH and fecal VFA concentration) compared with controls [178].

There is limited research evaluating probiotic use in horses with gastrointestinal disease [166]. Horses with acute enterocolitis administered *S. boulardii* had a shorter duration of diarrhea than those fed a placebo [179]. However, there was no significant difference in duration of hospitalization or outcome in the two groups. Treatment with *S. boulardii* was also evaluated in horses with antimicrobial-associated enterocolitis [180]. Although the organism could be successfully cultured from more than half of the horses receiving it, there were no statistically significant differences in any of the outcome measures. In other large animal species, probiotics have been used to help control *Salmonella* infection [181–183]. In horses, postoperative colic patients treated with two different probiotic products (containing combinations of organisms including *Lactobacillus* sp., *Streptococcus* sp., and *Bifidobacterium* sp.) had no difference in *Salmonella* shedding compared with placebo-treated horses [184]. Of note, no adverse effects were noted when probiotic administration exceeded the manufacturers recommended dose [184]. Horses treated with a multistrain probiotic had no difference in *Salmonella* shedding compared with control horses in a later trial [185].

Prebiotics that have been used in poultry to increase butyrate production include xylo-oligosaccharides (XOS) and other oligosaccharides. Work in poultry showed that XOS supplementation increased the conversion of lactate to butyrate [186]. In the horse, fructooligosaccharides (FOS) were shown to increase fecal butyrate concentrations [187], whereas Gürbüz et al [188] reported no effect of FOS on fecal pH, VFA composition, or immune status. Short-chain FOS supplementation mitigated an increase in lactate concentration following an abrupt introduction of barley (starch overload) [189].

Given the variability of results and the diversity of the equine microflora, additional studies are needed before excluding any potential beneficial effects of probiotics or prebiotics in horses with gastrointestinal disease.

6. Conclusion

There are many causes of altered intestinal permeability, and impairments in barrier function can have devastating consequences on the health of an individual (Fig. 1). To better understand the effects of leaky gut, clinicians and scientists must recognize the multiple factors that influence barrier function including the host immune response, barrier permeability, resident microflora, and others. A variety of tools are available in vitro, ex vivo, and in vivo to test intestinal permeability, and each offers unique advantages and disadvantages.

As our knowledge and understanding of normal and altered barrier function continues to expand, the ability to manipulate and modify intestinal permeability through the use of novel therapeutics remains promising. Most therapeutic research remains centered around experimental models of disease in rodents with limited work in the larger species, arguably narrowing the utility of these findings in horses on a clinical level. As reviewed above, alterations in intestinal permeability can be complex and multifactorial, offering researchers and clinicians several avenues to aid in disease recognition and diagnosis, investigate pathogenesis, and develop therapies to improve outcome and hopefully, in the future, prevent these diseases from occurring. With regards to the horse, additional research is critically needed and should use models of both healthy horses and those suffering from gastrointestinal disease. Several of the therapies discussed above, including SCFAs such as butyrate, essential nutrients including zinc and probiotics/prebiotics may offer horses similar benefits to those demonstrated in other species, ultimately improving the health and well-being of these animals. Before these therapies can be used clinically; however, studies on efficacy and safety are warranted and remain crucial to successful treatment of these complicated, often life-altering, conditions.

Acknowledgments

The authors wish to acknowledge Kenneth J. Kopp, DVM for his assistance in editing this review article, Lance H. Baumgard, PhD, Iowa State University, for providing his expertise and reviewing the final paper, and Alice Mac-Gregor Harvey, NC State College of Veterinary Medicine, for the graphical illustrations in this text. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Preparation of this paper was supported by Kemin Industries (Kemin Animal Nutrition - Equine Division).

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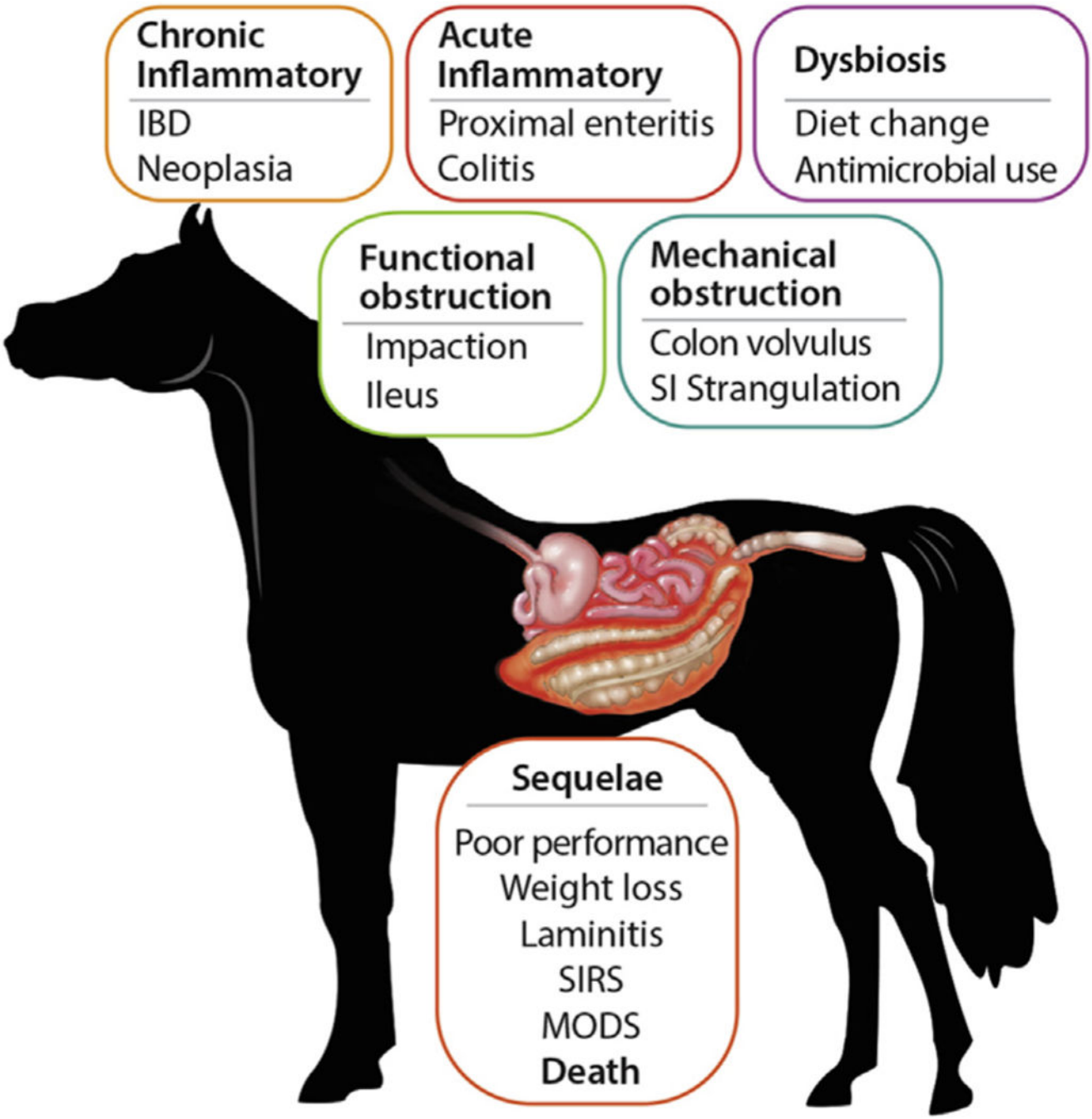

Fig. 1.

Altered intestinal permeability (leaky gut) in horses. Specific causes of leaky gut in horses range from alterations in the microbiota, acute and chronic inflammatory disease, and both mechanical and functional intestinal obstructions. These conditions alter permeability in several ways including disruption of normal blood flow to the intestine, increased production of proinflammatory cytokines, and disruption of the normal cell junctions, among others. Sequelae to leaky gut may include weight loss and poor performance in mild cases and SIRS, MODS, or death in severe cases. IBD, inflammatory bowel diseases; MODS, multiple

organ dysfunction syndrome; SI, small intestinal; SIRS, systemic inflammatory response syndrome.

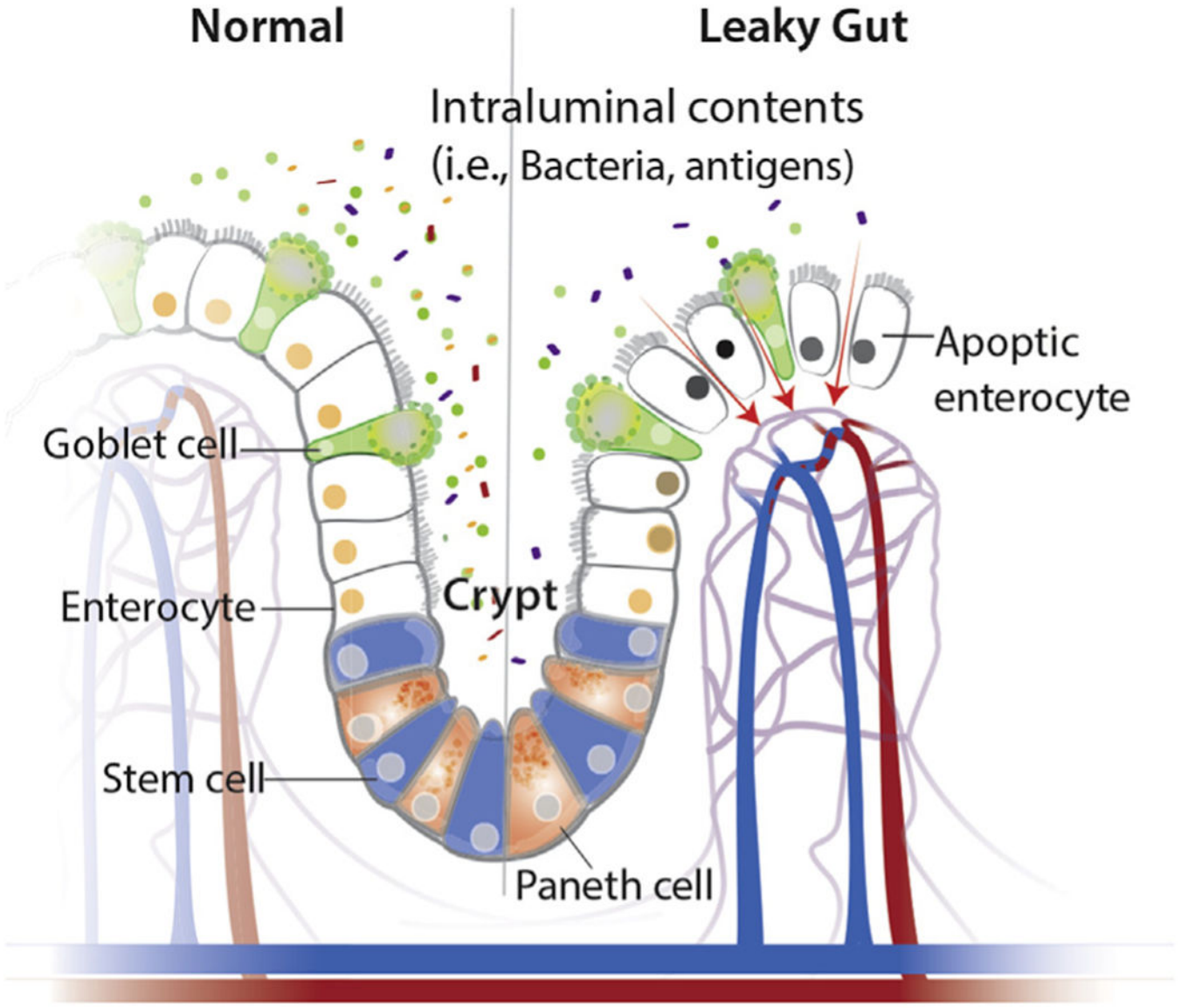

Fig. 2.

Components of the mucosal barrier in health and disease. The normal intestinal barrier is made up of a single layer of epithelial cells, with normal cell death (apoptosis) and turnover every 5–7 days. Undifferentiated stem cells are located at the crypt base and interspersed between post-mitotic Paneth cells. When intestinal permeability is altered, the junctions between the cells are disrupted and luminal contents can enter the surrounding tissues as well as systemic circulation. As a result, cells may undergo increased apoptosis and decreased barrier function.

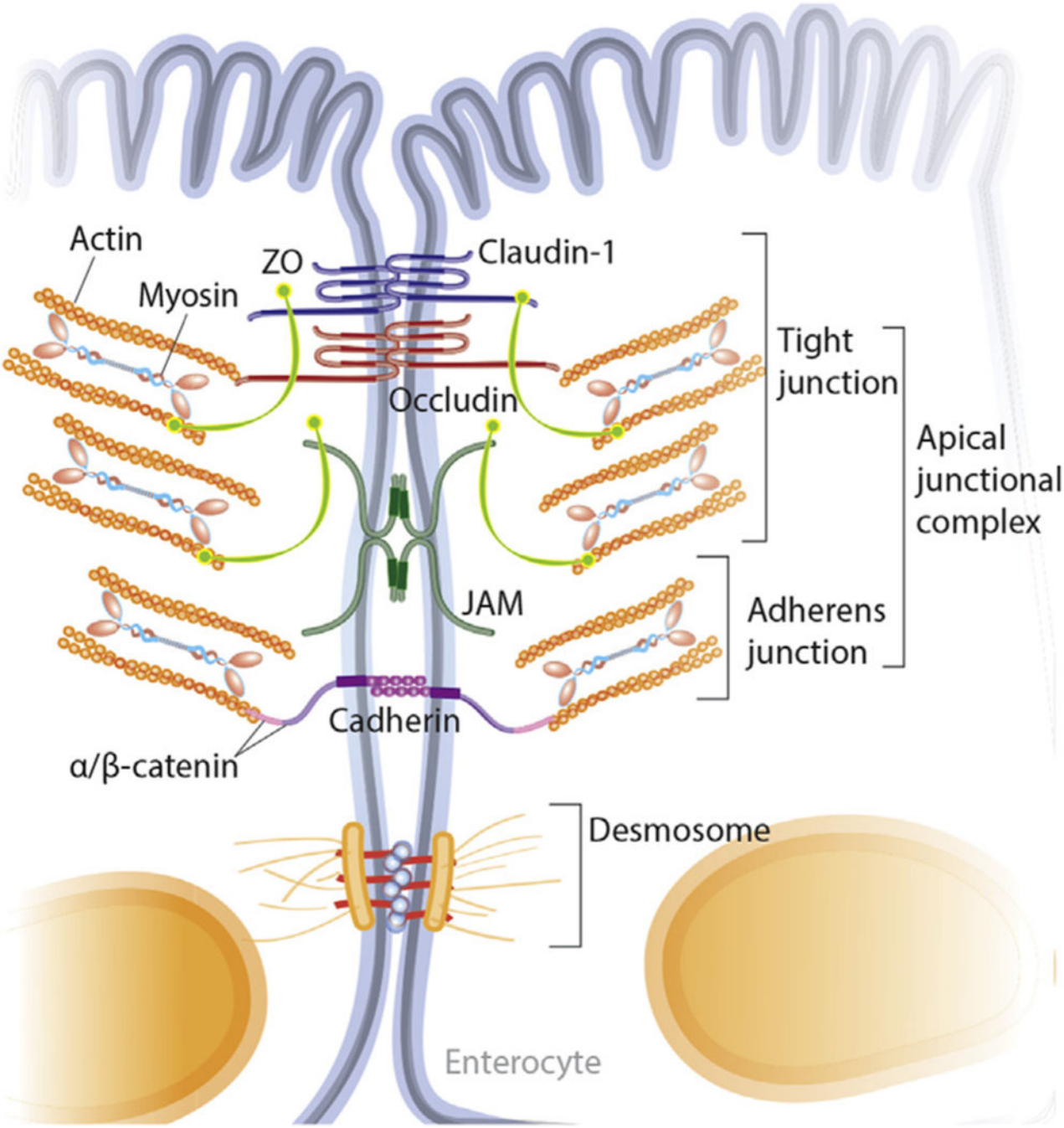

Fig. 3.

Detailed diagram of the intercellular junctions between intestinal epithelial cells. The intercellular junctions of intestinal epithelial cells are composed of several complexes including TJs, AJs, and desmosomes. The AJs and TJs together form the AJC. TJs are located at the apical end of the epithelial cells and are made up of multiple transmembrane proteins including occludins, claudins and JAM. Scaffolding proteins such as ZO in turn anchor the membrane proteins to the actin cytoskeleton. Simply, permeability is regulated at the TJ through actin and myosin contractility. Cell to cell contact at the AJ is mediated by

adhesion molecule complexes made up of protein families including the cadherins and catenins. AJs, along with desmosomes, provide strong connective bonds between epithelial cells. AJ, adherens junction; AJC, apical junctional complex; JAM, junctional adhesion molecules; TJs, tight junctions; ZO, zonula occludens.

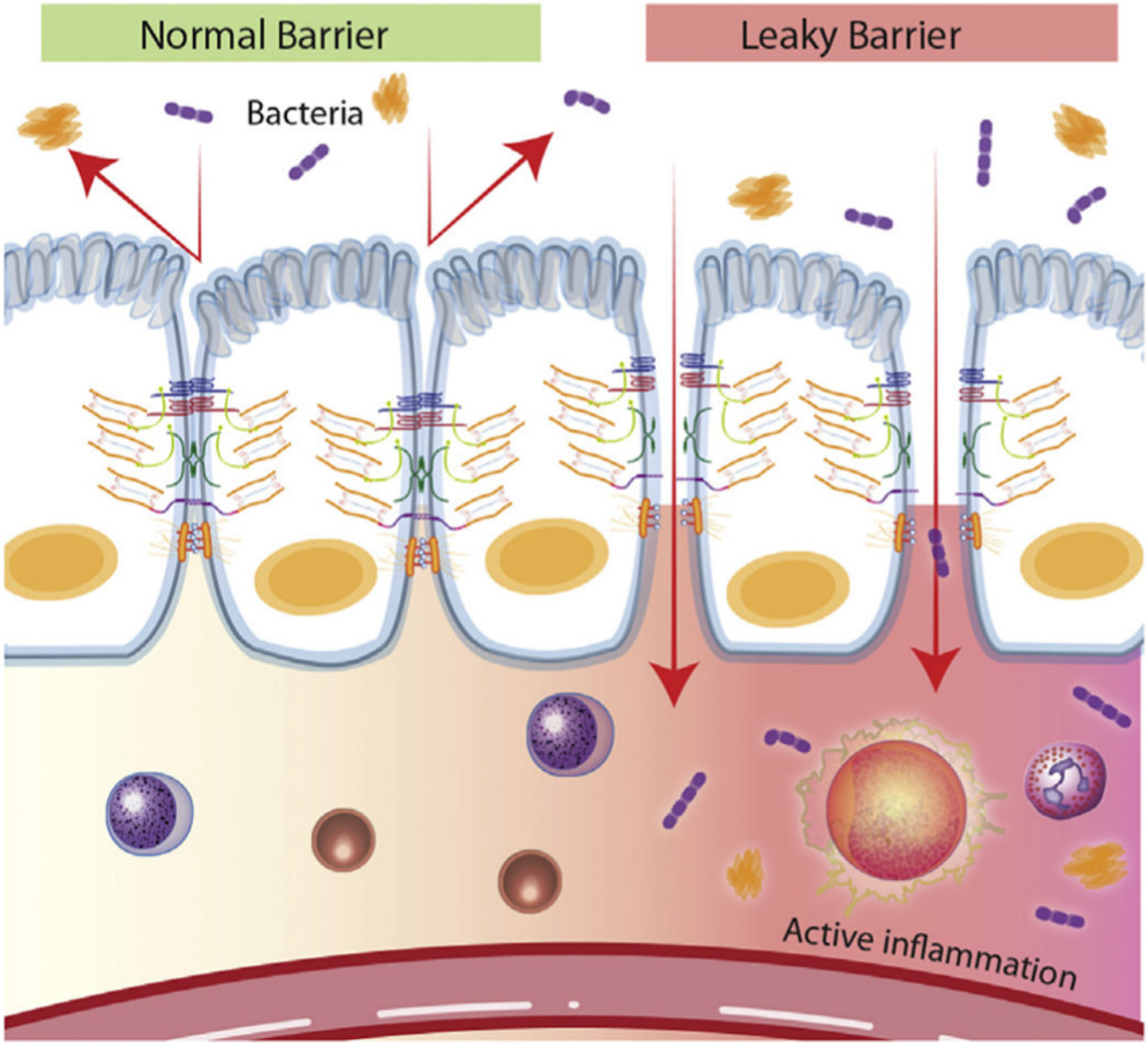

Fig. 4.

Normal versus impaired barrier function. The role of the intestinal epithelium is to provide a physical barrier against luminal contents such as bacteria. There are several important components of the barrier including tight junctions, adherens junctions, and desmosomes. Potentially harmful molecules cannot normally penetrate the barrier; however, when the barrier becomes compromised at any contact point, the passage of noxious molecules can occur and result in both an inflammatory and immune response. Disruption of the intestinal barrier may result in the development of local and systemic disease.