Rules of Engagement: Epithelial-Microbe Interactions and Inflammatory Bowel Disease

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Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex, multifactorial disorders that lead to chronic and relapsing intestinal inflammation. The exact etiology remains unknown, however multiple factors including the environment, genetic, dietary, mucosal immunity, and altered microbiome structure and function play important roles in disease onset and progression. Supporting this notion that the gut microbiota plays a pivotal role in IBD pathogenesis, studies in gnotobiotic mice have shown that mouse models of intestinal inflammation require a microbial community to develop colitis. Additionally, antimicrobial therapy in some IBD patients will temporarily induce remission further demonstrating an association between gut microbes and intestinal inflammation. Finally, a dysfunctional intestinal epithelial barrier is also recognized as a key pathogenic factor in IBD. The intestinal epithelium serves as a barrier between the luminal environment and the mucosal immune system and guards against harmful molecules and microorganisms while being permeable to essential nutrients and solutes. Beneficial (i.e., mutualists) bacteria promote mucosal health by strengthening barrier integrity, increasing local defenses (mucin and IgA production) and inhibiting pro-inflammatory immune responses and apoptosis to promote mucosal homeostasis. In contrast, pathogenic bacteria and pathobionts suppress expression and localization of tight junction proteins, cause dysregulation of apoptosis/proliferation and increase pro-inflammatory signaling that directly damages the intestinal mucosa. This review article will focus on the role of intestinal epithelial cells (IECs) and the luminal environment acting as mediators of barrier function in IBD. We will also share some of our translational observations of interactions between IECs, immune cells, and environmental factors contributing to maintenance of mucosal homeostasis, as it relates to GI inflammation and IBD in different animal models.

Keywords: IBD, microbiota, dog, mouse, intestinal permeability, epithelial barrier

INTRODUCTION

The inflammatory bowel diseases (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are complex, multifactorial inflammatory diseases affecting the gastrointestinal (GI) tract (1, 2). IBD is an immune-mediated disorder comprising two distinct phenotypes having varying clinical, endoscopic, immunologic and histopathologic features (3, 4). Crohn’s disease is characterized by
patchy, transmural inflammation that primarily affects the terminal ileum but can also involve the small intestine. Ulcerative colitis causes diffuse superficial mucosal ulcerative inflammation restricted to the rectum and colon. The cause for IBD remains unknown but it is likely that genetically susceptible individuals develop an aberrant immune response to their microbiota, leading to chronic inflammation and repetitive injury to the intestines (2). The onset of IBD typically occurs in the second or third decade of life but rising incidence worldwide suggest a prominent role for environmental factors (5).

The intestinal epithelium is composed of a monolayer of columnar epithelial cells that communicate continually with the luminal microbiota and an underlying network of innate and adaptive immune cells. This mucosal barrier normally prevents the entry of pathogenic microbes and toxins while regulating the absorption of nutrients, electrolytes, and water from the lumen into the systemic circulation (6). There is a growing body of data indicating that dysfunction of the intestinal barrier is a causative factor in the pathogenesis of IBD. For example, numerous IBD genetic risk loci affect pathways active in epithelial cells involved in essential functions such as innate immunity, autophagy and endoplasmic stress (7). Moreover, epithelial barrier dysfunction secondary to chronic inflammation and recurring “flares” is characteristic of IBD (8). During active disease, inflammatory mediators (cytokines/bacterial products) released in the intestinal mucosa progressively damage the epithelium and expose mucosal immune cells to luminal antigens that amplify the inflammatory response (3, 9). Finally, the intestinal epithelium is actively involved in repair mechanisms that promote mucosal healing through re-epithelialization to patch defects and maintain mucosal homeostasis (10, 11). Also contributing to maintenance of the mucosal barrier is the controlled replenishment of intestinal epithelial cell (IEC) subtypes (e.g., columnar cells, goblet cells, enteroendocrine cells and Paneth cells) from LGR5 intestinal stem cells (12). In this review, we will focus on the role of IECs and the luminal environment (including the microbiota) to act as mediators of barrier function in IBD. We will also share some of our translational observations of interactions between IECs, immune cells, and environmental factors (including the gut microbiota) contributing to loss of mucosal homeostasis as it relates to GI inflammation and IBD in different animal models.

### Intestinal Barrier and Mucosal Homeostasis

#### Structural Components of the Epithelial Barrier

The term *mucosal barrier* was first proposed by Cummings in 2004 and describes the complex structure that separates the luminal environment from the internal milieu (13). The intestinal mucosal barrier is a functional entity consisting of separate but interlinked components, including physical elements (e.g., the underlying vascular endothelium, epithelial cells, and the mucus layer), along with a chemical layer composed of digestive secretions, immune molecules, and cellular products (cytokines, inflammatory mediators, and antimicrobial peptides). Apart from these layers, the microbiota also contributes to barrier integrity along with immune functions and GI motility. The intestinal epithelium is composed of a single layer of columnar cells and different specialized cell subtypes: enterocytes, goblet cells, Paneth cells, enteroendocrine cells and immune cells, including intraepithelial lymphocytes (IELs) and dendritic cells (Table 1; Figure 1) (15). Three types of junctional complexes [tight junctions (TJ), adherens junctions (AJs) and desmosomes] provide mechanical cohesion to these columnar cells and seal the paracellular space to regulate the movement of water ions and small molecules across the intestinal mucosa (16–18).

Tight junctions form the most apical adhesive (JP) and are continuous around the IEC at the border between apical and lateral membrane regions (16–18). They function as a semi-permeable paracellular barrier that move ions and solutes through the intercellular space while excluding luminal antigens, bacteria and their toxins. Within TJ complexes are integral transmembrane proteins, occludin and members of the claudin family, that link adjacent cells to the actin cytoskeleton to regulate paracellular permeability (16). Claudins represent a family of TJ proteins that regulate the movement of water and electrolytes through sealing molecules and pores. Experimental

| TABLE 1 | Components of the intestinal epithelial barrier and their perturbation in IBD. |
|----------|-----------------|----------------------|
| **Components** | **Function** | **Known defects** |
| **Physical Barrier** | | |
| Mucus layer | Adherent and loose layers, contain AMPs and microbiota | Reduced thickness to mucus layer, bacterial biofilm with CD, altered composition to mucus layer |
| Enterocytes | Digestion, macromolecule transport, secrete β-defensins | Defective defense production, mucosal ulceration/erosions |
| Goblet cells | Secrete mucin and trefoil factors | Decreased number of goblet cells |
| Paneth cells | Secrete α-defensins, Reg3 proteins, lysozyme, BMPs for ISC niche | Reduced antimicrobial activity |
| Enteroendocrine cells | Produce serotonin and 5-HT; sense microbial metabolites | Altered enteroendocrine secretion |
| Intercellular junctions | Intercellular transport, regulate barrier function | Altered expression and localization |
| Intra-epithelial lymphocytes (IELs) | Immune surveillance, cytotoxic activity | Imbalance in IEL cytolytic and regulatory functions |
| Dendritic cells | Antigen sampling | Increased activation promoting inflammation |
| Plasma cells | Produce secretory IgA (sIgA), help maintain ISC niche | Increased in number, increased granzyme B and cytotoxic activities |
| **Chemical Barrier** | | |
| Digestive secretions | Degrade nutrients and bacteria | Altered secretions |
| Anti-microbial peptides (AMPs) | Bacterial degradation and exclusion | Reduced antimicrobial activity |
| Cytokines, inflammatory mediators | Promote inflammation | Increased production contributing to repetitive mucosal injury |

**BMPs**, bone morphogenic proteins; 5-HT, 5 hydroxytryptamin; ISC, intestinal stem cell; CD, Crohn’s disease.
studies indicate that differential claudin expression (either up- or down-regulation) is associated with impaired barrier function (19, 20). The important TJ adapter proteins, zonulin occludens (ZO) -1, -2 and -3, connect the cytoskeleton to the transmembrane TJ proteins. Underneath the TJs are the AJs that are important for cell-to-cell signaling and epithelial restitution, while desmosomes provide structural stability between the IECs (16, 21). Summarizing, the intestinal epithelium maintains its selective barrier function through the formation of complex protein-protein networks that mechanically link adjacent IECs to selectively seal the intercellular space.

The expression pattern of JPs is tightly regulated and varies by intestinal compartment (small vs. large intestines), villus/crypt location, and cell membrane location (apical, lateral or basolateral). The expression of AJ and TJ proteins is a dynamic process that is steadfastly regulated by phosphorylation causing both beneficial and harmful consequences (22–24). For example, phosphorylation can either promote TJ protein formation to enhance barrier function or alternatively it can disrupt and redistribute TJ and AJ proteins to increase intestinal permeability (25, 26).

The human intestinal epithelium constantly renews itself every 4–5 days under normal homeostasis, with the pace of renewal increasing following damage. Regulating this process are pluri-potential stem cells that give rise to all GI epithelial cell lineages and can generate whole intestinal crypts (12). At the tips of villi and along the epithelia of the colon, mature cells undergo apoptosis and are normally shed into the GI lumen. Intestinal stem cells (ISCs that express LGR5) can differentiate into four specialized cell types, including columnar cells (enterocytes and colonocytes), goblet cells, enteroendocrine cells and Paneth cells (the latter cell type found only in the small intestine) (15). Columnar cells are the most abundant epithelial cell found in the small and large intestines and are involved in absorption. Goblet cells produce and secrete mucin (e.g., mucin-2) which covers the surface of the intestinal epithelium. Antimicrobial peptides and lysozyme further fortify the antimicrobial properties of the mucus compartment to promote antigen elimination. Paneth cells produce lysozyme and several antimicrobial peptides to protect against microbial infection including α-defensins and Reg3 proteins (27, 28). They also reside adjacent to ISCs and provide the necessary growth factor (e.g., Wnt, EGF or Notch) signals to the ISCs and constitutes the stem cell’s niche (12). Epithelial cells secrete β-defensins in response to sensing of microbes by their pattern recognition as either commensal bacteria or pathogens. Secretory immunoglobulin
A (sIgA) is produced by plasma cells to mediate protection at mucosal surfaces by binding bacteria and viruses to prevent their attachment to or invasion of IECs (i.e., immune exclusion) (29). Finally, the resident bacteria provide a deterrent to microbial invasion and maintenance of mucosal homeostasis through competitive exclusion, nutrient utilization, niche localization and their production of bacteriocins (30).

**Intestinal Barrier Permeability Pathways**

The intestinal epithelium serves as the primary compartment of the mucosal barrier and uses both transcellular and paracellular mechanisms to transport substances from the lumen into the lamina propria. The transcellular pathway primarily transports nutrients and compounds having high molecular weight (>600 Da) by means of endocytosis or carrier-dependent transport systems. The protein complexes interconnecting enterocytes (i.e., TJ, AJ, and desmosomes) are dynamic key modulators that allow for the paracellular transport of water, small solutes and electrolytes between enterocytes while restricting the passage of microbes and large molecules (31, 32). Since paracellular transportation occurs through the space between cells, it is less selective as compared to the transcellular pathway which is regulated by membrane channels. Taken together, these two pathways selectively regulate the degree of permeability for substances having different physiochemical properties, such as variable size and ionic charge, into the lamina propria. Any impairment in the integrity or function of these transporting routes increases intestinal permeability which is implicated in the pathogenesis of several GI and extra-GI diseases (i.e., having local or systemic manifestations) such as IBD, celiac disease, type I diabetes, and emotional stress (33, 34).

The gut microbiota, which contains $10^{14}$ bacteria and 100-fold more genes than the entire human genome, has a pivotal role in development of the host immune system and metabolism (35). A well-balanced symbiotic relationship between the gut microbiota and the host is required for maintenance of mucosal homeostasis. There are approximately 1,000 different bacterial species within five dominant phyla (i.e., Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia) that comprise the healthy human fecal microbiota (36). In contrast, the core gut bacteria in the feces of specific pathogen free (SPF) mice contains 37 genera (37). In this group, Anaerostipes spp were present in all mice and are an important butyrate producing bacterial species contributing to mucosal barrier integrity. Another mucine microbe with high prevalence is *Parabacteroides* spp which are important in stimulating host immunity. The other dominant mucine bacteria include carbohydrate-utilizing and lactate and/or acetate-producing microbes such as *Bifidobacterium* spp and *Lactobacillus* spp. These observations suggest that the composition of a core microbiome within a species is essential for maintaining gut homeostasis and are reflective of overall host health to a variable extent.

**Methods to Investigate the Intestinal Epithelial Barrier**

The intestinal epithelial barrier remains selectively permeable if its integrity is not compromised. Following mucosal barrier disruption, intestinal permeability increases and delivers phlogistic dietary and/or microbial products to the mucosal immune system which provoke host responses. Therefore, the normally tolerogenic crosstalk between the host and the microbiota becomes perturbed resulting in the generation of an overactive immune response. Overtime, this continuous immune stimulation gives rise to intestinal inflammation which triggers the onset of chronic GI disease, such as IBD. Longitudinal studies in patients with IBD indicate that altered intestinal permeability precedes relapse of CD, suggesting a pathogenic role for barrier dysfunction in IBD as well as an indicator of impending symptoms (38). There are several methods for assessment of intestinal permeability via administration of oral probes, *in vitro* or tissue measures, and endoscopic evaluation of the intestinal epithelial barrier (mucosa) in humans (Table 2) (14, 21).

Our own work using a defined microbiota [colonized with the altered Schaudler flora (ASF)] mouse model shows that healthy ASF mice have increased intestinal permeability as compared to conventionally reared (CONV) mice. Using RNA *in situ* hybridization, we provide evidence that greater concentrations of bacteria (EUB probe) and/or their products translocate into the cecal lamina propria vs. bacterial products that translocate in CONV mice (Figure 2). Furthermore, ASF mice demonstrated greater IgG antibody response against members of their resident microbiota when compared to the antibody response directed against these same bacteria in CONV mice (unpublished observations). Our findings are in accordance with previously published data confirming that mice harboring a less diverse gut microbiota have an altered mucosal barrier and increased intestinal permeability (40).

**Microbiota Alterations in IBD**

**Observations in Human IBD**

Abundant clinical studies indicate a dysfunctional interaction between the gut microbiota and the host response in the onset and pathogenesis of IBD. Increased risk of IBD is associated with changes in composition/structure of the intestinal microbiota or genetic predisposition that impairs normal microbial sensing, both of which can cause altered host-microbe interactions (2, 3, 41–48). CD and UC are not considered single gene disorders, as over 240 susceptibility and IBD risk loci have now been identified (49, 50). Twin studies showed that while there is a genetic basis for IBD, it is not inherited in a simple Mendelian fashion (51). Genetic linkage analysis studies have identified nine disease loci, five of which meet the most stringent linkage analysis criteria, the remaining of which were at least suggestive (49). Mutations in several genes responsible for innate immune sensing of the intestinal microbiota, including NOD2/CARD15, IL-23R and ATG16L1, can also lead to increased risk for IBD (52–54). CARD15/NOD2 was the first IBD susceptibility gene that was identified emphasizing the importance of mucosal-microbial disturbances in the pathogenesis of IBD (53). CARD15 encodes an intracellular protein expressed in multiple immune system components including Paneth cells, monocytes, tissue macrophages and intestinal epithelial cells (52, 55–57). In Paneth cells, NOD2 mediates activation of NF-κB that leads to the
induction of defensins. With NOD2 mutations in CD, selective α-defensin production is attenuated which predisposes intestinal epithelial cells to microbial infection (58). Additionally, two autophagy genes, ATG16L1 and IRGM—both of which have roles in the processing of microbial antigens as part of the innate immune system—were identified as susceptibility genes (54, 59). Polymorphisms in these genes promote deranged innate immune responses leading to persistent intracellular bacterial infection that promote the development of IBD. IBD has also been linked to IL-10 deficiencies in humans. In the study by Glockler et al., investigators found that mutations in either IL10RA or IL10RB are associated with severe early onset enterocolitis in children (60). In a separate study, investigators reported NOD2 mutations in patients with IBD that were linked to inhibition of IL-10 in human monocytes (61).

The host microbiota plays an important role in the pathogenesis of IBD as evidenced by numerous clinical studies. Antibiotic use, both in early childhood and in adults, has been associated with increased risk for development of IBD (62). Moreover, the risk for IBD increases following an episode of infectious gastroenteritis (63). There are other observations implicating the microbiota including reports that mucosal inflammation is localized to gut segments with the greatest bacterial loads (2, 42). Furthermore, antibiotic treatment may be effective in a subset of IBD patients (post-surgical, CD and in pouchitis patients). Antibiotics have been used with varying degrees of success and longevity of response in patients with CD having luminal disease, fistulizing disease, and secondary septic complications such as post-operative infections (64). Results from large scale clinical trials and meta-analyses have been mixed with some analyses finding mild to moderate benefits in disease activity scores (65, 66) and others finding no benefit (67). Furthermore, probiotics and fecal microbiota transplant (in UC patients) may induce or maintain remission in some IBD patients (68–70).

Importantly, many studies have shown consistent alterations in microbial communities characterized by reduced microbial diversity in patients with IBD compared to controls (41, 71). The fecal microbiota of both CD and UC patients contains a depletion of Bacteroidetes and Firmicutes phyla (in particular Clostridium spp), which are the dominant normal fecal microbiota, and an increased abundance in Proteobacteria (42, 45, 72). Moreover, a metagenomic analysis of microbiomes demonstrated 25% fewer mucosal microbial genes from IBD patients compared with the microbiomes of healthy controls, suggesting that lower microbial diversity is present and contributing to disease (73). Several studies have found decreased abundance of Faecalibacterium prausnitzii (74), a major butyrate producing bacteria in the gut, and an increase in sulfate-reducing bacteria (SRB) which cause decreased expression of epithelial TJs to increase intestinal permeability in IBD (75). Still other studies have focused on the role of the mucosal microbiota that is different than the fecal microbiota between controls and patients with IBD. Using fluorescence in situ hybridization, high concentrations of bacteria were shown adherent to the epithelium of IBD patients as a thick biofilm, mainly composed of Bacteroides fragilis (43). In one seminal study, a depletion of Lactospiraceae and Bacteroides but increased abundance of Proteobacteria and Actinobacter were present in colonic biopsy specimens from both CD and UC patients, relative to control tissue samples (45). The distribution of operational taxonomic units (OTUs) was associated with disease state but not anatomy (small vs. large intestine) or gross pathology. Furthermore, the microbiome collected in multiple GI locations from a large cohort of treatment naïve patients with new-onset CD found

![FIGURE 2](image-url)
an increased abundance of Enterobacteriaceae, Pasteurellaceae, Veillonellaceae and Fusobacteriaceae and a reciprocal decrease of Erysipelotrichales, Bacteroidales and Clostridiales in pediatric IBD samples as compared to controls (45). These changes also correlated with disease status, that is, inflammation had a significant impact on microbial composition. Since several of the underrepresented bacterial phyla in IBD patients are butyrate-producing microbes, depletion of these organisms might reduce butyrate production, which is an important energy source for colonic epithelial cells and may enhance epithelial barrier integrity and mediate GI immune responses (42). Loss of significant quantities of these bacteria that provide key metabolic products (i.e., short chain fatty acids) to the host could exacerbate some forms of IBD (76–78).

**Table 2** | General means for assessment of intestinal permeability in humans and animals.

| Method                        | Human | Animal | Material needed | Comments                        |
|-------------------------------|-------|--------|-----------------|---------------------------------|
| **Orally administered probes**|       |        |                 |                                 |
| Lactulose/mannitol            | X     | X      | Urine           | Time consuming                  |
| FITC-dextran                  | X     | X      | Serum           | Time consuming                  |
| 51Cr-EDTA                     | X     | X      | Urine           | Time consuming; radiation hazard|
| **In vitro/tissue measures**  |       |        |                 |                                 |
| Ussing chamber                | X     | X      | Biopsies        | Invasive; requires specialized equipment |
| TEER                          | X     | X      | Biopsies        | Invasive; requires specialized equipment |
| Histology                     | X     | X      | Biopsies        | Invasive; permits specialized testing (IHC, confocal microscopy) for TJP expression |
| Scanning electron microscopy  | X     | X      | Biopsies        | Invasive; specialized fixative; expensive |
| DNA/RNA extraction            | X     | X      | Biopsies        | Invasive; permits qPCR for TJP expression |
| **Biomarkers**                |       |        |                 |                                 |
| LAL assay (LPS)               | X     | X      | Plasma          | May have technical limitations |
| Citrulline                    | X     | X      | Plasma          | Reliability in the dog is questioned |
| FABP                          | X     | X      | Plasma          | ELISA performed on plasma or urine |
| **Endoscopic measures**       |       |        |                 |                                 |
| Confoal endomicroscopy        | X     | X*     |                 | *As performed in dogs; specialized equipment; expensive |
| Endoscopic mucosal impedance  | X     |        |                 | Directly measures duodenal impedance; specialized equipment; expensive |

*Modified from references 20 and 38; TEER, trans-epithelial electrical resistance; LPS, lipopolysaccharide; FABP, fatty acid binding protein; IHC, immunohistochemistry; TJP, tight junction protein.

*Denote that this intervention is only used in dogs.

Pathobionts, such as adherent-invasive *Escherichia coli* (AIEC), are present within the mucosa in 21–62% of patients with ileal CD and 0–19% of healthy individuals (79, 80). Dysbiosis is associated with increased levels of oxygen in the intestinal lumen (81), possibly due to increased intestinal permeability and/or mucosal inflammation (82). In the inflamed gut, increased colonic oxygen levels restrict obligate anaerobic populations (e.g., Firmicutes) and increase the abundance of facultative anaerobes, including members of the family Enterobacteriaceae (83). Patients with CD have specific NOD2 variants that lead to defective innate sensing, autophagy, and immune responsiveness to CD-associated AIEC (7, 84). The adhesion molecule CEACAM6 is over expressed in ileal CD patients which also makes individuals more susceptible to mucosal colonization by AIEC (85). AIEC pathobionts strongly adhere to and invade IECs inducing robust pro-inflammatory cytokine secretion (e.g., IFN-γ, TNF-α) which causes direct damage to the intestinal barrier and promotes inflammation. Once within the ileal mucosa, AIECs can reside and replicate within macrophages, leading to an increased pro-inflammatory response (86). In contrast, AIEC colonization does not occur in colonic CD and the lack of AIEC mucosal translocation in UC patients would suggest that *E. coli* does not play a primary role in UC pathogenesis (87).

**Observations in Murine Models of Intestinal Inflammation**

Most different mouse models support a role for the microbiota in experimental intestinal inflammation. Early studies in mice treated with dextran sodium sulfate (DSS), a chemical irritant that disrupts the colonic intestinal epithelial barrier to contribute to the development of colitis, reported significant increases in intestinal *Bacteroidaceae* and *Clostridium* spp, in particular *Bacteroides distasonis* and *Clostridium ramosum*, in both acute and chronic colitis DSS models (88). In another study, increased numbers of colonic mucin-degrading *Akkermansia muciniphila* and Enterobacteriaceae were correlated to disease activity in DSS-treated mice resembling UC (89). Interleukin-10 knockout (IL−10−/−) mice develop spontaneous colitis that is entirely dependent on gut bacteria (90), and where colonic inflammation is attenuated when treated with antibiotics before disease onset (91) or is eliminated altogether in mice housed in a germ free environment (92). Animal models have also shown that intestinal inflammation is transferrable through the intestinal microbiota. Germ-free IL−10−/− mice colonized by the intestinal microbiota of IBD patients exhibit increased colitis as compared to mice colonized with the intestinal microbiota derived from healthy human controls (93). In IL−10−/− mice, loss of regulatory IL-10 secretion results in intolerance to their intestinal microbiota, unbalanced pro-inflammatory responses contributing to mucosal barrier disruption, and the development of spontaneous colitis. The administration of broad or narrow spectrum antibiotics shows different therapeutic activities in various regions of the colon in SPF colonized IL−10−/− mice. Narrow spectrum antibiotics, such as ciprofloxacin or metronidazole, prevented cecal and colonic inflammation in IL−10−/− mice following SPF colonization. Ciprofloxacin was most effective in treating cecal inflammation by reducing aerobic bacteria, including, *E. coli*.
and E. faecalis; whereas metronidazole was superior in reducing colitis and eliminated anaerobic bacteria (e.g., Bacteroides spp) in both the cecum and colon (94). Importantly, while ciprofloxacin and metronidazole prevented the induction of typhlocolitis in IL-10−/− SPF-colonized mice, these antibiotics had little effect after the onset of intestinal inflammation. In contrast, the broad-spectrum combination antibiotic vancomycin-imipenem decreased total luminal bacteria and prevented and treated both cecal and colonic inflammation. Taken together, these studies demonstrate that gut bacteria have differing inflammatory roles with some species initiating onset of intestinal inflammation while other microbe subsets drive chronic colitis (95).

Additional evidence supporting the role of the microbiota in colitis development is provided by studies using transfer animal models of colitis induced by deficiency of T-bet in innate immune cells. T-bet is a transcription factor that plays a crucial role in development of Th1 cells and in the regulation of innate and adaptive immunity (96). In certain murine models, loss of T-bet in mice lacking B and T cells (T-bet−/−/RAG1−/−) results in transmissible colitis in conventionally raised wild-type mice by co-housing, presumably caused by microbiota transmission (97). In similar fashion, Casp3/11-deficient mice, which are normally protected against DSS-induced colitis, lose this protection and become more sensitive to DSS on co-housing with WT mice (98).

Specific pathogenic bacteria have been associated with the development of intestinal inflammation in murine models. Proteus mirabilis and Klebsiella pneumoniae correlate with colitis in T-bet−/−/Rag2−/− mice, a mouse model resembling UC (97). Different Helicobacter spp, including infection with H. hepaticus and H. bilis or exposure to their antigens, trigger IBD-like disease in susceptible mice. For example, H. hepaticus induces chronic colitis in SPF-housed IL-10−/− mice accompanied by increased expression of pro-inflammatory biomarkers IFN-γ, TNF-α and nitric oxide (99). In a separate study, the combination of H. hepaticus infection and CD45RB high CD41 T-cell reconstitution resulted in marked disease expression in severe combined immunodeficiency (SCID) mice similar to that observed in human IBD (100). Still other experiments employing targeted infection with H. hepaticus were able to produce colitis and sometimes colonic tumors in different mouse strains having defects in immune function and/or regulation (101). Our group has previously shown that defined microbiota [i.e., altered Schaedler flora (ASF)] mice are a useful tool to investigate the impact of specific members of the Proteobacteria (e.g., E. coli, Helicobacter spp) on the development of colitis. The induction of typhlocolitis in ASF mice colonized with either H. bilis or Brachyspira hydysenteriae was accompanied by induction of ASF-specific antibody (102). Using a “multiple-hit” mouse model of colitis, we have shown that colonization of ASF mice with H. bilis increased host susceptibility to onset of severe colitis following low dose (1.5%) DSS administration (i.e., inflammatory trigger) (103). An analysis of the molecular/cellular mechanisms revealed increases in mucosal gene expression involving lymphocyte activation and inflammatory cell chemotaxis, with infiltration of more mucosal immune cells in H. bilis-colonized mice prior to DSS treatment vs. DSS treatment alone. A subsequent study with a similar experimental design used microarray analysis to demonstrate differential mucosal gene expression associated with alterations in fatty acid metabolism and detoxification in a time course following H. bilis colonization (104). This latter study provided preliminary evidence as to the types of factors or changes in the intestinal mucosa (i.e., alterations in housekeeper genes) that potentially predispose the host to the development of typhlocolitis.

Citrobacter rodentium is an attaching and effacing (non-invasive) bacterial pathogen that primarily causes acute typhlocolitis in mice, except when barrier function is impaired or in animals that are genetically susceptible to inflammation where infection can trigger chronic disease (105). The C. rodentium infection model was one of the first mouse models to show that composition of the intestinal microbiota influences susceptibility to infection (106), and that infection can alter the composition and spatial distribution of the resident microbiota post-infection (107). Finally, Fusobacterium varium isolated from the colonic mucosa of patients with UC was shown to induce experimental ulcerative colitis in mice (108). Collectively, these experimental studies provide compelling evidence that individual resident species are capable of inducing colitis in susceptible mouse models.

**Novel Animal Model Observations Implicating Epithelial Barrier Dysfunction in IBD**

Here we relate some of our own work utilizing different animal models to investigate host-microbe interactions mediating chronic intestinal inflammation and the role of the mucosal barrier in these different model systems.

**The Dog as a Naturally Occurring Model of Chronic Inflammatory Enteropathy**

Dogs represent a well-recognized large animal model that naturally develops CIE (also referred to as idiopathic IBD in the veterinary literature), sharing remarkable similarities in etiology, clinical course, histologic lesions and interventional strategies to human IBD (Table 3) (109–116). The obvious advantages of the dog in relation to other common animal models (e.g., rodents, zebra fish) include their large body size, longer life span, and they possess a GI tract of similar size, structure and function to that of humans. Of key importance for translational studies, pet dogs are exposed to the same environmental conditions and even share similar microbiota composition with their owners (117, 118). Clostridialis, Fusobacteria, Bacteroides and Proteobacteria are the dominant bacteria comprising the healthy canine fecal microbiota (119, 120). Metagenomic analyses in a small cohort of healthy dogs indicate that diet induced changes in microbial composition are not associated with changes in function, and that the fecal microbiota of dogs, mice and humans exhibit a high degree of metabolic and phylogenetic similarity (121). Considering the common microbiota and environmental exposures with humans, there is growing interest in whether similar mechanisms of CIE pathogenesis are shared between species (122).
Certain dog breeds show a predisposition to the development of CIE suggesting a role for host genetics in this disorder.

| Feature                  | Human | Dog | Rodent |
|--------------------------|-------|-----|--------|
| Genetic basis            | Yes   | Yes | Engineered |
| Etiology                 | Multifactorial and complex | Multifactorial and complex | +/- Multifactorial |
| Intact immune system     | Yes   | Yes | +/-    |
| Resident microbiota role | Yes   | Yes | Yes    |
| Blood in stool           | Yes   | Yes | +/-    |
| Diarrhea                 | Yes   | Yes | Yes    |
| Disease activity measures| Clinical indices, biomarkers | Clinical indices, biomarkers | Laboratory markers |
| Definitive diagnosis     | GI mucosal biopsy | GI mucosal biopsy | GI mucosal biopsy |
| Longitudinal studies     | Yes: endoscopy + histology | Yes: endoscopy + histology | Difficult to perform |
| Primary therapy          | Anti-inflammatory drugs | Diet + anti-inflammatory drugs | Anti-inflammatory drugs |
| Disease heterogeneity    | Yes   | Yes | Variable |
| Spontaneous GI flares    | Yes   | Yes | +/-    |

GI, gastrointestinal.

The German shepherd dog, Soft-coated wheaten terrier and Boxer dog/French bulldog have an increased incidence of CIE clinically that has been linked to mutations in innate immune genes, including TLR5, NOD2, and autophagy gene NCF2 (123, 124). Importantly, several of the same breeds (i.e., German shepherds, Boxer/French bulldog) show positive clinical response to administration of antimicrobials, indicating a potential interaction of host susceptibility with the intestinal microbiota in affected dogs. Intestinal biopsies are required to confirm histopathologic inflammation of CIE, with GI endoscopy being the preferred modality to visually inspect the GI mucosa and to acquire targeted biopsy samples. Mucosal lesions of erosions, friability and increased granularity are observed most frequently during endoscopy and correlate best to histopathologic inflammation (Figure 3) (113, 126). Lympho-plasmacytic enteritis of varying severity is the most common type of inflammation often accompanied by changes in mucosal architecture, including villous atrophy/fusion, erosions, ulceration, cryptal changes and/or depletion of colonic goblet cells (Figure 4) (127). Mixed cellular infiltrates are also observed in dogs with epithelial disruption (neutrophils) or in response to invasive mucosal bacteria (macrophages) as occurs with granulomatous colitis.

Like experimental models and human IBD, the intestinal microenvironment is implicated in the development of CIE in dogs. Numerous studies have shown that intestinal inflammation in dogs is accompanied by dysbiosis, where the proportions of Clostridiales, Fusobacteria, Bacteroidetes and Prevotellaceae are decreased, but the proportion of Proteobacteria, including...
Enterobacteriaceae, is significantly increased compared to healthy dogs (128–130). Mucosal associated E. coli are significantly increased with intestinal inflammation of CIE, granulomatous colitis and colorectal cancer (adenocarcinoma) in dogs (114, 131). Granulomatous colitis (GC) is a unique variant of CIE, causing chronic colitis with small volume diarrhea, straining, hematochezia and mucoid feces in predominantly young Boxer dogs. Here, a possible genetic defect in innate immune sensing confers increased susceptibility to E. coli invasion of colonic tissues (124). With this immune defect, ineffective respiratory burst impairs the host’s ability to eliminate intracellular pathogens, including catalase-positive bacteria. A diagnosis of canine GC is confirmed by mucosal culture and/or fluorescence in situ hybridization that identify invasive E. coli within the colonic mucosa of affected dogs (Figure 5). In Boxers with GC, long-term remission is observed with antimicrobial eradication of mucosally invasive E. coli, suggesting a causal relationship between this bacterial strain and clinical disease (131). Of interest, the observed phylotype of E. coli isolated from Boxer dogs with GC bears strong phylogenetic resemblance to the pathobiont E. coli strain isolated from CD patients (132).

The intestinal barrier of dogs with CIE has been investigated to a limited extent. Using duodenal biopsy samples obtained endoscopically from healthy dogs and dogs with CIE, the mucosal expression of claudin-1, -2, -3, -4, -5, -7, and -8; E-cadherin; and β-catenin was determined by immunoblotting and compared between dog groups (133). Results showed no difference in expression of each claudin and β-catenin between healthy dogs and dogs with CIE; while the expression of E-cadherin was reduced in dogs with CIE. Immunofluorescence microscopy (in a subset of CIE dogs) showed decreased intensity of E-cadherin labeling in the apical villi of dogs with CIE. In humans with IBD, a significant correlation between low E-cadherin expression and disease activity has been previously demonstrated (134). In another study, the ratio of IL-1β to IL-1 receptor antagonist (Ra), and the effect of IL-1β on occludin mRNA expression in the duodenal and colonic mucosa were investigated in healthy dogs and dogs with CIE (135). The ratio of IL-1β to IL-1Ra in the colonic mucosa was higher in dogs with CIE vs. healthy dogs. Ex vivo cultures of duodenal and colonic biopsies incubated with IL-1β showed reduced expression of occludin mRNA in colonic, but not duodenal, cultures of dogs with CIE. These findings are similar to observations in humans where both occludin mRNA and protein concentrations are reduced in the intestines of CD and UC patients (136). Finally, another study investigated intestinal pro- and active metalloproteinase (MMP) -2 and -9 activities in healthy dogs and dogs with chronic enteropathy (CE) using gelatin zymography.
In dogs with CE, there was a greater number of samples positive for pro- and active MMP2 and -9 in the duodenal, ileal and colonic mucosa as compared to healthy dogs (137). Similar findings of elevated matrix metalloproteinases have been reported in dogs with CIE and in humans with IBD (138, 139).

Clinical trials evaluating drug or probiotic therapy have provided indirect evidence on the role of the intestinal barrier in canine CIE. In one trial, the effects of a hydrolyzed diet and oral prednisone on the spatial distribution of mucosal bacteria in dogs with CIE was investigated using FISH (140). Medical therapy was associated with beneficial changes in microbial community structure and enhanced mucosal junctional protein expression in dogs with CIE. The spatial distribution of mucosal bacteria differed with increased numbers of Bifidobacteria, Faecalibacteria and Streptococci found within adherent mucus of dogs with CIE post-treatment compared to healthy dogs. Using immunohistochemistry (IHC), the expressions of occludin and E-cadherin were increased but zonulin decreased in dogs with CIE following prednisone therapy. Still other studies using multi-strain probiotics for the treatment of canine CIE have shown potential beneficial alterations in junctional proteins that are associated with remission. In one trial, probiotic therapy with VSL#3 was investigated in comparison to combination treatment with prednisone and metronidazole administered continuously to dogs with CIE for 90 days (115). Dogs treated with probiotic showed remission accompanied by changes in beneficial mucosal responses (i.e., increased numbers of FoxP3+ and TGF-β+ cells) and increased mucosal expression of occludin.

Another probiotic trial using FISH to investigate the mucosal microbiota showed that remission of dogs with CIE was associated with changes in beneficial bacterial species and up-regulated expression of junctional proteins following 6 weeks of probiotic therapy (141). Both probiotic and standard therapy for CIE (e.g., hydrolyzed diet + oral prednisone) were associated with rapid remission without improvement in histopathologic inflammation. Probiotic therapy was associated with increased expression (IHC) of junction proteins E-cadherin, occludin and zonulin vs. dogs with CIE that received standard therapy (Table 4; Figure 6). Collectively, these observations of increased barrier integrity in dogs receiving glucocorticoid or probiotic therapy for CIE are in broad agreement with studies in UC patients and experimental models of intestinal inflammation (142–145).

### TABLE 4 | Probiotic therapy modulates TJP expression in dogs with IBD.

|                | Colon | Claudin-2 | E-cadherin | Occludin | Zonulin |
|----------------|-------|-----------|------------|----------|---------|
| Healthy dogs   | 91*   | 1,031*    | 1,119*     | 371*     |         |
| Pre-VSL #3 IBD| 1,212^ | 575       | 131        | 61       |         |
| Post-VSL #3 IBD| 82    | 902^      | 859^       | 326^     |         |

*P < 0.05 for healthy dogs vs. Pre-VSL #3 IBD dogs; ^P < 0.05 for Pre-VSL #3 IBD dogs vs. Post-VSL #3 IBD dogs; TJP, tight junction protein. From reference 129.

**FIGURE 5** | Granulomatous colitis in a 2-year-old English bulldog. (A) Endoscopic image of severe colonic granularity (increased texture) involving the descending colon. (B) Colonic biopsy from this dog shows clusters (yellow fluorescence) of mucosal associated E. coli following fluorescence in situ hybridization. From: Jergens et al. (125), with permission.
Other Murine Model Observations

*Brachyspira hyodysenteriae* is a Gram-negative anaerobic spirochete and is the causative agent of swine dysentery. The pathogenesis of disease has been studied in mice and pigs and has been shown to rely on the presence of a resident microbiota (146, 147), production of a β-hemolysin (148), local inflammatory response of the host (149, 150), and recruitment of host inflammatory cells (151). With respect to the need for other resident bacteria, our own research has shown that the colonization of GF mice with *B. hyodysenteriae* failed to induce typhlocolitis in mice, even when mice were observed for 110 days post-colonization. The need for at least one member of the resident microbiota was demonstrated by administering *Bacteroides vulgatus* to GF mice previously colonized with *B. hyodysenteriae* (i.e., no disease) and typhlocolitis developed within 5 days. This result suggested that the presence of *B. vulgatus* either enhanced the virulence of *B. hyodysenteriae* or induced host innate immune responses that contributed to the resultant inflammatory response. Furthermore, treatment of mice with an antibiotic cocktail to which the spirochete was resistant in their drinking water for 7 days, prior to colonization with *B. hyodysenteriae*, prevented the onset of disease even though the numbers of spirochetes colonizing the cecum and colon were like that of untreated mice with typhlocolitis. In these conventionally reared mice, the role of the resident microbiota was further shown by replacing the antibiotic-containing drinking with normal drinking water and the severe typhlocolitis developed within 15 days. It was shown that the antibiotics significantly reduced the numbers of bacteria in the feces and cecal contents by six to seven log_{10} with the dominant bacterial types remaining being Gram-negative facultative anaerobes and strict anaerobes. One conclusion to be drawn from these results would suggest that the crosstalk between the host and the resident microbiota contributes to disease susceptibility and the severity of the inflammatory response (152, 153).

It has also been shown that disease caused by *B. hyodysenteriae* can be inhibited by treating mice orally with an extract (i.e., hypoxoside) from *Hypoxis hemerocallidea* corn (also known as *Hypoxis rooperi*, African Potato). Beginning seven days prior to challenge, the oral administration of hypoxoside did not prevent the colonization of *B. hyodysenteriae*, but prevented the onset of typhlocolitis as evidenced by the lack of inflammatory cell infiltration, absence of crypt hyperplasia, and reduction in the expression of cytokine-specific genes regulated by NF-κB activation (149). As with the administration of antibiotics mentioned above, the administration of hypoxoside prevented disease and expression of TNF-α-specific mRNA when treatment began at least 7 days prior to colonization with *B. hyodysenteriae*. The need to initiate treatment 7 days prior to colonization with *B. hyodysenteriae* coincides with the turnover of colonic epithelial cells and suggests that the host inflammatory set-point can be altered in the new epithelial cells by affecting which bacteria are present (i.e., antibiotic use) or by changing the responsiveness of the epithelial cells to phlogistic stimuli (i.e., hypoxoside).

In this regard, administration of hypoxoside also inhibited crypt epithelial cell hyperplasia following colonization with *B. hyodysenteriae* (Figure 7). The ability to affect epithelial cell responsiveness was further demonstrated by adding conjugated linoleic acid (CLA) to the diet of pigs prior to colonization with *B. hyodysenteriae*. It has been shown that CLA is a ligand for peroxisome proliferator-activated receptor gamma (PPAR-g) and that the activation of PPAR-g promotes mucosal epithelial health by suppression of inflammation and facilitating metabolic reprogramming (i.e., oxidative phosphorylation) of colonic epithelial cells associated with the use of SCFAs derived from microbial metabolism (150, 154). To further demonstrate that the interaction of *B. hyodysenteriae* with the colonic epithelium-induced inflammatory cell recruitment, mice that were treated with anti-CD18 or anti-CD29 to prevent extravasation of neutrophils from blood failed to develop typhlocolitis (151). Using *B. hyodysenteriae* as a model of bacterial induced colitis, these studies have demonstrated that the colonic epithelium in association with the resident microbiota is a key contributor of mucosal health or disease.

In the context of IBD, epithelial barrier function is a critical component of maintaining mucosal homeostasis and tissue health. It has been shown that mice (i.e., mdr1a<sup>-/-</sup>) lacking the multiple drug resistance gene P-glycoprotein 170 (Pg-170)
FIGURE 7 | Immunohistochemical detection of proliferating epithelial cells in mice treated with hypoxoside. Mice were either sham treated orally with sterile drinking water (Control) or hypoxoside (Colonized and treated). The mice treated daily with either saline or hypoxoside (15 mg) beginning 8 days prior to colonization with Brachyspira hyodysenteriae (B. hyo). Mice were necropsied 3 days after colonization. One hour prior to necropsy, mice received an IP injection of BrDU. Proliferating epithelial cells were identified by labeling their DNA with anti-BrDU using immunohistochemistry. From: (149), with permission.

develop spontaneous colitis between 8 and 30 weeks of age associated with epithelial barrier dysfunction. As an efflux pump, Pg-170 is highly expressed in colonic epithelial cells and contributes to the removal of xenobiotics and phlogistic compounds from the cytosol (155). As with many murine models of colitis, GF mdr1a−/− mice do not develop colitis and administration of metronidazole in the drinking water ameliorates the colitis, indicating a role for the resident microbiota in the disease process (156, 157). Like the studies performed using hypoxoside, we have shown that treating mdr1a−/− mice with botanical extracts from either Prunella vulgaris or Hypericum gentianoides prevented or significantly attenuated colitis in mdr1a−/− mice (158, 159). The reduction in colonic inflammation was consistent with the reduction of NF-κB regulated cytokines and chemokines (e.g., CXCL1, CXCL9, CCL2, CCL20, and TNF-α). In companion studies, we demonstrated that administration of caffeic acid to mice increased the expression to Cyp4b1 (i.e., cytochrome P450) in the colonic mucosal and ameliorated DSS-induce colitis (160). Analogous to Pg-170, CYP4B1 controls the metabolism of proinflammatory compounds in the GI epithelium and contributes to maintenance of the mucosal barrier. Again, this demonstrates the central role colonic epithelial cells have in the attenuation of mucosal inflammation induced by microbial compounds and in the maintenance of mucosal homeostasis and GI health.

As IECs are also able to take up antigen and PRR ligands, they contribute to the maintenance of mucosal immunity and intestinal health. The importance of the epithelial response to luminal antigens was elegantly demonstrated by examining the inflammatory response in MyD88−/− mice (161). Initially, the authors had reasoned that since much of the mucosal inflammation associated with IBD was associated with production of pro-inflammatory cytokines; the absence of MyD88 should reduce the severity of disease due to impaired recognition of MAMPs derived from the microbiota. However, these authors demonstrated that the MyD88−/− mice developed more severe colitis than the wild-type counterparts. These observations indicated that there is a cytoprotective aspect to the local inflammatory response that is key to mucosal homeostasis. As mentioned above, we had reported that the administration of anti-CD18 or anti-CD29 attenuated lesion severity in mice colonized with B. hyodysenteriae. However, if mice were administered a cocktail containing both anti-CD18 and anti-CD29 or neutrophils were depleted, lesions were more severe than in sham treated mice colonized with B. hyodysenteriae (151). Like the MyD88−/− mice, the inability to recruit inflammatory cells resulted in a more severe lesion supporting the importance of epithelial cell responses to inflammatory stimuli, at least in moderation. Similarly, the administration of hypoxoside likely had a beneficial effect in inhibiting the typhlocolitis associated with B. hyodysenteriae colonization because it attenuated the local inflammatory responses as opposed to inhibiting that response, thus, retaining the cytoprotective benefit of the residual inflammatory response.

The role of the epithelial cells to support antigen uptake and maintenance of mucosal tolerance is partially mediated by the induction of regulatory T cells (Tregs) and the secretion of IgA (sIgA) in the GI lumen. Functionally, one of the features of the sIgA is to provide for immune exclusion which would reduce, but not eliminate, microbial antigen interactions with epithelial cells and underlying immune cells (162). To this end,
we evaluated the ability of orally administered serum-derived bovine immunoglobulin (SBI) to inhibit DSS-induced murine colitis (163). The SBI would function to bind to bacterial antigens and reduce the innate and/or adaptive immune activation contributing to colitis. Results demonstrated that mucosal inflammation was significantly reduced, there was a decrease in secretion of pro-inflammatory cytokines and a reduction in intestinal fatty acid binding protein and serum amyloid A. As with the use of botanical extracts, dietary CLA and attenuation of neutrophil recruitment, the use of SBI to reduce mucosal inflammation by lessening the phlogistic potential of luminal content on the mucosa while allowing for the beneficial (i.e., cytoprotective) expression of host inflammatory responsiveness.

CONCLUSIONS

Host-microbe interactions play important roles in maintaining homeostasis of the mucosal epithelial barrier as well as contributing to the development of IBD. The concept that the intestinal epithelium serves as a "translator" between the intestinal microbiota and the immune system seems both logical and plausible (164). Here, the epithelium is responsive to signals from the microbiota by means of pathogen recognition receptors and translates these messages into signals that direct mucosal immune cells. Conversely, IECs receive signals from the underlying immune system and translate them into signals that shape intestinal barrier function and the structure and function of the gut microbiota. Dysregulation of the intestinal barrier is a salient feature of IBD in humans and animal models of inflammation, regardless of species. As such, treatment approaches that aim to support gut barrier function have been identified and are currently under review, including nutritional approaches (avoidance of Western-style diet, precision (FODMAP) diet, prebiotics/fibers); probiotic approaches (select probiotics, multi-strain probiotics, symbiotic preparations); and drug/other approaches (short chain fatty acids, metformin, fecal microbiota transplantation) (14, 21, 165, 166).

AUTHOR CONTRIBUTIONS

AJ, SP, JK, and MW: review design and input, data/narrative analysis, preparation, and review/editing the manuscript. AJ, SP, and MW: performance of experiments. All authors contributed to the article and approved the submitted version.

FUNDING

Funds received from Iowa State University, as a Frontiers institutional member, will be used for open access publication fees.

ACKNOWLEDGMENTS

This work was supported by grants from the NIH R01GM099537 and KO18618, USDA 85-CRSR-2-2583, Iowa State University Bailey Research Career Development Award, Canine Health Foundation 02002, and Kenneth Rainin Foundation Innovator 2014.

REFERENCES

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature.* (2007) 448:427–34. doi: 10.1038/nature06005
2. Sartor RB. Microbial influences in inflammatory bowel disease. *Gastroenterology.* (2008) 134:577–94. doi: 10.1053/j.gastro.2007.11.059
3. Podolsky DK. Inflammatory bowel disease. *N Engl J Med.* (1991) 325:928–37. doi: 10.1056/NEJM199109263251306
4. Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology.* (2011) 140:1756–67. doi: 10.1053/j.gastro.2011.02.016
5. Loddo I, Romano C. Inflammatory bowel disease: genetics, epigenetics, and pathogenesis. *Front Immunol.* (2015) 6:551. doi: 10.3389/fimmu.2015.00551
6. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol.* (2009) 9:799–809. doi: 10.1038/nri2653
7. Mccoe DF. IBD candidate genes and intestinal barrier regulation. *Inflamm Bowel Dis.* (2014) 20:1829–49. doi: 10.1097/MIB.0000000000000090
8. Koch S, Nusrat A. The life and death of epithelia during inflammation: lessons learned from the gut. *Annu Rev Pathol.* (2012) 7:55–60. doi: 10.1146/annurev-pathol-011811-120905
9. Bruzew M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, et al. Proinflammation cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol.* (2003) 171:6164–72. doi: 10.4049/jimmunol.171.11.6164
10. Beck PL, Rosenberg JM, Xavier RJ, Koh T, Wong JR, Podolsky DK. Transforming growth factor-beta mediates intestinal healing and susceptibility to injury in vitro and in vivo through epithelial cells. *Am J Pathol.* (2003) 162:597–608. doi: 10.1016/S0002-9440(10)63853-9
11. Paclik D, Lohse K, Wiedemann B, Dignass AU, Sturm A. Galectin-2 and—4, but not Galectin-1, promote intestinal epithelial wound healing in vitro through a TGF-beta-independent mechanism. *Inflamm Bowel Dis.* (2008) 14:1366–72. doi: 10.1002/ibd.20499
12. Sato T, Vries RG, Snippert HJ, Van De Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* (2009) 459:262–5. doi: 10.1038/nature07935
13. Cummings JH, Antoine, J.-M., Azpiroz F, Bourdet-Sicard R, Branditzaeg P, et al. PASSCLAIM1—Gut health and immunity. *Eur J Nutr.* (2004) 43:118–73. doi: 10.1007/s00394-004-1205-4
14. Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut.* (2019) 68:1516–26. doi: 10.1136/gutjnl-2019-318427
15. Yen TH, Wright NA. The gastrointestinal tract stem cell niche. *Stem Cell Rev.* (2006) 2:203–12. doi: 10.1007/s12015-006-0048-1
16. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol.* (2009) 124:3–20; quiz 21–2. doi: 10.1016/j.jaci.2009.05.038
17. Wada M, Tamura A, Takahashi N, Tsukita S. Loss of claudins 2 and 15 from mice causes defects in paracellular Na+ flow and nutrient transport in gut and leads to death from malnutrition. *Gastroenterology.* (2013) 144:369–80. doi: 10.1053/j.gastro.2012.10.035
18. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol.* (2001) 2:285–93. doi: 10.1038/35067088
19. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol.* (2002) 156:1099–111. doi: 10.1083/jcb.200110122
20. Turkens K, Troy TC. Permeability barrier dysfunction in transgenic mice overexpressing claudin 6. *Development.* (2002) 129:1775–84. doi: 10.1242/dev.129.7.1775
21. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulze, J.-D., et al. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterol. (2014) 14:189. doi: 10.1186/s12876-014-0189-7

22. Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorka KN. Intereukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. Inflamm Bowel Dis. (1999) 5:262–70. doi: 10.1097/00011327-199911000-00004

23. Arrieta MC, Madsen K, Doyle J, Meddings J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. Gut. (2009) 58:41–8. doi: 10.1136/gut.2008.150888

24. Lauketter MG, Nava P, Lee WY, Severson EA, Capaldo CT, Babb in BA, et al. CARD15/NOD2 functions as an antibacterial factor in patients with active inflammatory bowel disease. Nat Microbiol. (2017) 2:17004. doi: 10.1038/nmicrobiol.2017.4

25. Seth A, Basuroy S, Sheth P, Rao RK. L-Glutamine ameliorates intestinal permeability – a new target for disease prevention and therapy. Nat Microbiol. (2017) 2:17004. doi: 10.1038/nmicrobiol.2017.4

26. Manichanh C, Rigottier-Gois L, Bonniald M, Gasc M, Flechon A, et al. Reduced diversity of faecal microbiota in Crohn’s disease associated with susceptibility to Crohn’s disease. Nature. (2008) 453:48–51. doi: 10.1038/nature06974

27. Halpern J, Shapira Y, Geller B, Lapidot T, Drukker M, et al. Intestinal permeability test as a predictor of the pathogenesis of IBD: lessons from mouse infection models. Gastroenterology. (2002) 122:44–54. doi: 10.1053/gast.2002.30294

28. Swidsinski A, Ladhoff A, Perathaler A, Swidsinski S, Loening-Baucke V, Ortner M, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. (2002) 122:44–54. doi: 10.1053/gast.2002.30294

29. Hugot J-P, Chamaillard M, Zouali H, Lesage S, Cézard P, Belaiche J, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease. Nature. (2001) 411:603–6. doi: 10.1038/35079114

30. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. Nat Genet. (2017) 49:256–61. doi: 10.1038/ng.3760

31. Halme L, Paavola-Sakki P, Turunen U, Järvinen M, Farkkila M, Junttila M, et al. Family and twin studies in inflammatory bowel disease. World J Gastroenterol. (2008) 14:3688–72. doi: 10.3748/wjg.v14.i23.3686

32. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease. Nature. (2001) 411:603–6. doi: 10.1038/35079114

33. Hugot J-P, Chamaillard M, Zouali H, Lesage S, Cézard P, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn’s disease. Nature. (2001) 411:599–603. doi: 10.1038/35079107

34. Hapse J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. (2007) 39:207–11. doi: 10.1038/ng1954

35. Hisamatsu T, Suzuki M, Reinecker, H.-C., Nadeau WJ, McCormick BA, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology. (2003) 124:993–1000. doi: 10.1053/gast.2003.50153

36. Suzuki M, Hisamatsu T, Podolsky DK. Gamma interferon augments the intestinal permeability in patients with active inflammatory bowel disease. Gut. (2004) 53:685–93. doi: 10.1136/gut.2003.025403

37. Frank DN, St Amand AL, Feldman RA, Boedecker EC, Hapaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA. (2007) 104:13780–5. doi: 10.1073/pnas.0706625104

38. Swidsinski A, Ladhoff A, Perathaler A, Swidsinski S, Loening-Baucke V, Ortner M, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. (2002) 122:44–54. doi: 10.1053/gast.2002.30294

39. Swidsinski A, Weber J, Loening-Baucke V, Hare L, Lynch H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol. (2005) 43:3380–9. doi: 10.1128/JCM.43.11.3380-3389.2005

40. D’Arcy M, Green S, O’Donnell P, O’Donnell M, et al. Intestinal permeability and inflammation in Crohn’s disease patients. Clin Exp Immunol. (2003) 132:233–40. doi: 10.1046/j.1365-2176.2003.02068.x

41. Arumugam M, Raes J, Pelletier E, Le Paslier D, Qin J, Lugscheider E, et al. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Intern Med. (2008) 263:597–606. doi: 10.1111/j.1365-2969.2008.01962.x

42. Cooney R, Jewell D. The genetic basis of inflammatory bowel disease. Dig Dis. (2009) 27:428–42. doi: 10.1159/000234909

43. De Lange KM, Moutsianas L, Lee JC, Lamb CA, Luo Y, Kennedy NA, et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. Nat Genet. (2017) 49:256–61. doi: 10.1038/ng.3760

44. Hugot J-P, Chamaillard M, Zouali H, Lesage S, Cézard P, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn’s disease. Nature. (2001) 411:599–603. doi: 10.1038/35079107

45. Hapaz J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. (2007) 39:207–11. doi: 10.1038/ng1954

46. Hisamatsu T, Suzuki M, Reinecker, H.-C., Nadeau WJ, McCormick BA, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology. (2003) 124:993–1000. doi: 10.1053/gast.2003.50153

47. Suzuki M, Hisamatsu T, Podolsky DK. Gamma interferon augments the intestinal permeability in patients with active inflammatory bowel disease. Gut. (2004) 53:685–93. doi: 10.1136/gut.2003.025403

48. Frank DN, St Amand AL, Feldman RA, Boedecker EC, Hapaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA. (2007) 104:13780–5. doi: 10.1073/pnas.0706625104

49. Swidsinski A, Ladhoff A, Perathaler A, Swidsinski S, Loening-Baucke V, Ortner M, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. (2002) 122:44–54. doi: 10.1053/gast.2002.30294

50. Swidsinski A, Weber J, Loening-Baucke V, Hare L, Lynch H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol. (2005) 43:3380–9. doi: 10.1128/JCM.43.11.3380-3389.2005
66. Sutherland L, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, et al.

67. Su JW, Ma JJ, Zhang HJ. Use of antibiotics in patients with Crohn's disease.

68. Rutgeerts P, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, et al.

69. Abraham B, Quigley EMM. Antibiotics and probiotics in inflammatory bowel disease. World J Gastroenterol. 2016;22:1078–87. doi: 10.3748/wjg.v22.i13.1078

70. Arnold GL, Beaves MR, Prydyun VO, Mook WI. Preliminary study of ciprofloxacin in active Crohn’s disease. Inflamm Bowel Dis. (2002) 8:10–5. doi: 10.1097/00054725-200201000-00002

71. Noguchi E, Homma Y, Kang X, Netea MG, Ma X. A Crohn’s disease-associated NOD2 mutation suppresses transcription of human IL10 by inhibiting activity of the nuclear ribonucleoprotein hnRNP-A1. Nat Immunol. (2009) 10:471–9. doi: 10.1038/ni.1722

72. Theocari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. Scand J Gastroenterol. (2018) 53:1–7. doi: 10.1080/00365521.2017.1386711

73. Mann EA, Saeed SA. Gastrointestinal infection as a trigger for inflammatory bowel disease. Curr Opin Gastroenterol. (2012) 28:24–9. doi: 10.1097/00054725-200201000-00002

74. Su JW, Ma JJ, Zhang HJ. Use of antibiotics in patients with Crohn’s disease: a systematic review and meta-analysis. J Dig (2015) 16:58–66. doi: 10.11171/2990.121216

75. Rutgeerts P, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, et al. Controlled trial of metronidazole for prevention of Crohn’s recurrence after ileal resection. Gastroenterology. (1995) 108:1617–21. doi: 10.1016/S0016-5085(95)00212-3

76. Blanchaert C, Strubbe B, Peeters H. Fecal microbiota transplantation in ulcerative colitis. Acta Gastroenterol Belg. (2019) 82:519–28.

77. Abraham B, Quigley EMM. Antibiotics and probiotics in inflammatory bowel disease: when to use them? Front Gastroenterol. (2020) 11:62–9. doi: 10.3389/fgastro.2018-101057

78. Su JW, Ma JJ, Zhang HJ. Use of antibiotics in patients with Crohn’s disease: a systematic review and meta-analysis. J Dig (2015) 16:58–66. doi: 10.11171/2990.121216

79. Machiels K, Joossens MWE, Sabino J, De Preter V, Arijis I, Eckhaut VDI, et al. A decrease of the butyrate-producing species Roseburia hominis Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. GUT. (2014) 63:1275–83. doi: 10.1136/gutjnl-2013-304833

80. Qin J, Li S, Arumugam M, Burgdorf KS, Manichanh C, et al. A gut microbial gene catalogue established by metagenomic sequencing. Nature. (2010) 464:59–65. doi: 10.1038/nature08821

81. Rigottier-Gois L. Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis. Isme J. (2013) 7:1256–61. doi: 10.1038/ismej.2013.80

82. Rivera-Chávez F, Lopez CA, Baumler AJ. Oxygen as a driver of gut dysbiosis. Free Radic Biol Med. (2017) 105:93–101. doi: 10.1016/j.freeradbiomed.2016.09.022

83. Zeng MY, Inohara N, Núñez G. Mechanisms of inflammation-driven bacterial dysbiosis in the gut. Muc Immunol. (2017) 10:18–26. doi: 10.1038/mi.2016.75

84. Shawki A, Mc cole DF. Mechanisms of intestinal epithelial barrier dysfunction by adherent-invasive escherichia coli. Cell Mol Gastroenterol Hepatol. (2017) 3:341–50. doi: 10.1016/j.jcmgh.2016.10.004

85. Barnich N, Carvalho FA, Glasser A.,-L, Darcha C, Jantjeischf P, et al. CEACAM6 acts as a receptor for adherent-invasive E. Coli, supporting ileal mucosa colonization in Crohn’s disease. J Clin Invest. (2007) 117:1566–74. doi: 10.1172/JCI30504

86. Lapayotte P, Bringer MA, Darfeuille-Michaud A. Defects in autophagy favour adherent-invasive escherichia coli persistence within macrophages leading to increased pro-inflammatory response. Cell Microbiol. (2012) 14:791–807. doi: 10.1111/j.1462-5882.2012.01768.x

87. Rolhion N, Darfeuille-Michaud A. Adherent-invasive escherichia coli in inflammatory bowel disease. Inflamm Bowel Dis. (2007) 13:1277–83. doi: 10.1002/ibd.20176

88. Okayasu I, Hatakeyama S, Yamaida M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. (1990) 98:694–702. doi: 10.1016/S0016-5085(90)92890-H

89. Håkansson Å, Tormo-Badia N, Baridi A, Xu J, Molin G, Haglädt ML, et al. Immunological alteration and changes of gut microbiota after dextran sodium sulfate (DSS) administration in mice. Clin Exp Med. (2015) 15:107–20. doi: 10.1007/s10238-013-0270-5

90. Kühn R, Löhler J, Renneck D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. (1993) 75:263–74. doi: 10.1016/0092-8674(93)90068-P

91. Madsen KL, Doyle JS, Tavernini MM, Jewell LD, Rennie RP, Fedorak RN. Antibiotic therapy attenuates colitis in interleukin 10 gene knockout mice. Gastroenterology. (2000) 118:1094–105. doi: 10.1053/s00016-5085(00)7362-3

92. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. (1998) 66:5224–31. doi: 10.1128/IAI.66.11.5224-5231.1998

93. Nagao-Kitamoto H, Shreiner AB, Gíllilland MG, Kitamoto S, Ishii C, Hirayama A, et al. Functional characterization of inflammatory bowel disease–associated gut dysbiosis in gnotobiotic mice. Cell Mol Gastroenterol Hepatol. (2020) 15:107–20. doi: 10.1016/j.jcmgh.2016.10.004

94. Hoentjen F, Harmsen HJM, Braat H, Torrice CD, Mann BA, Sartor RB, et al. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut. (2003) 52:172–17. doi: 10.1136/gut.52.12.1721

95. Rath HC, Schultz M, Freitag R, Dieleman LA, Li F, Linde HJ, et al. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. Infect Immun. (2001) 69:2277–85. doi: 10.1128/IAI.69.4.2277-2285.2001

96. Peng SL. The T-box transcription factor T-bet in immunity and autoimmunity. Cell Mol Immunol. (2006) 3:87–95.

97. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. (2007) 131:33–45. doi: 10.1016/j.cell.2007.08.017

98. Brinkman BM, Becker AY, Hildebrand FW, Raes J, Huys G, et al. Gut microbiota affects sensitivity to acute DSS-induced colitis independently of host genotype. Inflamm Bowel Dis. (2013) 19:2560–7. doi: 10.1097/MIB.0b013e3182a8759a

99. Flatley N, Mitchell JM, Gorelick PL, Caporaso JG, Huleihel M, et al. Metabolic profiling of commensal bacteria reveals a distinct inter-5:0012.1712

100. Cahill RJ, Foltz CJ, Fox JG, Dangler CA, Powrie F, Schauer DB. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection. Muc Immunol. (2018) 13:1277–83. doi: 10.1002/imi.2018101
infection with helicobacter helpicus. *Infect Immun.* (1997) 65:3126–31. doi: 10.1128/iai.65.8.3126-3131.1997

101. Fox JG, Ge Z, Whary MT, Erdman SE, Horwitz BH. Helicobacter hepaticus infection in mice: models for understanding lower bowel inflammation and cancer. *Muc Immunol.* (2011) 4:22–30. doi: 10.1038/mi.2010.61

102. Jergens AE, Dorn A, Wilsen J, Dingbaum K, Henderson A, Liu Z, et al. Induction of differential immune reactivity to members of the flora of gnotobiotic mice following colonization with helicobacter bilis or brachyspiro hydropyderetina. *Microb Infect.* (2006) 8:1602–10. doi: 10.1016/j.micinf.2006.01.019

103. Liu Z, Ramer-Tait AE, Henderson AL, Demirkale CY, Nettleton D, Wang C, et al. Helicobacter bilis colonization enhances susceptibility to typhlocolitis following an inflammatory trigger. *Dig Dis Sci.* (2011) 56:2838–48. doi: 10.1007/s10620-011-1701-3

104. Liu Z, Henderson AL, Nettleton D, Wilson-Welder JH, Hostetter JM, Ramer-Tait A, et al. Mucosal gene expression profiles following the colonization of immunocompetent defined-flora CSH mice with helicobacter bilis: a prelude to typhlocolitis. *Microb Infect.* (2009) 11:374–83. doi: 10.1016/j.micinf.2008.12.013

105. Higgins LM, Frankel G, Dougan G, Dougan G, Macdonald TT. Citrobacter rodentium infection in mice elicits a mucosal Th1 cytokine response and lesions similar to those in murine inflammatory bowel disease. *Infect Immun.* (1999) 67:3031–9. doi: 10.1128/iai.67.6.3031-3039.1999

106. Mundy R, Macdonald TT, Dougan G, Frankel G, Wiles S. Citrobacter rodentium of mice and man. *Cell Microbiol.* (2005) 7:1697–706. doi: 10.1111/j.1462-5822.2005.00625.x

107. Hoffmann C, Hill DA, Minakh N, Kirk T, Troy A, Arts D, et al. Community-wide response of the gut microbiota to enteropathogenic citrobacter rodentium infection revealed by deep sequencing. *Infect Immun.* (2009) 77:4668–78. doi: 10.1128/iai.00493-09

108. Ohkusa T, Okayasu I, Oghara T, Morita K, Ogawa M, Sato N. Induction of experimental ulcerative colitis by fusobacterium varium isolated from colonic mucosa of patients with ulcerative colitis. *Gut.* (2003) 52:79–83. doi: 10.1136/gut.52.1.79

109. Jergens AE, Crandell J, Morrison JA, Deitz K, Pressel M, Ackermann M, et al. Comparison of oral prednisone and prednisone combined with metronidazole for induction therapy of canine inflammatory bowel disease: a randomized-controlled trial. *J Vet Intern Med.* (2010) 24:269–77. doi: 10.1111/j.1939-1676.2009.0447.x

110. Jergens AE, Evans RB, Ackermann M, Hostetter J, Willard M, Mansell J, et al. Design of a simplified histopathologic model for gastrointestinal inflammation in dogs. *Vet Pathol.* (2014) 51:946–50. doi: 10.1177/0300985813511123

111. Jergens AE, Garet Y, Moore FM, Niyo Y, Tsao C, Smith B. Colonic lymphocytosis and plasma cell populations in dogs with lymphoplastic plasmacytic colitis. *Am J Vet Res.* (1999) 60:515–20.

112. Jergens AE, Schreiner CA, Frank DE, Niyo Y, Ahrens FE, Eckersall PD, et al. A scoring index for disease activity in canine inflammatory bowel disease. *J Vet Intern Med.* (2003) 17:291–7. doi: 10.1111/j.1939-1676.2003.tb02450.x

113. Jergens AE, Simpson KW. Inflammatory bowel disease in veterinary medicine. *Front Biosci.* (2012) 4:1404–19. doi: 10.2741/e470

114. Cassmann E, White R, Atherly T, Wang C, Sung Y, Khoda S, et al. Comparison of oral prednisone and prednisone combined with metronidazole for induction therapy of canine inflammatory bowel disease. *Vet Pathol.* (2019) 56:2838–48. doi: 10.1177/0300985818813090

115. Hostutler RA, Lucia BJ, Johnson SE, Weisbrode SE, Sherding RD, Jaeger JQ, et al. Antibiotic-responsive histiocytic ulcerative colitis in 9 dogs. *J Vet Intern Med.* (2004) 18:499–504. doi: 10.1177/0192528X04t02574X

116. Simpson KW, Dogan B, Shrivastav M, Goldstein RE, Klaessig S, Mcdonough PL, et al. Adherent and invasive escherichia coli is associated with granulomatous colitis in boxer dogs. *Infect Immun.* (2006) 74:4778–92. doi: 10.1128/iai.1. A.00867-06

117. Karayiannakis AJ, Syrigos KN, Efstathiou J, Valizadeh A, Noda M, et al. Inflammatory bowel disease is associated with changes of enterocytic metabolism in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microb Ecol.* (2008) 66:567–78. doi: 10.1111/j.1574-6941.2008.00521.x

118. Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA, et al. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *ISME J.* (2011) 5:639–49. doi: 10.1038/ismej.2010.162

119. Pilla R, Suchodolski JS. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. *Front Vet Sci.* (2020) 6:648. doi: 10.3389/fvets.2019.00648

120. Suchodolski JS, Camacho J, Steiner JM. Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microb Ecol.* (2008) 66:567–78. doi: 10.1111/j.1574-6941.2008.00521.x

121. Jergens AE, Crandell J, Morrison JA, Deitz K, Pressel M, Ackermann M, et al. Comparison of oral prednisone and prednisone combined with metronidazole for induction therapy of canine inflammatory bowel disease: a randomized-controlled trial. *J Vet Intern Med.* (2010) 24:269–77. doi: 10.1111/j.1939-1676.2009.0447.x

122. Cassmann E, White R, Atherly T, Wang C, Sun Y, Khoda S, et al. Alterations in the ileal and colonic mucosal microbiota in canine chronic enteropathies. *PLoS ONE.* (2016) 11:e0147321. doi: 10.1371/journal.pone.0147321

123. Song SJ, Lauber C, Costello EK, Lovupane CA, Humphrey G, Berg-Lyons D, et al. Cohabiting family members share microbiota with one another and with their dogs. *Elife.* (2013) 2:e00458. doi: 10.7554/elife.00458.018

124. Makielski K, Cullen J, O'Connor A, Jergens AE. Narrative review of therapies for chronic enteropathies in dogs and cats. *J Vet Int Med.* (2019) 33:11–22. doi: 10.1111/jvim.15345

125. Karayiannakis AJ, Syrigos KN, Efistiathio J, Valizadeh A, Noda M, Playford RJ, et al. Expression of catenins and E-cadherin during epithelial differentiation in the duodenal and colonic mucosa of dogs with inflammatory bowel disease. *J Vet Intern Med.* (2018) 32:1019–25. doi: 10.1111/jvim.15117

126. Gassler N, Rohr C, Schneider A, Kartenbeck J, Bach A, Obermuller N, et al. Inflammatory bowel disease is associated with changes of enterocytic function.
