Introduction: paediatric inflammatory bowel diseases

The global prevalence of inflammatory bowel diseases (IBD) has been steadily increasing and is now greater than 0.3% (1). In some Western countries, such as Canada, rates are projected to be as high as 1% by the year 2030 (2). The prevalence in children has also been increasing (3), with up to 25% of diagnoses occurring in the paediatric population (4,5). There are two main subtypes of IBD, including Crohn disease (CD) and ulcerative colitis (UC). IBD consists of chronic intestinal inflammation, which presents with skip lesions occurring anywhere along the gastrointestinal tract from mouth to anus in CD, while in UC inflammation is restricted to the colon in a contiguous fashion. The
aetiology of IBD is not completely understood, and there is currently no cure; however, there is evidence that genetics, environment, immune response, and the intestinal microbiome interact to play a role in disease development and progression (6-14). Growth and development concerns are unique to paediatric IBD (pIBD), including failure to thrive, which is a common presentation that leads to an IBD diagnosis; furthermore, pIBD carries a risk of linear growth failure, delayed puberty, and reduced peak bone density (15-17). Children with IBD are also at an increased risk of psychosocial difficulties including depression and anxiety (18).

Younger age of diagnosis in paediatric CD (pCD) is associated with more complicated disease (19,20). Complicated disease is defined as strictureing and/or penetrating disease in pCD, and is associated with poorer outcomes and increased need for surgery (21). pCD patients are also more likely to have small bowel involvement than adults (42% compared to 15% of adults) (22). Paediatric UC (pUC) patients are more likely to have extensive inflammation in the form of pancolitis, ~43–81% (22-24) compared to ~20–35% in adults (22,25,26). Almost half of children diagnosed with pCD, and roughly 16% of children diagnosed with pUC require surgery in the ten years following diagnosis (27), and children with IBD have on average a lower health-related quality of life (28). In follow-up studies, diagnosis with IBD in childhood has been associated with increased risk of cancer and mortality in adulthood (29). To improve outcomes and prevent the complications of disease associated with a young age at diagnosis, there is a need to better define causes and predictors of response to therapy in pIBD. We present the following article in accordance with the narrative review checklist (available at http://dx.doi.org/10.21037/dmr-20-160).

Methods

The purpose of this literature review is to elucidate the host-microbe interactions in pIBD and describe how these interactions are influenced by diet and current disease therapies. Original, peer-reviewed, English language research published in the most recent five years was considered first and foremost, with the addition of older studies if they provided novel, foundational, or relevant information. Human studies were prioritized, followed by animal and then in vitro evidence, respectively.

Aetiology & pathophysiology: host, microbe, and diet

The pathogenesis of pIBD is complex, involving genetic and environmental factors. To clearly define how microbes and diet are involved in pathogenesis, and how they could therefore contribute to therapy response, we will first provide an overview of current knowledge of disease pathogenesis in pIBD in general. This will include details on genetics, microbes, the intestinal barrier, metabolites, and diet.

IBD-associated genes

Although genetics plays a role in pIBD, most patients are diagnosed without a family history (30), suggesting that genetic factors are less significant than environmental ones in the pathogenesis. Graham and Xavier provide a detailed review of IBD genetics (31). Associated genes are involved in intestinal epithelial barrier integrity, microbial recognition and clearance, and regulation of the host-immune response (32,33). The caspase recruitment domain family member 15 gene (CARD15), known as nucleotide-binding oligomerization domain two (NOD2), displays the strongest genetic association with IBD, and is specifically correlated with CD (34). NOD2 is a pattern recognition molecule in the cell cytosol that can recognize bacterial peptidoglycan (35,36). Individuals with the IBD-associated NOD2 variants have decreased intestinal antimicrobial peptides (e.g., α-defensins) (37), and the most common IBD-associated variant has no response in vitro to bacterial peptidoglycan and lipopolysaccharide (LPS) (38), along with a drastically reduced ability to prevent intracellular invasion (39). Patients with these variants also have lower circulating levels of anti-tumour necrosis factor-α (TNF-α) monoclonal antibody (MAb) (discussed below) between doses than patients with the wild-type gene, which may result in reduced treatment efficacy (40). Children with NOD2 variants are also more likely to present with growth impairment (41). Another IBD-associated variant is in the CARD9 gene, which results in impaired innate immune response to intracellular pathogens and increased pro-inflammatory signalling (including TNF-α) through increased nuclear factor-κB (NF-κB) signalling (42,43). Individuals with these variants also exhibit increased Malassezia restricta, a fungus capable of exacerbating colitis in animal models (44). Inability to adequately clear microbes, as may occur with the IBD-associated NOD2
and CARD9 variants, could result in chronic inflammation as microbes persist inappropriately in the intracellular or juxta-epithelial intestinal environment.

There are also IBD-associated genetic variants linked to increased intestinal permeability in IBD, such as Janus kinase (JAK)2 in CD (45). JAK2 is involved in cytokine signalling (46) and its inhibition can decrease T cell-mediated inflammation (47). The JAK inhibitor Tofacitinib has proven effective in some IBD populations (48), potentially by reducing JAK-driven intestinal permeability, reducing antigen translocation from the intraluminal environment, and production of T cell-mediated inflammation. Other examples of genetic associations with reduced barrier integrity include Prostaglandin E Receptor 4 (PTGER4) in CD and Hepatocyte Nuclear 4 Alpha (HNF4A) in UC (49), regulating junctional/cytoskeletal proteins and claudin-15 (important for epithelial tight junctions), respectively (50). Additional IBD-associated variants in genes involved in cell adhesion and tight junctions include C1orf106 and RNF186 (51-53). Suboptimal intestinal barrier function may result in increased antigen exposure and perpetuate a pro-inflammatory immune response (54-56).

Although a significant number of genetic associations have been identified in IBD, to date only 26% and 19% of the heritability of CD and UC, respectively, can be explained by genetics (57). Genetic associations also cannot explain the recent rises in incidence (2), highlighting the importance of assessing environmental factors as contributors to the pathophysiology of IBD.

**Microbial involvement in IBD**

The human gut microbiome is composed of bacteria, viruses, archaea, and eukaryotes, with approximately as many bacterial cells as there are human cells in the body (58). Alterations in the gut microbiome are thought to play important roles in numerous human diseases. The microbiome is also critical to health, and is essential for healthy immune development and certain metabolic functions, such as dietary fibre and protein fermentation, and generation of certain vitamins and neurotransmitters (59-61). The normal intestinal bacterial microbiota is composed mostly of obligate anaerobes from several main phyla, including: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and trace Verrucomicrobia, Fusobacteria, Cyanobacteria, and candidate division TM7 (62). The role of viruses, fungi, and other eukaryotes in the intestinal microbiome has been relatively understudied, but evidence suggests a dysbiosis of fungi and viruses also exists in pediatric (63-65) and adult (66,67) IBD. Interest in the role of fungi, which constitute a relatively small proportion of the total intestinal microbiota (68), was sparked by the increased prevalence of anti-fungal antibodies in patients with IBD relative to controls (69). The IBD mycobiome is characterized by reduced diversity and an increased total fungal load, with increased ratios of Basidiomycota to Ascomycota, and a decrease in the ratio of Saccharomyces cerevisiae to Candida albicans (44,63,67). There is also an increase in the previously mentioned M. restricta, seen with IBD-associated CARD9 variants (44,70). Alterations in fungi total and relative abundances and diversity, and potential related therapeutics were recently reviewed by Lam et al. (70). Changes in the intestinal virome in IBD are even less understood (71,72), but have been shown to trigger colitis in some animal IBD models (73), and likely influence complex relationships within the microbial ecosystem (74), including the effects of bacteriophage on the bacterial microbiota (75,76). Potential implications of the intestinal virome are discussed in several specific reviews (77,78).

Alterations in the intestinal bacterial microbiota are a well-documented hallmark of IBD, with somewhat different alterations in CD vs. UC, and more significant differences with more active disease, relative to controls (79). The microbial dysbiosis in IBD is likely, in-part, independent of associated host genetic factors, as demonstrated by numerous twin studies (80,81). Microbiota differences between twins concordant for CD supports a likely role for the influence by environmental factors in establishing and maintaining dysbiosis (82,83). Environmental factors known to play a role in influencing the intestinal microbiota include antibiotic exposure (84,85), infections (including COVID-19) (86), nutrition, and other environmental exposures such as tobacco smoke (87-93). Microbial changes in IBD show decreases in strict anaerobic species within the Firmicutes and Actinobacteria phyla, with increases in oxygen tolerant Proteobacteria and Bacteroidetes sp. (94). Other changes include lower levels of mucosal bacteria species richness in pCD that is not always reflected in stool analyses (95), reduced faecal microbiota diversity (96), and alterations in certain mucosal and bacterial taxa, including higher numbers of Enterobacteriaceae and lower abundances of strictly anaerobic Firmicutes, such as Clostridiales (95). Reduced levels of Clostridium sp., Faecalibacterium prausnitzii, and Bifidobacterium sp. are seen in IBD, with relative abundances reduced more in CD than UC (79). Reduced Bacteroides species are also seen in IBD, more...
so in active disease than remission (97), *F. prausnitzii*, an important fibre fermenter, has been shown to have anti-inflammatory effects, and its absence is predictive of disease relapse (98). Beneficial microbes such as *F. prausnitzii* that are reduced in IBD may improve mucosal barrier function through tight junction proteins, inducible heat shock proteins, immunomodulation, and even production of anti-inflammatory peptides (99-103). Microbial dysbiosis correlates strongly with disease severity, as measured by the paediatric Crohn disease activity index (PCDAI) (95). Microbes may even predict future disease activity: levels of *Enterobacteriaceae*, *Fusobacterium*, and *Haemophilus*, when combined with age of disease onset and PCDAI at diagnosis, have been found to be significantly predictive of future PCDAI (95). Unaffected siblings of CD patients also display an altered intestinal microbiome compared to controls that correlates with their increased risk for developing IBD, suggesting a causative role for the microbiome in IBD pathogenesis (104-106). Disease location is also associated with variations in microbial abundance: patients with predominantly ileal CD had lower abundances of *F. prausnitzii* and higher levels of *Escherichia coli* in intestinal mucosa specimens compared to patients with predominantly colonic/ileocolonic CD (81).

Although UC has shown less microbial distinction from non-IBD controls than CD (107), Shah *et al.* found that patient response to initial therapy was associated with differential abundances of certain microbial taxa (108). Certain microbes may predict or even mediate a patient's future response to treatment in pUC, as Shah *et al.* found that certain *Clostridium* and *Bacteroides* OTUs were only detectable at baseline in the pUC patients who went on to have no clinical response to treatment (108). Similarly, high *Candida* abundance at baseline was associated with clinical response and increased bacterial diversity following faecal microbiota transplantation in active UC (109). In severe pUC, phyllum level changes included lower levels of Firmicutes and higher levels of Proteobacteria compared to controls. The lower microbial richness in pUC was more prominent in children who failed to respond to glucocorticoid therapy (110). Additionally, fluctuations in the UC microbiome are associated with disease severity and need for colectomy (111), and significant depletion of *Ruminococcaceae* and *Lachnospiraceae* with an increased abundance of *Streptococcus anginosus* were found in patients with more severe disease.

While altered microbiota correlating with disease severity is well documented at various taxonomic levels (94), there is yet to be any specific microbial culprit linked with disease flares. Sequencing comparing mucosal microbiota in inflamed vs. non-inflamed bowel segments has not identified specific taxa associated with inflammation across individuals but does show significant composition changes in the non-inflamed terminal ileum of pUC patients, suggesting microbial alterations upstream of local inflammation (112,113). Likely, complex host-microbe interactions are specific to each individual. Roy *et al.* have shown that specific variations in microbial dysbiosis introduced in animal models lead to unique colitis phenotypes and pathologies specific to the microbial community introduced (114). Future techniques to identify ‘problematic microbiota’ unique to each patient with IBD may involve identifying levels of immunoglobulin (Ig) binding found on microbes isolated from patients. IgA and IgG have shown differential microbial binding in patients with IB, including differential binding between UC and CD (115,116). High levels of Ig coating has been used to identify bacteria from IBD patients that induce inflammation in animal and *in vitro* models, suggesting that these techniques could be used to identify culprit bacteria in specific patients (117,118). Mucosal IgA can help reduce microbial invasion (119). In some animal models certain antibodies against the intestinal microbiota protect against bacterial sepsis (120); the role of these antibodies in IBD remains unclear but has been explored as a marker of inappropriate immune responses to gut bacteria (121) or increased antigen exposure through intestinal barrier disruption with increased disease activity (115,122,123). Some antibodies directed against commensal microbes and fungi, such as anti-*Saccharomyces cerevisiae* (ASCA), have limited diagnostic and predictive roles in IBD (124).

**Altered intestinal barrier in IBD**

Impaired barrier integrity is another feature of IBD (125-129). pUC patients show reduced mucous barrier thickness, and fewer mucin-containing goblet cells, with bacteria colonizing closer to intestinal epithelial cells than controls (113). Similarly, bacterial colonization was in closer proximity to intestinal epithelial cells in pCD vs. controls (113). Confocal laser endomicroscopy has shown increased leakage of IV-administered fluorescein through the intestinal epithelial barrier in pCD and pUC compared to controls (125); in adults, increased fluorescein leakage was associated with disease relapse (55). As mentioned in relation to patient genetics, an impaired epithelial barrier...
may result in increased bacterial and antigen translocation that perpetuates chronic inflammation.

Alterations in the intestinal microbiota and host barrier integrity seen in IBD carry a number of potential consequences for the patient (130). Microbes can directly impact health by increasing or decreasing resistance to colonization by pathogens, producing vitamins and nutrients, and training the development of a balanced immune system (130-132). A balanced host-microbe interaction is essential for health; germ-free mice show aberrations in a number of body systems including: cardiovascular, respiratory, gastrointestinal, and immunity (131). Germ-free animals also show significant morphological and functional intestinal changes (131). Some of these aberrances can be corrected by colonization with specific bacteria, including improving intestinal barrier integrity with the addition of certain Lactobacillus sp. to germ-free animals (133-135). An unhealthy host-microbe relationship in IBD may perpetuate or trigger disease, and elucidation of this relationship can improve our understanding of the aetiologies of these diseases.

**Metabolites: linking diet to microbes in IBD**

Metabolites are compounds necessary for or formed during metabolism and represent dietary, host, and microbe factors reflecting the real-time microenvironment of the gut. Identification and quantification of metabolites can provide a concrete assessment of microbial function and human metabolism. In addition to altered microbial abundances, variations in urinary metabolites suggest altered microbial function in the IBD microbiome. In adults with IBD, serum and urine metabolites can distinguish active disease from remission (10). Urine metabolites can discriminate adult IBD patients from healthy controls, with a varying ability to distinguish CD from UC (136,137), while serum and faecal metabolites are capable of distinguishing pCD from pUC and healthy controls (138). A limited number of studies suggest that urinary metabonomics can also differentiate pIBD from healthy controls (139). Metabolomics of mucosal surface samples collected during colonoscopy in pIBD patients linked luminal succinate to bacterial invasion in vitro, highlighting the importance of the gut microenvironment in IBD (140). Lavelle & Sokol detail the groups of microbe-associated metabolites significant in IBD and their potential roles (141).

Metabolites commonly altered in paediatric and adult IBD belong to metabolic pathways suggesting variations in glutathione metabolism, the citric acid cycle (CAC), and importantly, microbial activity (136,139,142). Hippurate is defined as a co-metabolite, as it is produced by a combination of host and microbial metabolism (143); it is notably absent in germ-free animals (143), and significantly reduced in IBD (144). Hippurate has been positively associated with microbiome diversity, as well as increased fruit and whole grain intake (145). When potential dietary influences were excluded, hippurate persisted as a marker for IBD vs. controls (146). Supplementation with the hippurate precursor sodium benzoate does not increase urinary hippurate in CD or controls, and CD patients have persistently lower levels, suggesting an absence of the microbial metabolic function (147).

Other microbe-associated metabolites include short chain fatty acids (SCFA), which are produced from microbial fermentation of dietary fibres, and to a lesser extent amino acids; these are often significantly reduced in both adult and paediatric IBD (136,137,148,149). Stool from IBD patients typically has less acetate, butyrate, and propionate, while lactate and pyruvate are increased (148). Effects of SCFA include suppressing intestinal permeability and increasing the population of regulatory T-cells that can help to prevent chronic inflammation (150,151). SCFA can also activate anti-inflammatory signalling cascades by binding to intestinal G-protein coupled receptors such as GPR109A, GPR43, and GPR41 (152). Impaired metabolism of SCFA has been implicated in IBD; for example, experimental dextran sulphate sodium (DSS)-induced colitis is characterized by reduced butyrate oxidation and increased glucose oxidation (153). Additionally, in one study, GPR43 expression in the ileum was significantly reduced in CD compared to controls, regardless of disease activity (154). Inflammation impacts the response to butyrate in IBD; ex vivo organoids from IBD patients and controls respond similarly to butyrate, but when the pro-inflammatory cytokine TNF-α is added butyrate uptake is reduced in IBD organoids only (155). This could explain why exogenous administration of SCFAs has not consistently resulted in clinical improvement in the treatment of IBD (156). Previous studies examining direct administration of SCFA (using butyrate enemas) have shown mixed results, despite other promising in vitro, ex vivo, and animal studies showing decreased inflammatory markers and improved intestinal barrier (157,158).

Butyrate produced by bacterial fermentation is the primary fuel source for colonocytes (159); not surprisingly, colonocytes from germ-free mice have
fewer CAC intermediate enzymes (153). Butyrate has multiple physiologic effects, including reducing bacterial translocation in vitro (99), and can act as both an energy source and a histone deacetylase (HDAC) inhibitor (153). Butyrate concentrations are increased by some resistant starches (RS) (160), and SCFA production varies in response to diet and intestinal transit time (160). Stool SCFA content does not always reflect production through fermentation, as longer transit time is associated with reduced butyrate (160). Impacts of transit time on SCFA concentrations could be due to increased opportunity for absorption and utilization during slower transit, or related to delivery time of RS and dietary fibre to the large bowel and time for microbial metabolism and fermentation (160). A number of other factors are known to impact SCFA concentrations; antibiotics reduce SCFA and intake of different dietary fibres can increase SCFAs in varying amounts (161). Additionally, SCFA production can modify the intestinal microbiome: their acidity can select for more acid-tolerant bacteria (161). SCFA may play a role in patient response to treatment; Wang et al. found a significant reduction in SCFA-producing bacteria prior to Infliximab (IFX) therapy in pIBD, with a shift to a SCFA profile more similar to healthy controls after therapy, and that the abundance of SCFA-producing species was predictive of sustained remission in pIBD (158). However, it is important to recognize the limitations of metabolomics, especially as this relates to interindividual variation and lack of standardized protocols.

**Environment: focus on diet**

A “Western” diet that is low in dietary fibre and high in processed and fatty foods has been proposed to partially account for the recent rise in the incidence in IBD, particularly in the western world (162). Although a number of dietary risk factors and associations have been recognised, the majority have been identified through case-control or cohort studies. It is difficult to design and conduct experimental dietary studies that can prove causality, and in animal studies it can be challenging to approximate a human diet and consumption patterns. Assessing for associations with individual foods or nutrients may miss capturing the complexity of micro and macronutrient interactions. Additional complexities include the associations of dietary intake with culture and genetics, as well as numerous reporting biases.

Diet is associated with both increased and decreased risk in adult and paediatric IBD. CD has especially strong associations with dietary intake (163). Foods associated with increased risk of IBD have included fast-food (164) and increased protein intake that is potentially specific to animal protein (163,165–167). Protective factors include breast-feeding (163), and specifically for pCD: increased vegetables, fruits, fish, olive oil, grains, and nuts (168). Some sex-specific associations have been identified, and foods associated with increased risk for pCD in females included meat, sugary foods, and high fat foods (168). Many of these studies were conducted prospectively, for example E3N (166), EPIC (169), the Nurses’ Health Study I & II, and Health Professionals Follow-up Study (170), suggesting that dietary intakes may establish an intestinal environment that favours development of IBD. Diet is proposed to exert effects through alterations in host immunity, host barrier integrity, and alterations in the microbiota, including viruses (163,171). Potential mechanisms are discussed in depth in the review by Levine et al.; we will discuss them briefly here (163). Host variability in these factors, specifically the microbiota, likely plays a significant role in the complex associations seen with certain dietary components, such as meat or dietary fibres. In the absence of dietary fibres, some microbes display enhanced mucolytic activity – digesting and depleting the host intestinal mucous layer, compromising intestinal barrier integrity, and resulting in increased immune activation (163). Resulting epithelial barrier disruption, as evidenced by increased gap density, correlates positively with increased inflammation and disease activity (125). Dietary fibres and RS can increase SCFA production, potentially decreasing intestinal permeability (163). Other diet components may also impact the levels of SCFA receptors; in mice fed a high fat and sugar diet there was reduced GPR43 receptor expression (154).

Diet is one of the main determinants of the intestinal microbiome (172). Previous studies have found that dietary patterns are significantly more predictive of the intestinal microbiome than individual nutrients—likely as the complex interaction of whole foods cannot be predicted from isolated components (93). In mice, a Western style diet high in fat and sugar increased colonization with pathogenic organisms such as adherent invasive *E. coli* (AIEC) (173), which is a key pathobiont implicated in the pathophysiology of CD (174). Targeting these associations between host, diet, and microbe, a number of dietary therapies have been developed, and are described in the Treatment section.
Diet-host-microbe interactions in IBD

The genetic, microbial, dietary, and metabolic associations in IBD suggest that dysregulated host-microbe relationships, further influenced by diet, are important in the pathogenesis of IBD. This is additionally supported by evidence that microbiota-targeting therapies, like antibiotics and diet, can result in clinical improvement in IBD (99,163). Faecal microbiota transplant (FMT) is an emerging microbe-based therapy that has shown some promise, but experience in children is limited (175). A variety of protocols exist but evidence is conflicting on many aspects, such as: donor selection (universal may be beneficial) (176,177), fresh vs. previously frozen donor stool (178), or need for antibiotic pre-treatment (178-180). Baseline patient microbiota is likely a factor in the variability of outcomes with FMT; in adult UC, there is evidence that the baseline mycobiome, specifically increased Candida abundance, can be predictive of a positive FMT response (181). Much of the evidence for FMT is specific to treatment of a superimposed Clostridioides difficile infection (CDI) (182). FMT for CDI in IBD is less effective than without IBD and results in fewer microbial shifts (183,184). Although most adverse events with FMT in IBD are mild and include non-specific symptoms such as diarrhoea, nausea, and abdominal pain (176), some potentially serious disease flares may occur (182,185). FMT in IBD is further discussed in several recent reviews (179,186-189).

As another example, diversion of the faecal stream in pIBD through an ileostomy can result in clinical improvement, with disease severity often increasing again when the faecal stream is returned to the colon (190). Additionally, almost all colitis animal models, such as interleukin-10 (IL-10) knockout or TNFΔARE mice, do not develop intestinal inflammation under germ-free conditions (191,192).

Increased leakage of luminal antigens across the intestinal barrier in IBD, presumably due to genetic and environmental/diet factors, results in an excessive cytokine-dependent inflammatory response in mucosal tissue (193). These antigens can include bacterial products or components such as LPS or peptidoglycan. Microbial antigens can increase NF-κB signalling through increased production of TNF-α, which is recognized as an integral part of protection against invading pathogens (194). TNF-α is thought to play a role in the pathogenesis of IBD via fibroblast activation, enhanced production of pro-inflammatory cytokines, and enhanced T-cell resistance to apoptosis (193). TNF-α effects through NF-κB also result in Paneth cell death, damage to intestinal epithelial cells, and increased production of matrix metalloproteinases (MMPs) by myofibroblasts (193). Paneth cells are specialized secretory intestinal epithelial cells located in intestinal crypts of the small bowel (but also can be found in the large bowel in IBD) that are important for producing antimicrobial and immune-stimulating molecules (195). They have been shown to be important in defence against intestinal pathogens and play a role in regulating the abundance of certain intestinal microbiota such as Bacteroidetes and Firmicutes, two phyla significantly altered in IBD (110,195,196). In vitro, the addition of TNF-α to intestinal epithelial cell monolayers results in increased permeability (99).

Further evidence of imbalanced host-microbe interactions in IBD is the ability of increased antimicrobial antibodies in patient serum to predict disease phenotype in CD years before diagnosis (197). Patients with higher antimicrobial antibodies were more likely to have complicated CD, with higher titres predicting earlier complicated disease (197). In pCD, anti-Saccharomyces cerevisiae, anti-outer membrane porin C (an E. coli antigen), and anti-Cbib1 (a flagellin protein), are also predictive of complicated disease (198). The more antimicrobial antibodies a child with pCD was positive for, the higher the likelihood of an earlier progression to complicated disease and need for surgical intervention (198). Antimicrobial antibodies are generally less common in pUC (69), but associations with faecal calprotectin (a marker of mucosal inflammation) levels in pUC have been found (199). The reduced associations between antimicrobial antibodies in pUC compared with pCD may be reflective of the degree of dysbiosis, with the CD microbiome typically further distinguished from healthy controls than the UC microbiome (107).

Treatments in pIBD: links to microbes and diet

Traditional therapies

Despite the described advances in understanding the pathogenesis of IBD, most current treatments for IBD focus on reducing inflammation, mainly through suppression of the immune system. Treatment of pIBD has some variations between pCD and pUC, but both can include glucocorticoids (GCS), thiopurines, methotrexate, aminosalicylates (5-ASA), and biologic therapy (200,201).
Dietary therapy, which is unique in that it does not suppress the immune system, is a recommended therapy currently specific to pCD, with exclusive enteral nutrition (EEN) recognized as the first line therapy for luminal pCD (discussed in detail below) (201). 5-ASAs are anti-inflammatory drugs and are suggested as a first-line therapy to induce and maintain remission in mild or moderate pUC, and GCS are the second choice for therapy to induce remission in both pCD and pUC (200,201). GCS carry the risk of steroid-dependence, and have many side effects including increased risk for infections, diabetes, osteoporosis, and cataracts (202). Biologic therapy, usually anti-TNF, is recommended for chronically active or steroid dependent/resistant pUC when 5-ASA and thiopurines do not control disease, and is used for both induction and maintenance of remission (200). Biologics are compounds purified from another organism or virus (203). In pUC, FMT and antibiotics are not currently recommended; but probiotics like VSL#3 (a cocktail of several Lactobacillus, Bifidobacterium and Streptococcus salivarius subsp. thermophilus) or E. coli Nissle 1917 are approved as adjuvant therapy in mild pUC with limited effect. Well powered RCT studies are still needed to prove if probiotics are more effective than placebo (204).

Effects of therapy on host-microbe interactions remain poorly elucidated, even among therapies targeting the microbiota. Intestinal microbiome-altering effects of GCS in animals in both normal and inflammatory conditions include increased Bifidobacterium and Lactobacillus species with elimination of mucin degrader Mucispirillum (204). In pUC patients, GCS use was associated with increased Actinomyces abundance in patients who sustained remission, with an accompanying decrease in a Clostridium OTU (111). Thiopurines may increase mucosal bacteria numbers and adherence, decrease faecal bacterial diversity and richness, and in vitro inhibit Mycobacterium avium subspecies paratuberculosis (204), which is thought by some to play a causative role in CD due to disease similarities with mycobacterial enteritis (205). FMT, most commonly used in recurrent CDI, is being investigated for utility in IBD and shows some promise in UC and CD, albeit at a lower success rate than in CDI (187). Complexity in the host-microbe relationship of IBD when compared to CDI may explain the mixed results and weaker relationships so far observed between FMT and remission in IBD (204). As described above, understanding of the unique microbial dysbiosis present in each patient may be required to adequately modify the microbiome therapeutically (Figure 1).

Anti-TNF therapy

Anti-TNF-α therapy is recommended in pCD when, despite immunomodulatory therapy, the patient has persistent active luminal disease, active steroid-refractory disease, active perianal fistulizing disease, and with severe disease at presentation with growth retardation (201). In pUC, anti-TNF-α therapy is recommended for chronically active or steroid-dependent disease that is not controlled by 5-ASA and thiopurines (200). IFX and Adalimumab (ADA) are monoclonal antibodies (MAbs) that can recognize both membrane-bound and secreted TNF-α (206). Membrane-bound TNF may play more of a role than secreted, soluble TNF; agents that preferentially block soluble TNF have not been shown effective for IBD therapy (193). Blocking of TNF effects by IFX results in T-cell apoptosis and reduction in cytokines, chemokines, and adhesion molecules that promote the influx of immune cells into the gut (207). However, the relationship between TNF-α and mucosal healing is complex, as TNF-α knockout mice are more likely to develop colitis with DSS exposure (99). Furthermore, the ability of the probiotic VSL#3 to decrease intestinal permeability in inflamed ileal tissue ex vivo was shown to be TNF dependent in mice (208). Regardless, in vivo anti-TNF- therapy in pIBD patients has been shown to improve the intestinal epithelial barrier and promote mucosal healing (209,210). Risks and side-effects of anti-TNF-α include an increased risk of infection, paradoxical psoriasis in pCD, and infusion reactions (16). Infusion reactions can include the development of antibodies against IFX or ADA, which has been correlated with worsened drug effectiveness (211).

After eight weeks of anti-TNF therapy in pCD patients, faecal microbiota of treated patients more closely resembled healthy controls (65). pCD patients with active disease were found to have reduced relative abundance of almost one third of genera compared to healthy controls, including: Roseburia, Ruminococcus, Akkermansia, Prevotella, Coprococcus, and Eubacterium (65). Shannon diversity and OTU numbers increase with IFX therapy in pCD, with fewer differences in microbiota taxa comparing pCD and controls after IFX treatment than at baseline (212). Wang et al. found that pCD patients who would have a sustained response to IFX therapy (defined here as a PCDAI score of ≤10 throughout follow-up), also showed increased levels of SCFA-producing genera with IFX therapy, including: Blautia, Faecalibacterium, Lachnospira, and Roseburia (158). It remains to be seen, with these consistent changes in
**Active Disease.** Reactive oxygen species generated by immune cells in response to cytokines such as TNF-α inhibit anaerobic microbiota. An altered intestinal microbiome produces fewer beneficial metabolites and colonizes proximal to epithelial cells. A diet low in fermentable fibre with few SCFA producing bacteria results in fewer SCFA to promote barrier integrity and immune system tolerance.

Impact of treatment on microbiome: Anti-TNF-α, dietary therapies such as EEN (outlined in green), and others allow recovery of the microbiome. In patients where there is recovery of the commensal microbiota, resulting beneficial metabolites may help mediate the resolution of inflammation and improve barrier integrity (D).
Figure 1 Pathways from active disease to remission. Microbial dysbiosis is present in the context of chronic inflammation perpetuated by TNF-α, reactive oxygen species, and impaired epithelial barrier integrity (A). Treatment with first-line therapies, including EEN or anti-TNF-α, may resolve inflammation and improve barrier integrity despite the microbial dysbiosis (B); alternatively, normalizing the microbiome in response to these therapies or through microbe-altering treatments, including diet, may also promote remission (C). Ideally, a combination of pathways outlined in (B) and (C) will allow patients to achieve disease remission (D). Eukaryotic cell and anti-TNF-α/TNF-α images credit of: https://smart.servier.com/, edited by the authors.
microbiota with anti-TNF-α therapy, if these shifts are a cause or consequence of therapy success. Because IFX therapy is administered via intravenous infusion, and ADA subcutaneously, there is no direct interaction with the host intestinal microbiome. Resultant microbial changes may be due to modified host-microbe interactions in the form of decreased inflammation through mediators such as reactive oxygen species or antimicrobial peptides, or shifts may reflect other changes concomitant with therapy such as diet.

**Dietary therapy**

EEN therapy is currently the first line therapy for induction of remission in pCD and entails liquid meal replacement beverage and the exclusion of all other food and beverages except water for a period of typically six-eight weeks (213-215). The EEN formulas most commonly used include Modulen IBD®, Ensure/Ensure Plus®, Pediasure®, or Nutren/Nutren Junior®, formulas can be elemental or polymeric (216). Although the safety of this treatment is excellent, with no concern for side effects, EEN poses considerable challenges to patients. The most commonly described challenges involve the palatability and monotony of the diet, as well as cost (216). The exact mechanism of action of EEN remains unknown, but theories include immune and/or microbial modulation, removal of many dietary antigens and other ‘offending foods’ (such as preservatives and emulsifiers), and improved intestinal barrier function with decreased inflammation, as reviewed more specifically by others (163,213,215).

EEN therapy significantly impacts the microbiome within one week of initiation (65), and preliminary studies indicate differential changes in the microbiota of responders vs. non-responders to EEN. When comparing newly diagnosed pUC and pCD patients receiving either EEN or corticosteroids for induction therapy, achieving remission was found to better predict changes in patient microbiota than either the therapy or disease subtype (217). Kaakoush et al. found that responders to EEN had a greater decrease in OTUs than non-responders (response was defined as a PCDAI <10 after eight to 12 weeks of therapy) (218). Leach et al. found that patients with the greatest decreases in *Bacteroides* and *Prevotella* species with EEN treatment demonstrated improvement in PCDAI, with microbiota changes persisting even four months after EEN completion (219). In a study by Quince et al., EEN resulted in decreases in *Bifidobacterium, Ruminococcus*, and *Faecalibacterium*; Shannon diversity also decreased on EEN but returned to pre-treatment levels two months after EEN completion (220). These decreases in OTUs and diversity may reflect the lack of fibre in this liquid diet (219,221); decreasing numbers of dysbiosis-associated taxa present at baseline may be an important part of the therapy mechanism or a side-effect of the homogenous diet. Responsiveness of baseline microbes to this drastic change in diet may be a key determinant of therapy response (222).

Dietary therapies are rapidly expanding in pCD, with some in trial for pUC, but many of the microbial shifts associated with successful therapy to induce remission are consistent across the effective dietary therapies. CD-TREAT, another novel dietary therapy for pCD that attempts to recreate EEN dietary exclusions with food intake, induces similar microbial shifts when compared with EEN (223). The Crohn’s Disease Exclusion diet (CDED), which includes whole foods such as fruits, vegetables, carbohydrates, and meats, induced microbial shifts that include decreased *Bifidobacterium* and *Prevotella*, similar to EEN, and increases in *Roseburia*, which is typically decreased in pBD and is a known butyrate producer (65,158,224). Both EEN and CDED induce rapid response in >80% of pCD cases, seen within 3 weeks (225). It still remains unclear if these microbial shifts are in part responsible for the effectiveness of therapy, or result as a consequence of successful therapy and disease remission.

**Expanding horizons for dietary therapy**

Dietary interventions remain a safe treatment option for pCD patients; but much of the diet-host-microbe interactions remain poorly understood. Dietary fibres and proteins play an obvious role in this relationship as host microbes are responsible for the fermentation and breakdown of these components into some of the beneficial products discussed above (62,226-228). Williams et al. provide an in-depth review of the health-associated benefits of these products of fermentation (229). Research suggests that typical Western diets, where incidence of IBD is climbing most significantly (2), consists of only 10–20 g of dietary fibre daily, rather than the recommended 26–38 g, limiting the sources of carbon and energy for many intestinal microbes (230). More complexity is added when we closely examine the group of individual dietary fibres which have grown to encompass non-starch polysaccharides (e.g., cellulose, pectin), non-digestible oligosaccharides (e.g., fructooligosaccharides, galacto-oligosaccharides), non-
carbohydrate-based polymers (e.g., polyphenols, lignan), and carbohydrates considered to be of animal origin (e.g., chitin) (231).

Dietary fibres are commonly categorized based on their water solubility factor where soluble fibres (pectin, arabinogalactan, β-glucans, inulin, fructo-oligosaccharides, galacto-oligosaccharides, and xyloligosaccharides) are more readily available as a source of nutrition for microbes than their non-soluble counterparts (cellulose and lignin) (61,229,232). Microbial fermentation of dietary fibres involves a variety of species working in a coordinated community where each microbe plays a key role in the fermentation pathway, relying on partner organisms to complete the process (233-236). Many patients experience worsened symptoms following consumption of dietary fibres, and as discussed above, dysbiosis is a well-supported hallmark of IBD; it is possible that production of beneficial products of fibre fermentation are altered or reduced in IBD patients (118,237-240). This suggests that precision tailoring of fibre intake in IBD patients to ensure the capability of fermentation by their unique microbiomes may help to promote healthy levels of beneficial fibre fermentation products. Although some clinicians have promoted reduced fibre consumption, or fibre avoidance, in these patients, there is currently no evidence to support this as a broad therapy in IBD (241,242). When microbes capable of fermentation are present, fibre supplementation may have beneficial effects in IBD; animal models of IBD have demonstrated reduced C-reactive protein (CRP) levels and amelioration of disease activity and histology scores when given a probiotic/fibre-based prebiotic compared to prebiotic alone (243). Similar results have been shown in human studies of prebiotics in IBD patients (244,245). Currently, however, our understanding of the precise role of select fibre types in IBD remains poorly understood.

The hemicellulose fibre arabinoxylan (AX) is a large component of the fibre found in our diets, and is sourced from cereal grains like rye, wheat, oats, barley, rice, sorghum, and some legumes (246-251). A number of microbes ferment AX to SCFA such as acetate, propionate, butyrate, and ethanol (249,252-254). Studies of AX have demonstrated a protective role through stimulation of the caecal mucin layer, and reduced inflammatory markers in clinical trials where adult UC was ameliorated with no significant side effects (255-259). Lactobacilli, Enterococcus, and Bifidobacteria can ferment β-glucan fibres that are found in a variety of plant and fungal cell walls, including mushrooms, oats, and barley (260-263). The effects of β-glucan intake on IBD histological scores is highly dependent on the fibre source, with the most effective source to date from the mushroom species Pleurotus eryngii (264). Similarly, β-fructans, which are commonly found in roots, artichokes, banana, wheat, onion, and garlic are fermented primarily by acetate-producing and acetate-converting butyrate-producing strict anaerobic bacteria, which can lead to anti-inflammatory effects (265-267). The fibre pectin has received less attention as an anti-inflammatory therapeutic dietary fibre due to the variability in esterification and therefore by-product production, but is found in a wide variety of fruits and vegetables, and is easily accessible for fermentation by a number of microbes (Bacteroides, Prevotella, Bacillus, Agrobacterium, Pseudomonas, Ralstonia, Dickeya, and yeast) (268-276). Pectin is almost completely fermented to a variety of SCFAs (268-276). Although animal models have demonstrated beneficial effects with SCFA production from cellulose fermentation (277), succinate has been associated with promoting inflammation and bacterial invasion, suggesting a possible mechanism for patient described sensitivity to consumption of dietary fibres (140,141,278-280).

The unique interactions between the host microbiome and dietary fibres in each pIBD patient may help explain some of the mechanisms of the various dietary therapies, as well as highlight a potential avenue for future developments and dietary modifications. Appreciation of the current and desired diet-host-microbe interface in pIBD could allow better tailoring and development of modifying therapies. Novel approaches, building on these principles, would allow incorporation of a patient’s baseline microbiome/metabolome status in the consideration of therapy choice. For example, if specific SCFAs are reduced, microbes responsible for producing them could be added, or prebiotic-rich diets that would promote growth of these recommended microbes.

Conclusion and future perspective

As the global prevalence of pIBD increases, improved understanding of the pathogenesis of these diseases is warranted to prevent poor outcomes in these patients. The associated genetics, immune system changes, diet, and microbiome suggest that an altered-host microbe relationship is important to development and perpetuation of disease. Current and novel therapies modify this
relationship significantly, and in some cases improving this interface may be integral to achieving remission. Evaluation of the baseline host-microbe relationship in patients poses a novel arena for therapy development and dietary modification, as the metabolic capabilities of the patient’s microbiome may impact a patient’s response to therapy. As ongoing work clarifies this diet-host-microbe relationship, considering baseline microbiome status, targeting members of the microbiota and their capabilities through diet and other therapies may improve rates of disease remission and outcomes for paediatric patients with IBD.

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