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The intestinal microbiome, barrier function, and immune system in inflammatory bowel disease: a tripartite pathophysiological circuit with implications for new therapeutic directions

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***Abstract*:** We discuss the tripartite pathophysiological circuit of inflammatory bowel disease (IBD), involving the intestinal microbiota, barrier function, and immune system. Dysfunction in each of these physiological components (dysbiosis, leaky gut, and inflammation) contributes in a mutually interdependent manner to IBD onset and exacerbation. Genetic and environmental risk factors lead to disruption of gut homeostasis: genetic risks predominantly affect the immune system, environmental risks predominantly affect the microbiota, and both affect barrier function. Multiple genetic and environmental 'hits' are likely necessary to establish and exacerbate disease. Most conventional IBD therapies currently target only one component of the pathophysiological circuit, inflammation; however, many patients with IBD do not respond to immune-modulating therapies. Hope lies in new classes of therapies that target the microbiota and barrier function.

Keywords: barrier function, Crohn's disease, inflammatory bowel disease, leaky gut, microbiome

Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory condition of the gastrointestinal tract which includes two partially overlapping clinical entities: Crohn's disease (CD), characterized by patchy transmural inflammation that can involve the entire gastrointestinal tract, and ulcerative colitis (UC), characterized by mucosal inflammation limited to the colon [Feldman *et al.* 2015]. The incidence of CD and UC is rising worldwide [Molodecky *et al.* 2012] and despite current medical treatments which focus primarily on immunosuppression [Talley *et al.* 2011], over 20% of patients with CD still require surgery and over 10% of patients with UC still require colectomy [Rungoe *et al.* 2014]. The pathogenesis of IBD is multifactorial with genetic and environmental contributions believed to play a role in potentiating the immune system [Zhang and Li, 2014]. Recent work has also highlighted the importance of the intestinal microbiome and mucosal barrier function in disease pathophysiology [Kostic *et al.* 2014; Merga *et al.* 2014].

The intestinal microbiome has been likened to a virtual organ composed of microorganisms exhibiting complex bidirectional crosstalk with the environment and other organ systems [O'Hara and Shanahan, 2006; Sun and Chang, 2014]. The intestinal mucosal barrier is a virtual wall of tightly connected epithelial cells, buttressed by antimicrobial factors and mucus, that limits interaction between the microbiome and immune system [Turner, 2009]. In health, homeostasis exists between the intestinal microbiome, mucosal barrier, and immune system. In IBD, this homeostasis is disrupted leading to durable alterations in the intestinal microbiome (dysbiosis), disrupted barrier function (leaky gut), and immune system activation (inflammation) (Figure 1). Both genetic and environmental factors can influence transitions between health and disease. In this

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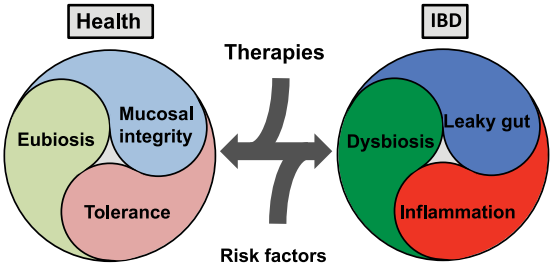

Figure 1. The tripartite pathophysiological circuit of inflammatory bowel disease (IBD). The microbiome, barrier function, and immune system all play critical roles in IBD pathophysiology with eubiosis, mucosal integrity and tolerance seen in health and dysbiosis, leaky gut, and inflammation seen in disease. Risk factors (environmental and genetic) push these pathophysiological components in the direction of disease. Therapeutic targets (microbiome, barrier function, and immune system based) push the components in the direction of health.

review, we discuss these factors with a focus on the microbiome and barrier function. While most current therapies modulate inflammation, we highlight new microbiome and barrier function based therapies under investigation for IBD.

The gastrointestinal microbiome

New research tools employed by initiatives like the Human Microbiome [Human Microbiome Project Consortium, 2012; Integrative HMP (iHMP) Research Network Consortium, 2014] and metagenomics of the human intestinal tract (MetaHIT) [Arumugam *et al.* 2011] have led to rapid advances in our understanding of the microbes present on and within our body. These microbes are collectively referred to as the *microbiota* and the complement of their genomic content is termed the *microbiome* [Ursell *et al.* 2012]. Massively parallel deep sequencing of bacterial 16S ribosomal RNA and yeast 18S ribosomal RNA has allowed taxonomic categorization of the *microbiota* without the need to grow individual organisms, the majority of which remain uncultured [Rajilic-Stojanovic *et al.* 2007; Hamady and Knight, 2009; Metzker, 2010]. Metagenomics, metatranscriptomics, metaproteomics, and metabolomics have helped us understand the metabolic pathways present within the microbiome [Lepage *et al.* 2013]. Model systems like gnotobiotic mice [Faith *et al.* 2010] and *ex vivo* systems [Roeselers *et al.* 2013] are allowing us to investigate host microbe interactions and understand the contributions of isolated microbes under controlled conditions to health and disease.

Within the gastrointestinal tract, the microbiota varies lengthwise (mouth to rectum) and cross sectionally (lumen to mucosa) [Eckburg *et al.* 2005; Wang *et al.* 2005]. It contains all divisions of life: archaea, prokarya, eukarya (mostly fungi) as well as viruses (mostly bacteriophage) [Ley *et al.* 2006; Scanlan and Marchesi, 2008; Ianiro *et al.* 2014; Scarpellini *et al.* 2015]. The majority of studies to date have focused on the 10–100 trillion bacterial cells present throughout the gastrointestinal tract [Eckburg *et al.* 2005]. Ninety percent of the bacteria fall into the two phyla: *Bacteroidetes* and *Firmicutes* [Eckburg *et al.* 2005]. Other phyla, including *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, are also present in lower quantities [Eckburg *et al.* 2005]. Diversity estimates place the total number of species between 1000 and 5000 [Zoetendal *et al.* 2008], and only a fraction of these, the 'core' microbiota, are commonly present in most individuals [Turnbaugh *et al.* 2009; Sekelja *et al.* 2011]. Individual microbiomes have been classified into different enterotypes (or faecotypes) [Arumugam *et al.* 2011; Jeffery *et al.* 2012], characterized by predominant species and metabolic pathways that correlate with long-term dietary preferences (high protein and animal fat *versus* high carbohydrate) [Wu *et al.* 2011].

The intestinal microbiome is shaped by both genetic and environmental factors [Spor *et al.* 2011]. Interestingly, the microbiomes of monozygotic twin pairs are more similar than mother–child pairs, which are more similar than unrelated pairs irrespective of physical separation [Turnbaugh *et al.* 2009]. From the time of birth, environmental factors like mode of delivery (cesarean section *versus* vaginal) and feeding preference (breast feeding *versus* formula feeding) shape the gut microbiome [Penders *et al.* 2006; Fallani *et al.* 2010]. This microbiome is highly dynamic in the first year of life with relative stabilization in the transition to an adult diet [Koenig *et al.* 2011]. Short- and long-term food preferences including vegetarian *versus* meat-based diets have significant effects on the microbiome [Wu *et al.* 2011; David *et al.* 2014]. Environmental factors such as level of hygiene, exposure to infections, antibiotics, and other drugs can also modify the microbiome [Spor *et al.* 2011].

In health, the microbiome plays key roles in metabolism of food and drugs, development of

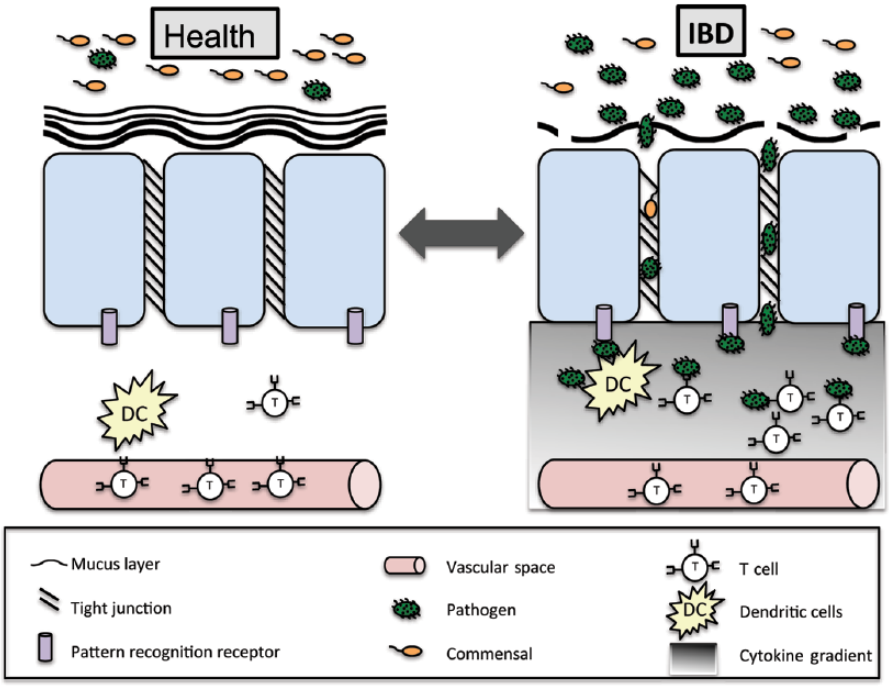

Figure 2. Mechanisms of inflammatory bowel disease (IBD) pathophysiology. IBD involves three pathophysiological components (dysbiosis, leaky gut, and inflammation) that are mutually dependent. In health, the mucosal barrier including two mucus layers, epithelial cells, and tight junctions separate the microbiota from the immune system. Breakdown of the mucosal barrier due to environmental and genetic factors leads to translocation of gastrointestinal organisms and activation of the innate and adaptive immune system. Genetic and environmental factors also contribute to dysbiosis and immune system activation leading to further breakdown of the mucosal barrier.

the gastrointestinal epithelium, development and modulation of the immune system, and protection from infections [Sekirov *et al.* 2010]. A healthy microbiota, established at an early age, exhibits resilience; multiple environmental inputs are likely necessary to effect a sustained and clinically relevant change [Lozupone *et al.* 2012]. Alterations in the microbiome have been associated with a surprising range of conditions, including neuropsychiatric diseases [Collins *et al.* 2012], asthma and atopic diseases [Van Nimwegen *et al.* 2011], obesity and metabolic syndrome [Nicholson *et al.* 2012], colorectal cancer [Louis *et al.* 2014], enteric infections [Kamada *et al.* 2013], irritable bowel syndrome [Dupont, 2014], and IBD [Manichanh *et al.* 2012].

The microbiome in IBD

The microbiome in IBD is known to be different from that of healthy individuals [Ott *et al.* 2004; Manichanh *et al.* 2006; Frank *et al.* 2007; Michail *et al.* 2012; Nagalingam and Lynch, 2012; Rajilic-Stojanovic *et al.* 2013; Bellaguarda and Chang, 2015; Sheehan *et al.* 2015] (Figure 2). The extent to which these changes are a cause or a consequence of inflammation remains a debate, but both may be accurate in that dysbiosis and inflammation are likely to be mutually reinforcing in patients with IBD. Similar to other forms of inflammatory diarrhea, there is a loss of diversity and stability of the microbiota in IBD. One of the most consistent findings is a decrease in the commensal spore-forming and butyrate-producing *Clostridium* clusters IV and XIVa (*Firmicutes* phylum) [Manichanh *et al.* 2006; Frank *et al.* 2007; Sartor, 2008]. These species are known to stimulate regulatory T cells (Tregs), leading to immune tolerance and reduction in gastrointestinal inflammation [Atarashi *et al.* 2011]. One member of this cluster, *Faecalibacterium prausnitzii*, is decreased in IBD [Fujimoto *et al.* 2013; Machiels *et al.* 2014] and predicts the recurrence of disease after ileal resection in CD [Sokol *et al.* 2008]. Similar decreases in *Clostridium* clusters IV and XIVa are seen in *Clostridium difficile* infection and *C. difficile*-negative nosocomial diarrhea and are therefore likely to be both a generic effect of and

predisposing factor for inflammatory diarrhea [Antharam *et al.* 2013].

Increases in certain bacteria are also observed in IBD. It remains unclear to what extent these specific increases are the driving forces of the inflammatory process (keystone pathogen) [Hajishengallis *et al.* 2012] *versus* opportunistic contributors to an already established inflammatory process (pathobiont) [Chow *et al.* 2011]. Some of the most consistently elevated bacterial species in IBD are members of the family Enterobacteraceae (phylum *Proteobacteria*). These include the iconic gut pathogens *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Escherichia coli*. Indeed, there is an extensive line of research linking adherent-invasive *E. coli* to ileal Crohn's disease [Darfeuille-Michaud *et al.* 2004]. The gut pathogen *C. difficile* is also increased in prevalence in IBD [Clayton *et al.* 2009; Berg *et al.* 2013]. A large multicenter study of patients with new-onset patients demonstrated increases in *E. coli, Fusobacterium nucleatum, Haemophilus parainfluenzae*, and *Veillonella parvula*; this increase in combination with a decrease in other species and an overall decline in species diversity correlated strongly with inflammation [Gevers *et al.* 2014]. Other studies have found increases in the intracellular bacteria *Mycobacterium paratuberculosis* in CD [Mcnees *et al.* 2015] and adherent invasive bacteria, *Fusobacterium*, in UC [Strauss *et al.* 2011].

While most research has focused on bacteria, work has begun to interrogate the role of fungal and viral components of the microbiota and unlike the bacterial microbiota, the diversity of the mycobiome [Richard *et al.* 2015] and virome [Norman *et al.* 2015; Ray, 2015] appear to be increased. The pathophysiological significance of these changes is an area of active investigation. Related immune system studies are also ongoing, including evaluation of the role of C-type lectin receptor dectin 1 (CLEC7A); a polymorphism of this receptor, which appears to interact with the mycobiome, may be linked to severe UC [Iliev *et al.* 2012].

Intestinal mucosal barrier function in IBD

The intestinal mucosal barrier separates the microbiota, food, and other luminal contents from the innate and adaptive immune system (Figure 2). It is composed of inner and outer mucus layers impregnated with antimicrobial factors and underlying intestinal epithelial cells stitched together with connecting protein networks called tight junctions [Turner, 2009]. In a healthy gut, the microbiota does not touch epithelial cells and is sampled in a controlled manner *via* specialized microfold (M) cells located in Peyer's patches along the distal small intestine [Hooper and Macpherson, 2010]. Depending on the microbe and the immune system, this can lead to either immune tolerance or activation. In IBD, this mucosal barrier is disrupted, resulting in translocation of the intestinal microbiota and potentiation of the immune system [Merga *et al.* 2014]. As with dysbiosis, it is debated whether changes seen in barrier function are the result or the cause of the disease.

The inner mucus layer while devoid of bacteria in healthy controls [Johansson *et al.* 2008], shows increased permeability in IBD allowing interaction of the microbiota with the normally inaccessible epithelial surface [Schultsz *et al.* 1999; Swidsinski *et al.* 2005; Johansson *et al.* 2014]. The increased permeability may be due to altered composition of the mucus components secreted by goblet cells, including decreased mucin [Moehle *et al.* 2006], decreased glycosylation products [Theodoratou *et al.* 2014], decreased trefoil factor [Aamann *et al.* 2014] or due to decreases in antimicrobial factors secreted into the mucus by epithelial cells (Reg3γ), Paneth cells (defensins) and plasma cells [immunoglobulin A (IgA)] [MacDermott *et al.* 1989; Ramasundara *et al.* 2009; Hooper and Macpherson, 2010]. In UC but not CD, the mucus layers are thinner or absent and the goblet cells responsible for mucus production are depleted [Johansson *et al.* 2014]. Certain members of the IBD-associated microbiota use mucus as an energy source and tightly regulate its production, thus there is evidence that the mucus changes may be as much the result of dysbiosis as a cause [Deplancke and Gaskins, 2001; Derrien *et al.* 2004; Png *et al.* 2010].

The network of proteins called tight junctions connecting epithelial cells also show increased permeability in IBD [Michielan and D'Inca, 2015]. Both environmental (microbes, diet) and genetic factors can influence tight junction integrity [Ulluwishewa *et al.* 2011]. Disruption allows microbes to translocate beyond the mucosal surface resulting in access to the immunologically active submucosa and systemic space. Endotoxemia (lipopolysaccharide) is well documented in IBD [Pastor Rojo *et al.* 2007] and other microbial components (flagellin, pilli, and

lipoteichoic acid) are also likely responsible for stimulating the immune system [Klapproth and Sasaki, 2010].

Immune system in IBD

The immune system plays a critical role in the development of IBD and it is likely that invading microorganisms are necessary for potentiating its effects [Geremia *et al.* 2014] (Figure 2). Microorganisms that invade epithelial cells, the submucosa, or systemic space can stimulate various components of the immune system, including autophagy [Parkes, 2012], innate immunity [Abraham and Medzhitov, 2011], and adaptive immunity [Kato *et al.* 2014]. Dysfunction in these pathways plays a role in IBD pathogenesis.

Autophagy, the regulated mechanism by which cells process and destruct organelles and intracellular pathogens, is disrupted in some forms of CD. Mutations in key autophagy genes like nucleotide oligomerization domain 2 (*NOD2*) and *ATG16L1* are associated with terminal ileal Crohn's, and certain intracellular pathogens are able to manipulate autophagy to form autophagic vacuoles where they remain protected from immune responses [Parkes, 2012].

Microorganisms that reach the submucosa through disrupted tight junctions can interact with the basolateral surface of epithelial cells that are covered in pattern recognition receptors such as toll-like receptors that recognize various components of microbial pathogens [Man *et al.* 2011]. They stimulate the release of inflammatory cytokines recruiting phagocytic cells and components of the adaptive immune system. Toll-like receptors and regulating molecules have been shown to have altered expression in both active and inactive IBD [Fernandes *et al.* 2015].

IBD risk factors

Both genetic and environmental risk factors influence the development of IBD (Figure 3). Understanding how these factors impact disease susceptibility, onset, and exacerbation can guide future investigations aimed at identifying targets for disease treatment and prevention.

Genetic risks

Monozygotic twin concordance rates are only 15–20% in UC and less than 50% in CD [Halme

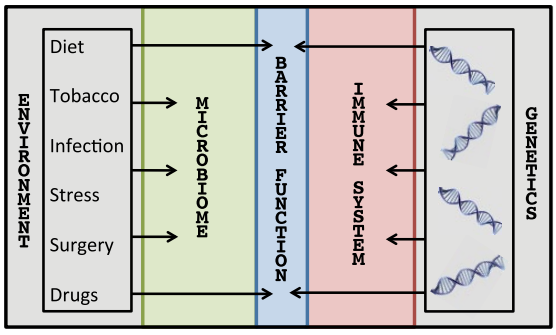

Figure 3. Risk factors for inflammatory bowel disease (IBD). There are multiple risk factors, both genetic and environmental, that mutually contribute to the development and exacerbation of IBD. Genetics directly affect barrier function and the immune system, whereas environmental factors (e.g. diet, tobacco use, infections, stress, surgical procedures, and medications, including antibiotic exposure) directly affect barrier function and the microbiota.

et al. 2006], indicating that although genes confer increased susceptibility to IBD, they are not sufficient for the development of disease. Twins and family members of patients with IBD often demonstrate abnormal gut parameters, including leaky gut [Buhner *et al.* 2006; D'Inca *et al.* 2006] and altered microbiome without developing IBD, suggesting that genetics predispose people to gut dysfunction but other environmental factors are necessary for triggering disease onset [Willing *et al.* 2010; Halfvarson, 2011; Hedin *et al.* 2014].

Genome-wide association studies (GWAS) have identified over 200 IBD-associated loci [Ogura *et al.* 2001; Jostins *et al.* 2012; Knights *et al.* 2013; McGovern *et al.* 2015]. All gene variants carry a low penetrance with the highest odds ratio of 7 conferred by *NOD2*, a gene coding for an intracellular receptor that recognizes a component of bacterial cell walls. Other gene associations have odds ratios of less than 1. A mutation in the *IL23* receptor has been shown to offer a two- to threefold protection against developing IBD [McGovern *et al.* 2015]. Genes likely influence all three components of IBD pathophysiology (microbiome, barrier function, and immunity) but primarily affect barrier function and immunity through their impact on mucus production, tight junctions, autophagy, and the innate and adaptive immune response [Jostins *et al.* 2012; Dalal and Chang, 2014; Van Limbergen *et al.* 2014; Bianco *et al.* 2015].

Environmental risks

Epidemiologic studies have described a rising incidence of both UC and CD as countries undergo industrialization and demographic transition [Bernstein and Shanahan, 2008]. These epidemiologic changes are brisk beyond the speed of genetic alterations at the population level, suggesting there is likely a strong environmental component [Sheehan *et al.* 2015]. There are multiple environmental factors that may synergistically contribute to the rising incidence of IBD, including hygiene, diet, medications, tobacco use, surgical practices, and stress [Shanahan, 2012]. These environmental factors are likely to have effects on all three components of IBD pathophysiology (microbiome, barrier function, and immunity), but may exert a disproportionate effect on the gut microbiome and barrier function.

Hygiene

The hygiene hypothesis has been discussed as a contributing factor to the rise of autoimmune diseases in the developed world [Rook, 2012]. Central to this hypothesis is the idea that developed countries see less pathogenic burden (e.g. viral infections, *Helicobacter pylori*, and helminths) due to improved cleanliness in comparison to developing countries. Lack of exposure to infectious antigens during early immune development in childhood may have lasting impact on the immune system by switching the predominance of T lymphocyte subtypes (T helper 1 to T helper 2) that potentiate autoimmune phenomena including IBD [Koloski *et al.* 2008].

Diet

Several studies describe a potential association between the rising incidence of IBD and a 'Western' diet composed largely of processed foods that are high in fat and protein and low in fiber (fruits and vegetables) [Chapman-Kiddell *et al.* 2010; Hou *et al.* 2011]. This may be due to direct effects of diet on the microbiota [Wu *et al.* 2013] and barrier function [Martinez-Medina *et al.* 2014]. Diets low in fiber have consistently been linked to IBD perhaps due to decreases in short chain fatty acid (SCFA) production by commensal bacteria (*Clostridium* clusters IV and IVXa) whose preferred energy source is fiber [Van Immerseel *et al.* 2010]. The SCFA butyrate is critical for colonic health as the preferred energy source for colonocytes [Van Immerseel *et al.* 2010]. It also contributes to tight junction integrity and is a regulator of Treg cells [Smith *et al.* 2013].

Associations between high fat, protein, and sugar diets have been less consistent in epidemiologic studies [Hou *et al.* 2011], although animal studies have shown a strong link between high fat, high simple sugar diets [Martinez-Medina *et al.* 2014]. It may be that particular fats *versus* overall fat intake is important for disease development. For example, saturated milk fat, but not polyunsaturated fat, leads to the expansion of the sulfite reducing pathobiont *Bilophila wadsworthia* and to the development of colitis in a mouse model of IBD [Devkota *et al.* 2012]. Interestingly, mode of feeding after birth (breastfeeding *versus* formula) influences the microbiota and may have a long-term effect on IBD incidence [Barclay *et al.* 2009; Guaraldi and Salvatori, 2012].

Food additives may also be linked to the development of IBD. These include common dietary emulsifiers, carboxymethylcellulose (CMC) and polysorbate 80 (P80), which induce low-grade inflammation and metabolic syndrome in wild type mice and promote a robust colitis in genetically predisposed mice. The emulsifiers altered the microbiota to have more inflammatory potential and increased the number of mucolytic bacteria causing erosion of the mucus layer [Chassaing *et al.* 2015]. Maltodextrin, another common food emulsifier, has shown similar effects in animal models [Nickerson *et al.* 2015]; however, the effect of these additives in humans is less clear. Other food components such as gliadin (a glycoprotein and major component of gluten) can disrupt tight junctions and may also be critical for IBD pathophysiology [Ulluwishewa *et al.* 2011; Chassaing *et al.* 2015; Lerner and Matthias, 2015].

Medications

Different patterns of medication use have been postulated to play a role in the rising incidence of IBD *via* an effect on the microbiota or barrier function. Antibiotics are known to cause shifts in microbial composition and use in infancy [Shaw *et al.* 2010], childhood [Kronman *et al.* 2012], and adult life [Shaw *et al.* 2011] has been associated with an increased risk of IBD [Modi *et al.* 2014]. Tetracycline in the treatment of acne has been associated with increased risk of CD [Margolis *et al.* 2010; Alikhan *et al.* 2011].

Nonsteroidal anti-inflammatory drugs promote intestinal barrier disruption [Ananthakrishnan, 2013], alter the microbiota composition [Rogers and Aronoff, 2015], and have been associated with increased risk of IBD development and clinical relapse [Takeuchi *et al.* 2006; Chan *et al.* 2011; Ananthakrishnan *et al.* 2012]. Oral contraceptives have effects on the vaginal microbiota [Achilles and Hillier, 2013], also likely affect the gastrointestinal microbiota, and are variably associated with an increased risk for developing Crohn's disease [Timmer *et al.* 1998; Cosnes *et al.* 1999; Alic, 2000]. Other medications too are associated with microbiota changes (e.g. proton pump inhibitors) [Freedberg *et al.* 2015] and improvements in tight junction integrity (e.g. β blockers) [Reiberger *et al.* 2013] and should be evaluated for their association with increased and decreased risk of IBD.

Tobacco use

Nicotine interestingly has a beneficial effect on tight junction integrity and it is possible that different modes of action of tobacco on the microbiota *versus* gut barrier function may explain the differential effects of tobacco on CD and UC [McGilligan *et al.* 2007]. Tobacco use is associated with an increased risk for CD and reduced risk for UC and tobacco cessation reverses this effect [Cosnes, 2008; Parkes *et al.* 2014]. Smokers with active CD have a clinically relevant gastrointestinal dysbiosis, and smoking cessation induces profound changes in the microbiota [Benjamin *et al.* 2012; Biedermann *et al.* 2013].

Surgical practices

Appendectomies are associated with a decreased risk for UC [Kaplan *et al.* 2008; Radford-Smith, 2008; Cheluvappa *et al.* 2014] and possibly an increased risk for CD [Andersson *et al.* 2003; Kaplan *et al.* 2007]. Patients with UC, after appendectomies, experience fewer flares, fewer colectomies and a decreased need for immunosuppressive therapy [Naganuma *et al.* 2001; Radford-Smith *et al.* 2002; Radford-Smith, 2008]. Studies evaluating the microbiota of the resected inflamed appendix show increases in pathogenic organisms, including the adherent invasive bacterium *Fusobacterium* [Swidsinski *et al.* 2011; Guinane *et al.* 2013]. Similar organisms have increased incidence in active IBD and thus appendectomies may be protective in UC by eliminating a pathogen reservoir [Strauss *et al.* 2011]. Cesarean sections *versus* vaginal births are also associated with alterations in the microbiota and an increased risk for IBD [Dominguez-Bello *et al.* 2010; Bager *et al.* 2012] perhaps due to differences in early microbial colonization of the gastrointestinal tract.

Stress

Stress correlates with IBD relapse [Singh *et al.* 2009] and improvements in stress *via* counseling correlate with decreased IBD symptoms [Wahed *et al.* 2010]. Stress has also been shown to have profound effects on the microbiota [Lutgendorff *et al.* 2008; Bangsgaard Bendtsen *et al.* 2012], barrier function [Soderholm *et al.* 2002; Camilleri *et al.* 2012], and intestinal inflammation [Melgar *et al.* 2008; Singh *et al.* 2009; Matsunaga *et al.* 2011] and thus may contribute significantly to IBD pathophysiology.

IBD therapies

The majority of IBD therapies to date have focused on modulating inflammation *via* the immune system. These have enabled great leaps forward in medically treating a disease that historically only had surgical treatment options. Despite therapeutic advances, many patients with IBD still require surgery. Short-circuiting IBD pathophysiology may ultimately require a therapeutic approach that integrates all three pathophysiological components (dysbiosis, leaky gut, and inflammation). Therapies will likely be highly individualized based on future diagnostics that point toward keystone dysfunction in one or more of these pathophysiological components. To this end, in addition to immune-based therapies, therapies targeting dysbiosis and leaky gut are currently being explored (Figure 4).

Immune-based therapies

Current IBD treatments include the use of medications that modulate the immune system, including aminosalicylates, corticosteroids, immunomodulators (e.g. methotrexate, azathioprine), antitumor necrosis factor (TNF) agents, and integrin inhibitors. Newer biologic agents that focus on other inflammatory cytokines, their receptors, and downstream pathways are also in various stages of development [Peng *et al.* 2014].

Immune-based therapies as their name implies have direct effects on the immune system, but

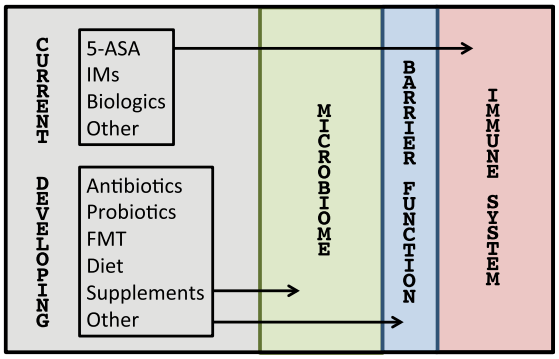

Figure 4. Therapies for inflammatory bowel disease (IBD). Conventional therapies like aminosalicylates (5-ASA), immunomodulators (IMs), and biologics are traditionally thought to work *via* the immune system but may also have direct and indirect effects on the microbiota and barrier function (not depicted). Vice versa, developing therapies like antibiotics, probiotics, fecal microbiota transplantation (FMT), diet, and supplements that are thought to target the microbiota and barrier function may have direct and indirect effects on the immune system (not depicted).

some studies have also demonstrated either direct or indirect effects on the microbiota and mucosal barrier function. Mesalamine and other salicylates for example have been shown to change the intestinal microbiota and this may in part be due to direct effects on microbes [Andrews *et al.* 2011]. Indeed, mesalamine and other salicyaltes decrease the expression of microbial adherence factors and biofilm formation [Damman, 2013]. Unlike immunomodulators, which lead to proliferation of mucosally associated bacteria, mesalamine leads to a decrease in mucosally associated bacteria in UC [Swidsinski *et al.* 2007]. Studies of biologics show an ameliorating effect on the microbiome and on tight junctions [Edelblum and Turner, 2009; Busquets *et al.* 2015]. Indeed, TNF and other inflammatory cytokines directly disrupt tight junctions and thus a biologic's primary mechanism of action may in large part be due to reestablishing barrier function [Li *et al.* 2008; Edelblum and Turner, 2009].

Microbiota-based therapies

Microbiota-based therapies include antibiotics, probiotics, fecal microbiota transplantation (FMT), and diet. These have been investigated as treatments for IBD with varying results. Therapeutic manipulation of the microbiota offers theoretical advantages over immune system and barrier function based therapies as a treatment strategy since the microbiota is more malleable than host factors under greater genetic influence.

Antibiotics

The antibiotics that have been most studied as treatments for IBD include metronidazole, rifaximin, ciprofloxacin, and antimycobacterial agents [Bejaoui *et al.* 2015]. Most efficacy has been demonstrated in CD, particularly in inducing remission (with less consistent data showing maintenance of remission), treatment of perianal disease, and treatment of pouchitis [Khan *et al.* 2011; Cammarota *et al.* 2015]. Antibiotics are less effective in the treatment of adult UC (Khan *et al.* 2011), although there may be some efficacy in the pediatric population [Turner *et al.* 2014]. Rifaximin and ciprofloxacin by one meta-analysis may have the greatest benefit in inducing remission in CD [Arnold *et al.* 2002; Prantera *et al.* 2006; Khan *et al.* 2011]. Metronidazole has particular benefit in the treatment of perianal disease [Brandt *et al.* 1982; Sutherland *et al.* 1991; Khan *et al.* 2011; Mowat *et al.* 2011].

Rifaximin's effect on the microbiota includes decreases in certain pathogens with reciprocal increases in *Bifidobacteria* and *F. prausnitzii* in CD [Maccaferri *et al.* 2010; Guslandi, 2011]. Antibiotics are not without risk and while there may be some studies demonstrating benefit, there is also the potential for antibiotic-related side effects, including resistance, reduction in biodiversity, and risk for *C. difficile* infection.

Probiotics

Probiotics have shown some efficacy in UC and pouchitis with less efficacy in CD [Cammarota *et al.* 2015]. Generalizations about the efficacy of probiotics is complicated by the variability of the formulations and specific strains studied. The two probiotic formulations that have been studied most extensively in IBD are *E. coli* Nissle 1917 and VSL#3. *E. coli* Nissle 1917 has been shown to be comparable to mesalamine in maintaining remission [Kruis *et al.* 2004; Henker *et al.* 2008]. VSL#3 is a mixture of eight different bacteria (four strains of lactobacilli, three strains of *Bifidobacteria*, and one strain of *Streptococcus*) and has been shown to be effective for induction and maintenance of remission in pouchitis [Gionchetti *et al.* 2000; Mimura *et al.* 2004] in both pediatric UC [Miele *et al.* 2009] and adult UC [Sood *et al.*

2009]. VSL#3 when used in combination with conventional therapy has also demonstrated some efficacy in decreasing disease activity [Tursi *et al.* 2010].

Several mechanisms of action have been proposed, including promoting the growth of anti-inflammatory bacteria and inhibiting the growth of pathogenic bacteria [Dalal and Chang, 2014]. Some strains of bacteria are also able to produce SCFAs that are the preferred energy source of colonocytes. Probiotics are often formulated with prebiotics, indigestible fibers that help promote their growth. Despite some beneficial results overall in IBD, there are no current guidelines recommending the routine use of probiotics in the induction or maintenance of IBD. Additionally, one must consider the potential risk of bacterial translocation of probiotics in patients who are critically ill or immunocompromised, which can very rarely lead to sepsis and multiorgan failure [Theodorakopoulou *et al.* 2013].

Fecal microbiota transplantation

FMT involves the infusion of donor stool into an individual with the aim of restoring a 'healthy' microbiota and treating disease. It has been used most extensively and effectively (>90% cure rate) as a treatment for recurrent *C. difficile* infection [Gough *et al.* 2011; Van Nood *et al.* 2013] and in the wake of this success has been evaluated for other diverse indications [Borody *et al.* 2013].

Several case series [Colman and Rubin, 2014] and placebo-controlled trials [Moayyedi *et al.* 2015; Rossen *et al.* 2015] have evaluated its efficacy in IBD with mixed results [Damman *et al.* 2012; Hansen and Sartor, 2015]. Many of these studies have evaluated its effect in UC [Angelberger *et al.* 2013; Kump *et al.* 2013; Kunde *et al.* 2013; Damman *et al.* 2015], although some studies have also investigated the treatment for CD [Zhang *et al.* 2013; Cui *et al.* 2015; Suskind *et al.* 2015]. While induction of remission appears to be possible in a subset of patients with both UC and CD, this effect is neither universal, nor sustained. Several studies have measured whether engraftment of the donor stool correlates with efficacy with mixed results [Angelberger *et al.* 2013; Kump *et al.* 2013; Damman *et al.* 2015]. It is likely that repeated infusions are necessary for maximum efficacy and sustained effect [Damman *et al.* 2015]. It is possible that pretreatment with antibiotics and adjunctive treatment with diet may also augment efficacy [Damman *et al.* 2015].

The enthusiasm exploring FMT as a treatment for IBD has been partially tempered by observed and theoretical side effects [Rubin, 2013]. While case series have demonstrated that FMT may be safe in a diverse array of immunocompromised patients, this remains a concern in patients with IBD on immunosuppressive therapy [Kelly *et al.* 2014; Di Bella *et al.* 2015]. Fever and elevations in inflammatory markers have been observed in patients with IBD following FMT [Rubin, 2013]. Despite screening measures, there are also concerns that FMT may transmit infectious agents that may not manifest in disease for years [Bourlioux and Workgroup of the French Academy of Pharmacy, 2015]. To help mitigate this risk, several groups are evaluating the role of a rationally designed 'artificial stool' that contains a subset of clinically active microbes with more limited infectious risks [Petrof *et al.* 2013; Petrof and Khoruts, 2014].

Diet

Various diets have been proposed to prevent and treat IBD. Given the strong benefit of fiber intake, a well balanced, healthy diet with fruits and vegetables is recommended. Since a protein-rich diet with excess meat and alcohol has demonstrated increased relapse rates in UC, avoidance of these items may be beneficial [Tilg and Kaser, 2004].

Exclusive elemental nutrition (EEN), a formula-based therapy, with an efficacy rate of 85% and low side-effect profile, is recommended as first-line therapy for induction of remission [Critch *et al.* 2012] and helps maintain remission in pediatric CD [Wu *et al.* 2013]. It is equivalent to corticosteroid therapy in inducing clinical remission and superior to corticosteroids in inducing histologic remission [Gorard *et al.* 1993; Borrelli *et al.* 2006]. Efficacy of EEN in adult patients with CD appears to be less perhaps as a result of poor compliance or greater prior exposure to immunosuppressive therapies [Lee *et al.* 2015]. Partial enteral nutrition, a diet in which table foods are added to EEN, has also been shown to be efficacious in both adult and pediatric CD [Sigall-Boneh *et al.* 2014]. EEN is hypothesized to be effective by limiting antigen exposure (due to rapid transit), enhancing nutritional status, and altering the microbiome and immune response [Voitk *et al.* 1973; Rajendran and Kumar, 2010].

Other nonformula-based dietary interventions have also been studied. The specific carbohydrate diet (SCD), an elimination diet which removes grains, milk, and sweeteners (except honey), has been shown in small case series to be effective for inducing and maintaining remission in CD [Cohen *et al.* 2014; Suskind *et al.* 2014; Kakodkar *et al.* 2015] and UC [Obih *et al.* 2016]. The mechanism by which the SCD works may come from alteration of the microbiome or barrier function *via* differences in macronutritients or removal of certain dietary exposures such as emulsifiers [Martinez-Medina *et al.* 2014; Chassaing *et al.* 2015; Nickerson *et al.* 2015]. Interestingly, the effect of EEN and SCD on the microbiota is divergent, with microbial diversity decreasing with EEN [Gerasimidis *et al.* 2014; Quince *et al.* 2015] and increasing with SCD [Walters *et al.* 2014]. Other diet therapies including individually tailored exclusion [Riordan *et al.* 1993], IgG4 targeted exclusion [Rajendran and Kumar, 2010], lacto-ovo-vegetarian fiber rich diet [Chiba *et al.* 2010] and low FODMAPs (fermentable oligo-di-monosaccharides and polyols) diet [Gearry *et al.* 2009] have reported primarily symptomatic improvement in patients with IBD.

Barrier function based therapies

Restoring mucosal barrier integrity holds promise as a future treatment approach for IBD. It may indeed be a common denominator in all IBD therapies, including those that target the immune system or microbiota. As noted above, inhibition of TNFα and other inflammatory cytokines leads to improvement in barrier function. Remodeling the microbiota has also been shown to improve barrier function [Cani *et al.* 2009; Ulluwishewa *et al.* 2011], and exclusionary diets may improve barrier function *via* effects on the mucus layer [Martinez-Medina *et al.* 2014; Chassaing *et al.* 2015; Nickerson *et al.* 2015]. Even the mechanism of action of helminth therapies in which parasites are introduced to patients in a controlled fashion may be grounded in improving barrier function [Wolff *et al.* 2012].

Several drugs are known to act directly on restoring barrier function. A delayed release phosphatidylcholine, 'LT-02', recently completed phase II clinical trials for UC and is believed to work by helping restore the mucus layer [Stremmel *et al.* 2010; Karner *et al.* 2014]. Teduglutide is a glucagon-like 2 peptide analog that is indicated for the treatment of short gut syndrome [Jeppesen, 2012]. It has strong trophic effects on intestinal mucosa with restoration of the mucosal barrier and pilot studies have shown efficacy in the treatment of moderate to severe CD [Buchman *et al.* 2010; Blonski *et al.* 2013].

Some supplements have also been investigated for their ability to improve tight junctions [Ulluwishewa *et al.* 2011]. The amino acid, L-glutamine, has been studied extensively in nutrition for critically ill patients and burn victims in which tight junction disruption is known to contribute to bacteremia [Wischmeyer, 2007; Bollhalder *et al.* 2013], although in patients in the intensive care unit with multiorgan failure, glutamine increased mortality [Heyland *et al.* 2013]. *In vitro* cell culture studies show a direct effect on improving tight junction function [Bertrand *et al.* 2015]. Only limited studies have been performed in treating active IBD with negative results [Akobeng *et al.* 2000; Ockenga *et al.* 2005], but further studies are being considered to investigate the role of L-glutamine in maintenance rather than induction of remission.

Natural products often used by alternative practitioners for the treatment of IBD have shown some efficacy and may also exert their effect *via* modulation of tight junctions [Gilardi *et al.* 2014]. Curcumin (turmeric extract) and boswellia have been shown to promote improvement in tight junctions in cell culture based assays [Wang *et al.* 2012; Catanzaro *et al.* 2015]. Other dietary components (macro- and micronutrients), in addition to the microbiota effects described above, may directly impact tight junctions in IBD [Ulluwishewa *et al.* 2011].

Conclusion

The microbiome, barrier function, and immune system play an integrated role in the development of IBD, and all three components are likely critical for perpetuating the disease process. Genetic risk factors may be disproportionately responsible for immune system dysfunction, environmental risk factors may be disproportionately responsible for dysbiosis, and both factors likely contribute to barrier dysfunction. While currently available therapies targeting the immune system have made great strides in IBD therapy, many patients remain refractory to treatment. There is great promise in targeting the other two pathophysiological components of IBD, the microbiota and barrier function, as new primary or adjunctive therapies for IBD.

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