We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

The Role of the Microbiota in Gastrointestinal Health and Disease

Anne-Marie C. Overstreet, Amanda E. Ramer-Tait, Albert E. Jergens and Michael J. Wannemuehler

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53920

1. Introduction

Antony van Leeuwenhoek's observation of "animalcules" in the tarter of teeth in 1683 marked the beginning of humans trying to understand their relationship with microbes. Now commonly referred to as "bacteria" or the "microbiota", we share our inner and outer space with these single-celled organisms. They are present all around us in the oceans, soil, leaves, and even air [1]. One of the microbial niches that has recently garnered much attention is the human body. In this niche, we share the table with 10^{14} microbial cells, which outnumber our eukaryotic cells ten to one [1, 2]. These organisms colonize most body sites, including the skin, oral cavity, gastrointestinal, urogenital and respiratory tracts. Additionally, there is 150 times more genetic material associated with our resident bacteria when compared to our own DNA and comprise what is known as our body's "second genome," a concept recently reviewed by Zhu et al. [3]. In an effort to understand the role that these organisms have on human development, health and disease, the National Institutes of Health launched the "Human Microbiome Project" in 2007 in an effort to elucidate this host-microbe interaction [4-6].

The role of bacteria is most obvious in the gastrointestinal tract (GIT). Bacteria in this niche have the ability to break down substances that otherwise could not be digested by GIT cells; they also produce metabolites needed by the body such as vitamin K (menaquinones), folate, B12, and riboflavin [7-12]. The GIT has a surface area the size of a tennis court and is home to 10^{11}-10^{12} organisms per gram of colonic content [1, 13]. The predominant bacterial phyla present in the GIT include the Firmicutes and Bacteroidetes with low levels of Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia [14-16]. It has been noted that members of the predominant factions (the Firmicutes and Bacteroidetes) belong to only three groups, *Bacteroides*, *Clostridium coccoides* (cluster XIVa) and *Clostridium leptum* (cluster IV) [15, 17, 18].

There are also Archaea present in the GIT and have been found to belong to one phylotype, *Methanobrevibacter smithii* [14, 19].

1.1. Initial colonization of the gastrointestinal tract

Infants are born sterile and establishment of the GI microbiota begins shortly thereafter. Studies in mice have revealed that the first organisms to colonize include lactobacilli,fFlavo-bacteria and Group N Streptococci [20]. These three groups were found in the stomach and the small and large intestine. When the mice were 12 days of age, the flavobacteria disappeared from all three sites. Concurrently, there was a rapid increase in the presence of enterococci and slow lactose fermenting coliform bacilli reaching 10^9 bacteria per gram of tissue in the large intestine. This spike was transient however, and as the mice aged, a shift toward a more strict anaerobic population occurred in the large intestine. This ordered colonization occurs because aerotolerant organisms are able to colonize the GIT early in the presence of oxygen. Through their metabolism, they reduce the redox potential and allow for the later colonization and replication of strict anaerobes [21].

1.2. Impact of the method of delivery on colonization

In humans, the method of delivery impacts the initial microbiota of the infant [22-24]. In a study of Venezuelan women, vaginal delivery was associated with initial colonization by *Lactobacillus*, *Prevotella*, *Atopobium*, and *Sneathia* spp.; in contrast Cesarean delivery was associated with colonization of *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* spp. (organisms typically found on skin). It was also noted that the initial bacteria present in infants born vaginally were vertically transferred from the mother. This was not the case for those born via Cesarean. A Finnish study analyzed infant fecal samples collected between three days of age and six months of age to evaluate temporal colonization [23]. The results revealed that at three days of age, all but one infant, regardless of the delivery method, were colonized with aerobic bacteria. However, a higher percentage of vaginally delivered infants were colonized with *Bifidobacterium*-like (BLB) and *Lactobacillus*-like (LBB) bacteria as compared to Cesarean delivered infants. These differential colonization patterns were corrected by day ten for LBB and by day 30 for BLB. Additional characterization of the fecal microbiota revealed that at one month of age, infants born via Cesarean had a significant increase in *Clostridium perfringens* (25% to 56%); an increase not seen in vaginally delivered infants. This is an important obser-vation, as *C. perfringens* has been thought to play a role in sudden infant death syndrome (SIDS) [25]. Also of note, significant differences in the levels of *Bacteroides fragilis* were observed between the two methods of delivery [23]. Specifically, this bacterium was present at statisti-cally higher numbers in vaginally delivered infants as compared to Cesarean delivered infants at all time points throughout the six months of the study.

These differences in initial colonizers have been associated with adverse effects, as infants born via Cesarean are more susceptible to skin infections with methicillin resistant *Staphylococcus aureus* (MRSA). A 2004 report indicated that 82% of infant MRSA cases in Chicago and 64% in Los Angeles were delivered via Cesarean , all of which involved healthy, full-term infants [26]. An additional prospective cohort study indicated a link between Cesarean delivery and

Clostridium difficile infection, which was further associated with the development of wheeze, asthma, eczema, and food allergies throughout the first seven years of life [27]. Consistent with these findings, a Norwegian study documented an association between asthma and Cesarean delivered children [28]. Although the epidemiological connections between Cesarean delivery, the composition of the microbiota, and disease are still being studied, strong evidence exists that early colonization of the intestines with aerobic skin microbes is associated with health risks.

1.3. Impact of feeding method on colonization

Another factor affecting early colonization of the human gut is determined by feeding method [29]. In one study of vaginally delivered infants, the fecal microbiota of breast-fed compared to formula-fed infants within the first month of life was analyzed using fluorescence *in situ* hybridization (FISH) to evaluate 11 separate groups of organisms [29]. *Bifidobacterium* spp. were the most prominent organisms found to colonize infants fed breast milk, comprising 69% of the bacterial population, with the next most prevalent group being the *Bacteroides* and *Prevotella* spp. making up 12%. With respect to the fecal microbiota of formula-fed infants, the microbiota was actually more diverse than that found in the breast-fed babies. Formula-fed infants had *Bifidobacterium* spp. as the most predominant group, however, they only comprised 32% of the bacterial population while *Bacteroides* and *Prevotella* spp. comprised 29% of the composition. Additionally, *Atopbium* spp. were found to account for 8% of the microbiota in formula-fed infants, but only comprised 1% of the community in the breast-fed infants.

1.4. Microbial changes that occur after weaning

The next major shift in the microbial population occurs during weaning. In a study consisting of infants from five European countries, the composition of the fecal microbiota at four weeks post-weaning was analyzed via FISH using 10 different probes [30]. When considering the results from all of the infants, it was noted that the microbial communities consisted primarily of *Bifidobacterium* (37%), *C. coccoides* (14%) and *Bacteroides* (14%). When samples were obtained from infants that were separated by geographic location, differences among the groups were noticeable. Infants from Spain had significantly greater *Lactobacillus* spp. and less *Bifidobacterium* spp. as compared to infants from the UK, Sweden, Germany, and Italy. The Spanish fecal samples also had significantly greater presence of *Bacteroides* spp. and *C. leptum* compared to those from the UK, Sweden, or Italy. The numbers of *C. leptum* was highest in samples obtained from German infants. The microbiota of infants from Spain also had significantly greater amounts of enterobacteria when compared to those from the UK, Sweden, or Germany, and higher numbers of *Streptococcus* spp. compared to those from Germany. The infants with the highest amount of *Bifidobacterium* spp. were from Sweden and the UK. Further separation of the data revealed that breast-fed infants had an increased percentage of *Bifidobacterium* spp. while *Bacteroides* spp. and *C. coccoides* were reduced post-weaning. Additionally, delivery method also influenced the microbiota composition, with vaginally delivered infants having higher numbers of *Bacteroides* present.

This study also reported results from infants who provided samples before (at six weeks of age) and four weeks after weaning [30]. Switching to solid food during this time caused a significant reduction in the presence of bifidobactera, enterobacteria, and *C. difficile*/*C. perfringens* proportions. These reductions were accompanied by significant increases in both *C. leptum* and *C. coccoides* clusters. Again, location also played a significant role in the microbial differences at weaning. Spanish infants had the greatest increase in both *C. coccoides* and *C. leptum* post-weaning compared to the other four countries, while enterobacteria was significantly decreased in the Spanish samples compared to those from the other countries. The samples from Spanish infants also had significant increases in *Atopobium* spp. compared to those from Sweden and Germany and *Streptococcus* spp. compared to Germany and Italy. The authors also report significant decreases post-weaning in the enterobacteria present in the Italian infant samples as compared to samples from Sweden and the UK. As mentioned previously, feeding and delivery method alter the composition of the microbiota at birth; this is also true after weaning. Formula-fed infants had higher proportions of *C. leptum* in their feces compared to breast-fed infants post-weaning. Infants born via Cesarean section possessed increased levels of *Bacteroides* spp. after weaning, whereas there was no change in the abundance of this group in vaginally delivered infants. Additionally, *Atopobium* spp. decreased in vaginally delivered children but increased in Cesarean delivered children post-weaning [30].

Studies describing development or maturation of the human microbiota reveal that as an individual ages, the microbiota shifts from predominantly facultative anaerobes to strict anaerobes, just as previously observed in rodents [20]. Notably, variations in initial colonization associated with different delivery and feeding methods seems to profoundly impact the microbial community post-weaning. Additionally, geographic location plays an important role in the initial colonization of the (GIT). In spite of all these variables, several themes for colonization can be described. Initially, *Bifidobacterium* spp. are the primary colonizers of the infant GIT along with other species such as *Bacteroides*, *Lactobacillus*, *Prevotella*, and *Atopobium*. As the infant matures, the oxygen presence in the gut is reduced and the infant begins eating solid food, which promotes a transition towards the growth and maintenance of the anaerobes found in the *C. coccoides* and *C. leptum* clusters.

1.5. The microbiota and gastrointestinal homeostasis

The commensal microbiota has a tremendous influence on the development and functional capabilities of the GIT of its host. Numerous studies have documented the effects that the microbiota has on GIT development in mice. Specifically, the morphology of the intestines differs significantly in animals devoid of microbes (i.e., germfree) compared to conventionally-reared (CONVR) mice [31]. The mucus layer is thinner and epithelial cells have a slower rate of turnover compared to those from conventional animals, primarily because of extended time in the S and G_1 phases of the cell cycle [32]. This can be corrected by bacterial colonization. In Figure 1, panel A shows intestinal tissue from germfree (GF) mice and panel B shows tissue from a mouse colonized with *Brachyspira hyodysenteriae*. Colonization with *B. hyodysenteriae*

elicits the production of goblet cells and increases the heights of the mucosa, resembling what is seen in CONVR mice.
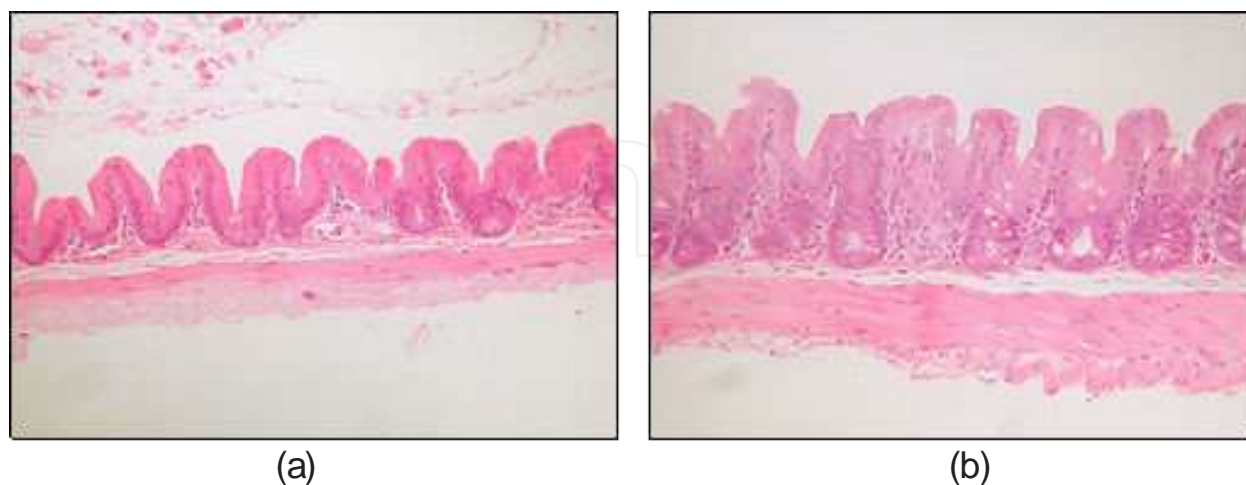

(a)   (b)

Figure 1. Photomicrographs of cecal tissue from a) a germfree mouse and b) a mouse monoassociated with *Brachyspira hyodysenteriae.* Note the changes in the cellularity, presence of goblet cells, and height of the mucosa in the ceca from the monoassociated mouse 28 days after colonization with *B. hyodysenteriae*.

In the small intestine, this change in epithelial cell transit time is doubled, going from 53 hours in a CONVR mouse to 115 hours in a GF mouse [33]. The most noticeable gross change in GF mice is the enlargement of the cecum, which can comprise up to 19% of the mouse's body weight [34]. The cecum of a GF mouse can assume a more normal size (4.5% body weight) following colonization with a mixture of *Lactobacillus* spp., Group N streptococci , *Bacteroides* spp., enterococci and coliform bacilli recovered from CONVR mice [34]. As shown in Figure 2, monoassociation with *B. hyodysenteriae* also returns the cecum to a more normal size.

Of interest, not all bacteria tested were able to return the cecum to a normal size. It was noted that colonizing mice with *Lactobacillus* spp. and Group N streptococci only was not sufficient to change the morphology of the cecum. However, when *Bacteroides* spp. colonized the mice, the morphology assumed a more normal size. Other differences between GF and CONVR mice include the consistency of the cecal contents, with the contents from a GF mouse being more liquid, hypotonic and alkaline than in a CONVR mouse [35]. Additionally, GI transit time is slower in GF mice, which are also prone to vitamin deficiency due to a lack of bacterial metabolism [36-38]. In GF rats, monoassociation with either a rat-derived *Escherichia coli* strain or a sarcina-like micrococcus was able to reverse the vitamin K deficiency. Research identifying the response to *Salmonella enteritis* serovar Typhimurium challenge indicated that there was a 50-fold greater translocation of bacteria to the mesenteric lymph node, and organisms were present in the blood of GF mice, something not seen in CONVR mice [37]. This phenomenon occurred due to the decreased transit time in the small intestine of GF mice, thereby allowing the *Salmonella* to multiply in the host and translocate to other tissue sites.

Figure 2. Physical and morphological changes associated with microbial colonization. Shown here are ceca from a germfree mouse (left), monoassociated mouse (center), and conventional microbiota mouse (right). Note the increased size of the cecum from the GF mouse. The distal end of the cecum (appendix) of the monoassociated mouse has begun to undergo morphological changes, including the development of a lymphoid tissue known as a cecal tonsil.

1.6. Effect of bacterial colonization on immune development

In addition to its importance in maintaining gut homeostasis, the GI microbiota is also critical for normal priming and development of the immune system [39-41]. GF mice have reduced numbers of immune cells present in the lamina propria and have smaller Peyer's patches compared to CONVR mice. They also have a diminished capacity for antibody production, fewer plasma cells, smaller mesenteric lymph nodes, and reduced numbers of germinal centers compared to CONVR mice [39]. However, this underdeveloped immune system is fully capable of mounting a response comparable to that of a CONVR mouse when stimulated with bacterial antigen or protein [40, 41]. Macrophages from CONVR mice process antigen faster than those from GF mice, likely because the continued exposure of CONVR macrophages to bacterial antigen allows them to be "primed" for antigen degradation; a phenomenon that does not occur in GF mice [41]. The presence of bacteria in the GIT promotes decreased immunoreactivity towards commensal organisms. The barrier between the gut microbiota and the underlying gastrointestinal associated lymphoid tissue consists of a single layer of epithelial cells covered by two layers of mucus [42, 43]. The inner layer of mucus is approximately 100 μm thick while the outer layer is approximately 700 μm in the rat colon [43]. The inner layer is firmly attached to the epithelial cells and devoid of bacteria, while the outer layer is "loose"and contains commensal organisms [44]. The major constituent of these layers is a

protein known as Muc2 [42]. The importance of Muc2 has been highlighted by the development of Muc2$^{-/-}$ mice. These mice fail to gain weight, have diarrhea by seven weeks of age, occult blood present in their feces by eight weeks of age, and the majority of mice had gross bleeding and reversible rectal prolapse by nine weeks of age [45]. These mice also develop microscopic evidence of colitis as early as five weeks of age.

Underneath the mucus layers lay the intestinal epithelial cells, which are held together by tight junction proteins such as occludins, claudins, and junctional adhesion molecules [46]. These proteins seal the paracellular junction between the cells and regulate the entry of nutrients, ions and water. They also act as a barrier against bacterial entry. Loss of epithelial barrier function promotes a break in immunological tolerance and facilitates immunoreactivity towards the normal commensal microbiota (Figure 3). This phenomenon has been demonstrated in mouse studies employing 2,4,6 trinitrobenzensulfonic acid (TNBS), a chemical that disrupts the epithelial barrier to allow translocation of bacteria, resulting in the induction of both innate inflammatory responses and antigen-specific immune responses [47]. Subsequent to the loss of epithelial barrier function, these mice develop severe gastrointestinal inflammation, which can be ameliorated by pretreating with antibiotics to reduce the microbial load [47].

The role of the commensal bacteria in regulating the immune response was elegantly demonstrated in a study by Wlodarska et al. [48]. Mice administered metronidazole were more susceptible to subsequent infection with the pathogen, *Citrobacter rodentium*. Metronidazole-treated mice had increased submucosal edema, ulcerations, mucosal hyperplasia and decreased numbers of goblet cells. The author also noted enhanced expression of *IL-25* and *Reg3γ* mRNA and an increased presence of NK cells and macrophages in the lamina propria of these mice, indicating an increase in microbial stimulation. The inner mucous layer of metronidazole-treated mice was significantly thinner and mRNA expression of genes encoding for the goblet-cell specific proteins Muc2, TFF3, and RelmB was also decreased in these mice [49, 50]. The thinning of the inner mucous layer following metronidazole treatment allowed for *C. rodentium* to more closely associate with the epithelium and promote production of the pro-inflammatory cytokines and chemokines TNF-α, IFN-γ, and MCP-1. This study highlights the contribution of the commensal microbiota in the production of the mucus barrier and subsequently in maintaining mucosal homeostasis [48].

It has also been noted that specific members of the resident microbiota are able to illicit specific immune functions. Colonization with *Bacteroides fragilis* expressing polysaccharide A (PSA) suppressed development of T helper 17 (T$_h$17) CD4$^+$ T cells by promoting the development of Foxp3$^+$ T regulatory (T$_{reg}$) CD4$^+$ T cells and inducing IL-10 expression via TLR2 signaling [51]. Additional studies have demonstrated that purified PSA is sufficient to expand the numbers of Foxp3$^+$ T$_{reg}$ cells [52]. *B. fragilis* inhabits colonic crypts. This niche puts the organism in close contact with the immune system but its production of PSA ensures that no inflammatory response is induced. Both prophylactic and therapeutic treatment of mice with PSA has been shown to ameliorate TNBS-induced colitis in mice [52]. More recently, work from Atarashi et al., eloquently demonstrated a role for indigenous *Clostridium* species in the induction of colonic T$_{reg}$ cells [53]. Colonization of GF mice with a cocktail of 46 *Clostridium* strains enriched for clusters IV and XIVa promoted TGF-β production from intestinal epithelial cells and

increased the number of Foxp3$^+$ T$_{reg}$ cells in the colon. Furthermore, inoculation of young CONVR mice with *Clostridium* species facilitated resistance to both dextran sodium sulfate (DSS)- and oxazolone-induced colitis as well as increased systemic Immunoglobuin E (IgE) production as adults.
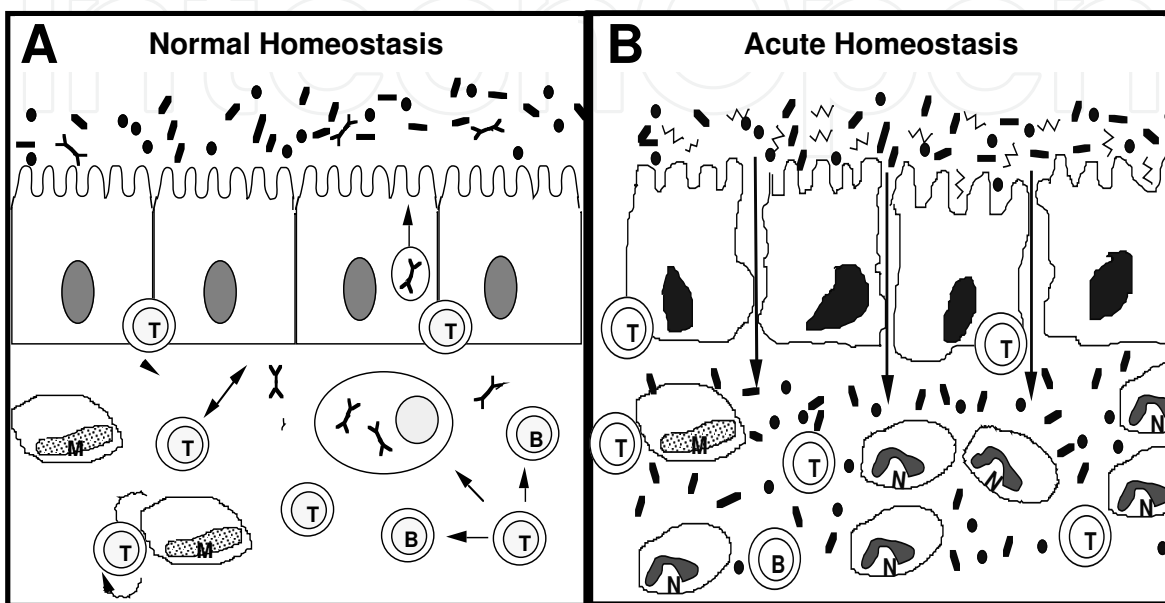

Figure 3. Panel A shows normal mucosal homeostasis. The epithelial cells provide a barrier between the commensal bacteria and the underlying immune cells. Panel B depicts acute inflammation. The epithelial cell barrier has been breached, allowing bacteria to be readily detected by the immune system and promote a pro-inflammatory immune response.

The segmented filamentous bacteria (SFB) is another microorganism of interest that has been implicated in the induction of T$_h$17 responses [54]. Ivanov et al. found that C57BL/6 mice purchased from the Jackson Laboratory had significantly less IL-17-producing CD4$^+$ T cells in the small intestine compared to those from Taconic Farms. This discrepancy could be corrected via intragastric gavage of the Jackson mice with contents from the small intestines of the Taconic mice. Using a 16S ribosomal RNA PhyloChip analysis, SFB were identified in the microbiota of mice from Taconic Farms but not in those from the Jackson Laboratory. Monoassociation of mice with SFB induced the production of T$_h$17 cells and up-regulated genes encoding antimicrobial peptides and serum amyloid A (SAA). SAA co-cultured with naive CD4$^+$ T cells and lamina propria derived dendritic cells induced T$_h$17 cell differentiation *in vitro*. The authors concluded that SFB, which tightly adheres to and imbeds itself among the microvilli on the epithelial cell surface, induces the production of SAA by intestinal epithelial cells. In turn, the SAA acts on lamina propria dendritic cells to stimulate the induction of T$_h$17 cells. Colonization with SFB has also been shown to protect against *C. rodentium* infection. Th17 cytokines such as

IL-22 likely stimulate intestinal epithelial cells to secrete antimicrobial peptides to limit the growth of the pathogen and its infiltration into the colonic wall [54].

1.7. Effect of bacterial colonization on other aspects of the body

In addition to promoting immune maturation, the commensal microbiota also helps to regulate fat storage. GF mice eat 29% more food than conventional mice yet have 42% less body fat and a decreased metabolic rate [55]. Colonization of GF mice with cecal contents from conventional mice for 14 days caused a 57% increase in total body fat with a concomitant 27% reduction in food intake. These "conventionalized" mice also had increases in leptin, fasting glucose, and insulin levels compared to GF mice; they also developed insulin resistance. Levels of mRNA specific for the transcription factors, SREBP-1 and ChREBP, were also elevated leading to increased production of lipogenic enzymes. Fat formation is aided by the regulator lipoprotein lipase (LPL) and is inhibited by the *fiaf* gene product whose expression is suppressed in microbiota-bearing conventional mice. The authors suggest that the bacteria in the gut breakdown dietary polysaccharide into monosacchaVaides that are then transported to the liver to activate lipogenic enzymes. This process promotes fat formation in the peripheral tissues due to the suppression of *fiaf*. In addition to the production of white adipose tissue, the microbiota may also play a role in eye health [56]. A recent study revealed differences in the lipid profiles in the lens and retinas of GF mice as compared to CONVR mice. The CONVR mice had reduced concentrations of multiple phosphatidylcholines and an overall reduced presence of phospholipids in the lens. The authors postulate these changes may be due to the increased exposure of the CONVR mice to more oxidative stress than their GF counterparts [56]. Together, these studies help us realize the effects that the gut microbiota may have on host systems that, at the surface, seem to have limited or no connection to the GIT.

1.8. Bacterial production of short chain fatty acids

Another health benefit that the microbiota provides its host revolves around the production of short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate as end products of anaerobic fermentation [57]. These SCFA (predominantly butyrate) can be utilized as energy sources by eukaryotic cells. Members of the *Clostridium* clusters IV (e.g., *Faecalibacterium prausnitzii)* and XIVa (e.g., *Roseburia* spp. and *Eubacterium rectale*) are the primary butyrate producers in the GIT, and they comprise approximately 2-15% of the total gut microbiota [14, 58-61]. Butyrate, a four-carbon fatty acid, is produced by bacteria via one of two metabolic pathways. The first pathway utilizes the enzymes phosphotransbutyrylase and butyrate kinase to form butyrate from butyryl-CoA to yield one ATP per one molecule of butyrate produced [62, 63]. The second pathway, which is utilized by most organisms in the gut, uses butyryl-CoA acetate CoA transferase to form butyrate and acetyl-CoA from butyryl-CoA [64, 65].

Once produced, butyrate has multiple effects on gut health. A primary use is as a preferred energy source for colonocytes, and the mechanisms by which butyrate is utilized in the GIT is summarized in Figure 4 [66-69]. Two biomarkers of energy homeostasis, ATP

and NADH/NAD$^+$ levels, are both significantly reduced in only the colonic tissues of GF mice as compared to CONVR mice [70]. This observation indicates that GF mice have a reduction in TCA cycle activity, and subsequently less ATP is generated for cellular energy. Moreover, this reduction in ATP was correlated with increased signs of energetic stress in colonocytes, including increased expression of 5′-adenosine monophosphate-activated protein kinase (AMPK). Consistent with previous reports describing a role for AMPK in inducing autophagy [71], GF coloncytes also expressed elevated levels of the autophagosome marker LC3-11. Transmission election microscopic analysis revealed that significantly more GF colonocytes were undergoing autophagy than colonocytes from CONVR mice. Colonizing GF mice with a conventional microbiota reversed these effects, as did incubation of isolated colonocytes with butyrate. Additional experiments employing the fatty-acid oxidation blocker, etomoxir, demonstrated that colonocytes consume butyrate as an energy source and not as a histone deacetylase (HDAC) inhibitor, another known function of butyrate [72, 73].

Butyrate functions as an HDAC inhibitor by blocking cellular deacetylase activity and allowing histone acetylation [72, 73]. Histone modification causes changes in cellular gene expression patterns; compounds and molecules that can elicit these modifications are being studied as potential anti-cancer therapeutics [74]. A comparative study of gene expression patterns in HT-29 cells (a colon carcinoma-derived cell line), treated with either butyrate or trichostain A (a known HDAC inhibitor) revealed that both substances had similar effects on gene expression [75]. Upregulated genes (21 total) were found to regulate the cell cycle, signal transduction, DNA repair and genome transcription. Only two genes were down-regulated—lactoferrinδ and MAPKAP kinase. It is also important to note that both butyrate and trichostain A inhibited the growth of HT-29 cells by creating an arrest in the G_1 phase of the cell cycle [76]. Butyrate may also down-regulate pro-inflammatory responses via its HDAC activity, as incubation of butyrate with inflamed biopsy samples or LPS-induced peripheral blood mononuclear cells (PBMCs) reduced the mRNA expression of IL-6, TNF-α, TNF-β, and IL-1β [77]. Additionally, in murine studies of TNBS- and DSS-induced colitis, and in ulcerative colitis (UC) patients, administration of butyrate enemas ameliorates disease activity via NFκB inhibition [77-79]. Butyrate has been shown to decrease both COX-2 and PGE$_2$ expression in HT-29 cells stimulated with TNF-α [80].

The information presented in this section clearly defines a central role for the gut microbiota in many physiological processes; the microbiota even has a tremendous impact on the host's health status. Causal links likely exist between the methods by which infants are delivered, their gut microbial colonization patterns and subsequent health concerns, including asthma, eczema and, food allergies. Associations between gut bacterial communities and obesity, diabetes, and cardiovascular disease have also been documented [81-83]. A significant body of literature also links changes in the composition of the gut microbiota with inflammatory bowel disease (IBD), which is discussed in detail in the following section [84-89].
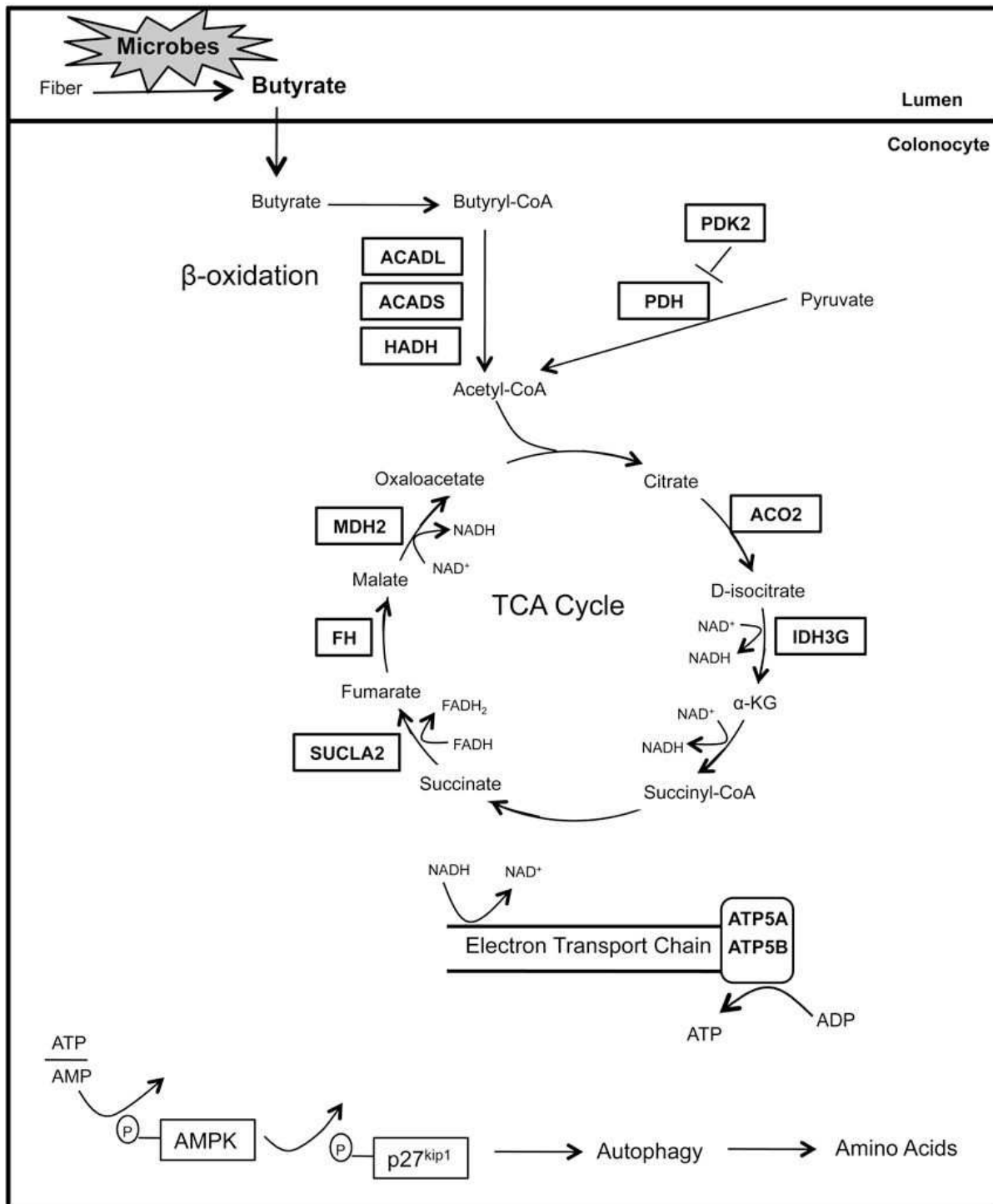
Figure 4. Microbial regulation of colonocyte metabolism. Schematic depicting how dietary fiber is fermented by microbes into butyrate in the lumen of the colon, which is then transported into the colonocyte. In the colonocyte, butyrate promotes oxidative metabolism and inhibits autophagy. Based on transcriptome and proteome experiments, enzymes regulated by microbes are shown in boxes. In all cases, boxed enzymes that function in β-oxidation and the TCA cycle are downregulated in GF colonocytes, revealing that microbes positively regulate their expression. Diminished ATP results in phosphorylation of AMPK and p27, which culminates in autophagy. (Cell Metab. 2011 May 4;13(5): 517-5) Reprinted with permission from the publisher license number 2977121495018

2. Introduction to inflammatory bowel disease

In 1932, three physicians, Burrill Crohn, Leon Ginzburg, and Gordon Oppenheimer described a disease of unknown etiology in the terminal ileum of young adults [90]. The disease was characterized as being similar to UC (fever, diarrhea and weight loss) and having ulcerations of the mucosa that would eventually lead to stenosis of the lumen and formation of multiple fistulas. Eventually, surgical intervention was used to resect the affected portion of the intestinal tract and patients recovered with little signs of the disease persisting. In their 1932 report, Crohn and colleagues discuss observations from other physicians regarding observations of granulomas in the small and large intestine of unknown etiology being classified under the umbrella term "benign granulomas". Still today Crohn's disease (CD) and UC are both included under the umbrella term "inflammatory bowel disease." Crohn and colleagues described the disease as beginning at the ileocecal junction with lesions separated by normal mucosa [90]. They described inflammation of the submucosa and the muscularis resulting in a markedly thickened bowel wall. The presence of "giant cells" was also noted, which they attributed to vegetable matter becoming entrapped in the ulcers and become encapsulated during healing. The authors concluded that the presence of these giant cells in the granulomatous lesion led others to believe that the inflammation was due to an unusual form of tuberculosis. However, the authors could not find any evidence of tuberculosis in their 14 patients.

Clinically, the patients with IBD were characterized as young adults with fever, diarrhea, dull abdominal pain, and vomiting; they were also weak, anemic and had poor appetite. Upon physical examination, the author noted five commonalties: 1) a mass in the right iliac region, 2) evidence of fistula formation, 3) emaciation and anemia, 4) approximately half of the subjects had undergone an appendectomy, and 5) evidence of intestinal obstruction. Treatment was supportive and surgical resection of the affected portion of the intestinal tract was recommended; 13 of their 14 patients had no symptoms post-operatively. For the one patient that developed recurrent symptoms, it was later determined that not all of the affected intestinal tissue had been resected during the first surgery.

Eighty years after the first published article of what would become known as "Crohn's disease," there is still no cure for this disease nor do we fully understand its etiology. Unfortunately, the number of people being diagnosed with IBD is increasing yearly [91]. There is not one specific, causative agent of IBD; instead the etiology is thought to be multifactorial in nature, with host genetics, environmental factors, the induction of aberrant immune responses, and the gastrointestinal microbiota all contributing to disease pathogenesis.

2.1. Genetic factors affecting IBD

As of 2011, genome-wide meta-analyses had identified 71 susceptibility loci associated with CD, 47 with UC and 28 associated with both, some of which are shown in Figure 5 [92-95]. Here, we focus on genetic variants within specific genes involved in mediating host responses to microbial components.

Inflammatory bowel disease susceptibility loci.
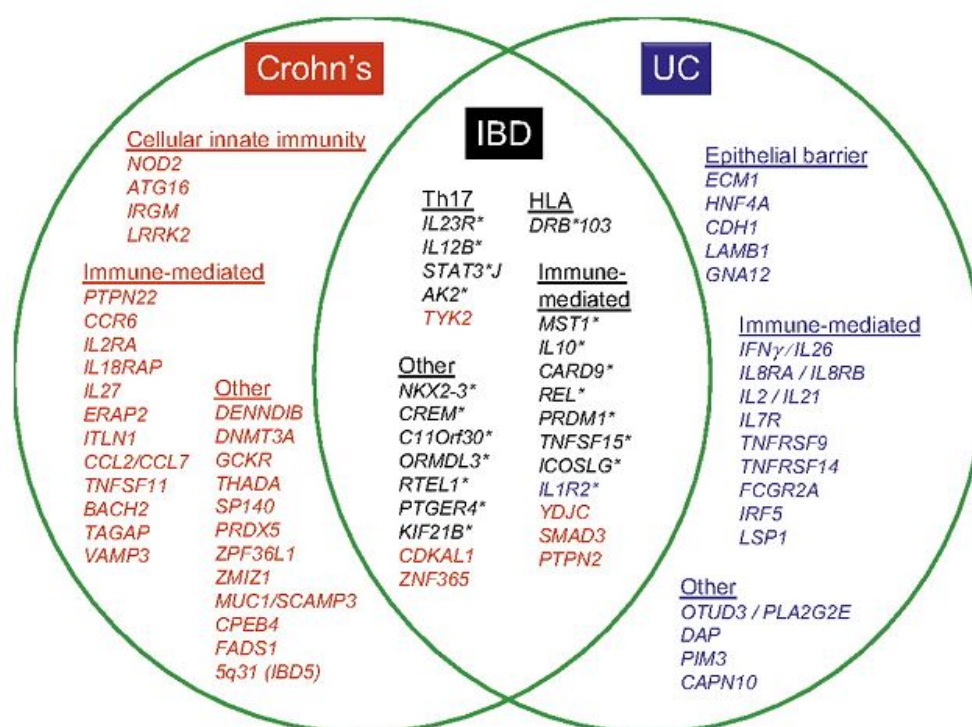
Lees C W et al. Gut 2011;60:1739-1753

Figure 5. Inflammatory bowel disease susceptibility loci. The loci (depicted by lead gene name) attaining genome wide significance (p < 5x10^{-8}) are shown for Crohn's disease (CD, red), ulcerative colitis (UC, blue) and IBD (black where p < 5x10^{-8} in CD and UC; red were p < 5x10^{-8} in CD and p < 5x10^{-4} in UC; blue where p < 5x10^{-8} in UC and p < 5x10^{-4} in CD. Reprinted with permission from the publisher license number: 2977121069437

2.1.1. Nod2

One of the first candidate genes linked to IBD susceptibility was *Nod2*. Located on chromosome 16, the defective allele affects the activation of the transcription factor NF-κB. NOD2 is expressed in antigen-presenting cells, macrophages, lymphocytes, ileal Paneth cells, and intestinal epithelial cells and functions as an intracellular microbial recognition molecule [96-100]. NOD2 senses muramyl dipeptide (MDP), a minimally bioactive motif of peptido-glycan from both Gram-negative and Gram-positive bacteria. Once activated by its ligand, NOD2 undergoes a conformational change to expose its CARD domains and recruits the kinase RIP2 to the complex which associates with NOD2 via homophillic CARD-CARD interactions [97]. This process leads to the activation of IKK and the subsequent degradation of IκB and ultimately results in the translocation of NF-κB to the nucleus [96, 101].

Petnicki-Ocwieja et al. demonstrated that MDP stimulation of ileal crypts induced bactericidal secretions capable of killing *E. coli* [102]. Moreover, the same authors showed that NOD2 was

required for the bactericidal activity of crypt secretions of the terminal ileum. The composition of the ileal microbiota of the NOD2$^{-/-}$ mice was significantly altered in comparison to their WT counterparts. Specifically, NOD2$^{-/-}$ mice had increased numbers of Firmicutes, specifically *Bacillus* species and *Bacteroides* species. Wild-type mice were able to clear an infection of *Helicobacter hepaticus*, an opportunistic mouse pathogen, within seven days while NOD2$^{-/-}$ mice remained colonized with *H. hepaticus* for at least 14 days post-infection [102]. Also of note, the presence of a gut microbiota was required for the expression of NOD2. GF mice possessed decreased levels of NOD2—a phenotype that could be reversed following monoassociation with either Gram-negative or Gram-positive organisms. Together, these results outline a reciprocal regulatory relationship between NOD2 expression and the gut microbiota.

2.1.2. ATG16L1

Another gene implicated in the pathogenesis of IBD that regulates cellular autophagy is *ATG16L1* [95, 103]. Autophagy is a process utilized by cells to recycle cellular compo-nents. This process may also be utilized by cells as a non-apoptotic method of program-med cell death [104]. *ATG16L1* is expressed by CD4$^+$ and CD8$^+$ T cells, CD19$^+$ B cells, epithelial cells, and macrophages [105, 106]. Studies by Caldwell et al. utilizing mice en-gineered to express low or hypomorphic levels of *ATG16L1* revealed that this gene is in-deed an autophagy-related protein. Elevated levels of cytosolic proteins normally degraded by a rapamycin-induced degradation pathway were found in the cells of the *ATG16L1* hypomorphic (ATG16L1^{HM}) mice [95]. Additionally, cells from ATG16L1^{HM} mice possessed fewer autophagosomes following rapamycin treatment or nutrient deprivation. The small intestines and colons of ATG16L1^{HM} mice had no morphological defects in crypt height or villus length but did possess abnormalities found in their Paneth cells. Specifically, lysozyme within Paneth cells was found to be either depleted/absent or dif-fuse, which is in contrast to the normal and orderly packaging of lysozyme within gran-ules. Decreased levels of lysozyme were also observed in the ileal mucus layer of ATG16L1^{HM} mice. However, there were intact granules observed in the crypt lumen, in-dicating a potential role of *ATG16L1* in the maintenance of the Paneth cell granule exocy-tosis pathway. The authors observed no differences between WT and ATG16L1^{HM} mice in terms of resistance to a challenge with *Listeria monocytogenes*. However, others have re-ported a reduction in intracellular bacteria targeted to autophagic vacuoles following in vitro infections of HeLa or Caco-2 cells with *S. typhimurium* in conjunction with siRNA to reduce the expression of *ATG16L1* [105, 107]. Additional work by Caldwell et al. re-vealed that 100 % of ileocolic resection specimens obtained from humans with the *ATG16L1* risk allele had abnormal Paneth cells that looked similar to those found in the ATG16L1^{HM} mice [95].

Using siRNA to reduce *ATG16L1* expression in THP-1 macrophages revealed an increase in intracellular adherent and invasive *E. coli* (AIEC) as compared to normal THP-1 infected macrophages [106]. AIEC promote enhanced production of TNF-α and IL-6 from macrophag-es. Silencing of *ATG16L1* expression resulted in significant increases in both cytokines. The authors postulate that bacterial clearance is less efficient in individuals with the risk alleles for

ATG16L1. When AIEC are present, this reduction in bacterial clearance likely leads to increased production of pro-inflammatory cytokines, which could tip the immune balance to an inflammatory state. Thus, it appears that the presence of bacterial provocateurs (e.g., AIEC) in the microbiota increases the risk of developing IBD in individuals possessing polymorphisms in genes such as *ATG16L* or *NOD2.*

2.2. The immune response and IBD

The mucosal immune system is charged with the monumental task of balancing responsiveness and tolerance to a tremendous number of environmental antigens, including those from both food and bacteria. Although the exact cause of IBD remains elusive, significant evidence supports the hypothesis that GIT inflammation is initiated and perpetuated by a dysregulated immune response directed against the gut microbiota resulting in deleterious responses in genetically susceptible individuals following an environmental trigger. The host with a genetic predisposition for IBD may possess defects in epithelial permeability and/or altered regulation (i.e., NOD2 deficiency) of commensal bacteria. Potential environmental triggers may include smoking, certain medications, or a gastrointestinal illness that induces a break in homeostasis. Regardless of the specific genetic or environmental trigger, what ensues is an exaggerated, inappropriate mucosal immune response characterized by chronic activation of T cells and the production of cytokines and other inflammatory mediators.

The success of cytokine-targeted immunotherapies for a subset of IBD patients supports the idea that these chemical messengers of the immune system play an important role in disease pathogenesis. However, the nature of the immune phenotypes observed in CD and UC patients do differ [108]. The immune response of CD is typically associated with a T helper 1 (Th1) phenotype while UC is characterized by a T helper 2 (Th2) phenotype [108]. Isolated lamina propria (LP) CD4$^+$ T cells from CD patients produce IFN-γ when stimulated via the CD2/CD28 pathway; in contrast, LP CD4$^+$ T cells from UC patients secreted mostly IL-5 [109]. Another cytokine with a strong link to CD is IL-12 [110]. Messenger RNA for IL-12p40 in LP mononuclear cells (LPMC) was found in 85 % of CD patients, a percentage significantly higher than that found in healthy and UC GIT tissue samples. Similarly, IL-12p35 mRNA was detectable in the LPMC of 92 % of CD patients; expression was again significantly higher than for healthy and UC patients. Elevated levels of IL-12 were also detectable in the serum of CD patients [110], and this potent Th1-promoting cytokine is able to induce IFN-γ production from LP lymphocytes (LPL) isolated from CD patients [111]. Additionally, studies employing a murine model of TNBS-induced colitis have revealed that administration of anti-IL-12 antibodies ameliorated disease severity, presumably by decreasing IFN-γ secretion from LP CD4$^+$ T cells [112].

In UC, production of the cytokines IL-5 and IL-13 appear to mediate the disease process. Using LPMC from IBD patients, those with UC had increased production of IL-13 and IL-5 upon *in vitro* restimulation as compared to LPMCs recovered from either CD patients or healthy individuals [113]. Levels of IFN-γ produced by LPMC from UC patients were similar to those produced by cells from healthy controls. The authors specifically identified that CD4$^+$ CD161$^+$

NK T cells were the primary source of IL-13 in UC patients, as these cells produced 30-fold more IL-13 than NK T cells from CD patients following *in vitro* restimulation.

More recent work has implicated the T helper 17 (Th17) lineage of CD4$^+$ T cells in the chronic inflammation observed in IBD [114, 115]. The development of Th17 T cells is dependent upon the presence of both IL-6 and TGF-β [115]. Colonic biopsies from UC and CD patients expressed higher levels of IL-17A mRNA as compared to those from healthy controls [115-117]. Immunohistochemical analysis of these tissues identified increased numbers of IL-17A$^+$ cells in both the LP and the epithelium of UC and CD patients as compared to controls [116]. Additional work also noted the presence of IL-17$^+$ cells in patients with active IBD [114]. However, the IL-17$^+$ cells were found predominantly in the LP of UC patients but in the submucosa and muscularis propria of CD patients [114]. These different locations of IL-17$^+$ cells complements prior observations that CD lesions often present as transmural while those in UC are superficial. Also of note, a comparison of biopsies from patients with active versus inactive disease revealed that IL-17$^+$ cells were only increased in numbers during active disease. The authors also determined that both T cells and monocytes/macrophages are a source of IL-17, and that IBD patients have elevated levels of IL-17 in their sera as compared to undetectable levels of this cytokine in the sera of healthy individuals.

The maintenance of Th17 T cells requires the presence of IL-23 [115]. Of interest, a gene variant significantly associated with CD encodes for the IL-23R, which, along with IL-12Rβ1, comprises the IL-23 receptor complex [118, 119]. This receptor complex interacts with IL-23 (a heterodimer of IL-12p40 and IL-23p19) to direct the production of a T_H17 immune response [120-122]. Anti-IL-23 antibodies have been shown to both prevent and ameliorate established disease in a T-cell transfer model of murine colitis [123]. Studies of human tissues demonstrate that IL-23R expression is upregulated not only on IL-17 producing CD4$^+$ T cells, but also on IFN-γ$^+$ T cells in both UC and CD patients [115]. Stimulation of LP CD4$^+$ T cells from UC patients with IL-23 significantly increased IL-17 production; in contrast, IL-23 stimulation of LP CD4$^+$ T cells from CD patients resulted in enhanced IFN-γ secretion [115]. In addition to promoting the production of T_H17 cells, IL-23 also inhibits the production of Foxp3$^+$ T_{reg} cells [124] and suppresses production of IL-10, a key regulatory cytokine [125].

Another important hallmark of the immune response observed in IBD is the production of IgG antibodies against the normal commensal microbiota [126]. Increased intestinal permeability, be it through genetic predisposition or environmental trigger, promotes enhanced immunoreactivity to bacterial antigens. The mucosal immunoglobulin profile of healthy adults consists predominantly of IgA with only a small amount of IgG. In contrast, patients with active IBD presented with significantly higher amounts of IgG in their mucosal secretions with no difference in amounts of IgA as compared to controls. The IgG antibodies present in IBD patients were identified as binding to non-pathogenic bacterial commensals, including *E. coli*, *B. fragilis*, *C. perfringens*, *Klebsiella aerogenes*, and *Enterobacter faecalis*. Patients with CD had significantly higher titers than UC patients, while antibody titers to these commensal bacteria were at or below the limit of detection in serum samples from healthy controls. Notable differences were also observed in the isotypes of IgG present in UC versus CD patients. Specifically, UC patients produced predominantly IgG$_1$ and IgG$_3$ antibodies to bacterial

antigens, while IgG_1, IgG_2, IgG_3 were the predominant isotypes reacting with the bacterial antigens in serum samples from CD patients [126]. Conversely, other reports have described an increase in IgG_1 antibodies in UC patients and an increased in IgG_2 antibodies in CD patients [127-130]. Additional analyses have determined that IgG antibodies in CD patients are primarily directed towards bacterial cytoplasmic proteins rather than membrane associated proteins [126]. Despite the presence of many types of antibodies in both the mucosal secretions and serum of IBD patients, no direct evidence for their involvement in IBD immunopathogenesis has been reported, indicating that they may simply be a marker for immune responsiveness and/or dysregulation [131].

2.3. Environmental factors affecting IBD

Although there are allelic differences in many people with IBD, genetics alone cannot completely account for the development of IBD nor the increase in the incidence of IBD worldwide. Studies of monozygotic twins best highlight this concept, as there are disease concordance rates of only 50 % for CD and only 20 % for UC [132, 133]. Of interest, a British study assessing discordant twins with CD found an association with mumps infection, smoking, and oral contraceptive usage with the development of CD [134]. Additionally, the twin(s) with CD had suffered both a medical illness, more episodes of gastroenteritis, and spent more time with animals. Smoking is one confounding environmental factor that is of special interest, because it appears to have a protective effect on UC, but increases the risk of CD [132, 135-137]. Another factor that appears to be protective for UC is an appendectomy; its effect on CD is not as evident [138-140]. The equivalent procedure performed in mice has been shown to ameliorate colitis in both chemically-induced and genetically-engineered murine colitis models [141, 142]. Modest associations between oral contraceptive use and IBD have been documented, while others have found breast-feeding to be protective against both UC and CD [143, 144]. A meta-analysis performed by investigators in New Zealand identified other potential environmental factors, including being an only child, using antibiotics prior to and during adolescence (four or more courses in year), and having a pet in the house during childhood [145]. IBD also tends to occur in extended families as first and second degree relatives of IBD patients reported the occurrence of disease with a significantly higher frequency than the general population [146]. These finding strengthen the interconnection between genetics, environmental factors, and the incidence of IBD.

2.3.1. Antibiotic usage

Antibiotics are used in the treatment of IBD to reduce microbial load and dampen inflammatory immune responses. However, their use prior to disease diagnosis is now being identified as a risk factor for developing IBD. A Danish study of IBD patients revealed that antibiotic users were 1.84 times more likely to develop IBD, which correlated to a 12 % increase in disease risk for each course of antibiotics taken [147]. Further analysis revealed that antibiotic users were 3.41 times more likely to develop CD than UC, which correlated to an increased risk of 18 % per course of antibiotics used. Specifi-

cally, usage of penicillin V and extended spectrum penicillins were associated with the greatest disease risks. A Swedish study focused on the use of antibiotics from birth to age 5, a time when the microbiota and immune response are still developing and/or maturing, as a risk factor for IBD. They reported an association between a diagnosis of pneumonia, subsequent antibiotic treatment and the onset of both pediatric and adult CD [148]. Another study by Card et al. revealed a statistically significant association between antibiotic usage 2 to 5 years prior to diagnosis of CD in patients from the United Kingdom [149]. This finding was confirmed in a Canadian study, which reported that the more antibiotics taken within 2 to 5 years of diagnosis, the greater the risk of developing IBD [150]. That same study found that disease risk was weakly associated with penicillin use and greatly associated with metronidazole use, which was prescribed primarily for "non-infectious gastroenteritis." This association with antibiotic usage may be explained by the failure of the gut microbiota to reestablish its normal community structure and function following a course of antibiotics [151]. This alteration in the microbiota or dysbiosis may be a predisposing factor to the onset of IBD in a susceptible individual.

2.3.2. MAP

Another potential environmental risk factor for the development of IBD is a chronic pathogenic infection in the GIT. To date, no one particular organism has been found to be the causative agent of IBD. Although many microbial pathogens have been implicated as causative, only two have been significantly investigated. The first organism hypothesized to be associated with IBD was *Mycobacterium avium* subsp. *paratuberculosis,* which causes Johne's disease in cattle [152, 153]. The granulomatous inflammation observed in CD patients led many early researchers to investigate various types of *Mycobacterial* spp. in CD pathogenesis but confirmation was never achieved (reviewed in [154]). The first report of *M. paratuberculosis*-like organisms isolated from patients with CD in was published 1984. Now known as *M. avium* subsp. *paratuberculosis* (MAP), there is still no consensus as to whether or not it plays a role in the onset of IBD in a subset of genetically susceptible patients [155]. Using PCR to amplify the DNA insertion element IS*900*, which is specific to MAP, in biopsy sections, Sanderson et al. reported that 65 % of adult CD patients examined were PCR positive [156]. Dell'Isola et al. reported that 72 % of pediatric CD patients examined were PCR positive for the presence of MAP [156, 157]. While many studies have reported the presence of MAP DNA in CD patients, just as many have found MAP DNA in samples from healthy individuals and UC patients [156-162]. Moreover, multiple studies have failed to detect MAP-specific DNA in any CD patient sample examined [163-169]. The ability to detect MAP in some tissue samples and not others may be due, in part, to the organism's fastidiousness, slow growth, and/or the presence of PCR inhibitors in fecal/tissue samples that inhibit the detection of IS900. Serological evidence also fails to provide definitive answers for the involvement of MAP in the pathogenesis of IBD. Some studies assessing the presence of serum antibody titers against MAP-derived antigens find an association with IBD [170-176] while others do not [177-182]. More recent work has identified a possible link between variants in the *Nod2*

gene and the presence of MAP [183]. In this study, 68 % of CD patients were positive for MAP DNA based on PCR analysis as compared to 21 % of the healthy individuals. Fifty-one percent of the CD patients were carriers of NOD2 polymorphisms as compared to 21 % of healthy individuals, and 74 % of the CD patients possessing one of the mutated NOD2 alleles were positive for MAP DNA. Even with of all of the data collected, the debate continues as to whether or not MAP plays an important role in the pathogenesis of CD [184, 185].

2.3.3. Adherent-invasive Escherichia coli

Another organism implicated in IBD pathogenesis is adherent-invasive *Escherichia coli* (AIEC). First described in 1998, AIEC isolates were recovered from the ileal mucosa of CD patients [186]. These organisms are found in higher numbers in CD patients (22 %) as compared to healthy controls (6 %) [187]. These isolates were able to adhere to Caco-2 cells and did not posses any of the virulence genes associated with known *E. coli* pathotypes (e.g., ETEC, EHEC) [186]. A characterization of expressed adhesions in AIEC strains revealed the *Pap* and *Sfa* adhesion or colonization factors, both of which are found in uropathogenic *E. coli* strains; however many of the AIEC strains did not possess any of the known adhesions associated with pathogenic *E. coli* (e.g., intimin). Some of the cytotoxic strains were found to express the *hly* operon, and it was subsequently demonstrated that these organisms express a type 1 pili similar in sequence to an *E. coli* strain associated with avian colisepticemia and meningitis [188]. Type 1 pili are involved in both the adherence and internalization of *E. coli* into host cells. They specifically bind to the CEACAM6 receptor on ileal enterocytes, which is expressed at significantly higher levels in the inflamed small intestinal epithelium of CD patients as compared to healthy controls [189].

Further research involving the prototypic AIEC strain, LF82, has shown that this pathogenic group of *E. coli* invades epithelial cells via engagement of actin microtubules and microfilaments and replicates intracellularly even without possessing any of the known invasive determinants found in other invasive *E. coli* strains [190]. AIEC strains are also able to survive and replicate within macrophages without causing apoptosis; they also induce production of the pro-inflammatory cytokine TNF-α [191]. LF82 is also able to induce *in vitro* aggregation of peripheral blood mononuclear cells (PBMCs), an observation reminiscent of the granulomas observed in the colonic tissue of CD patients [192].

In addition to being detected with increased frequency in human IBD patients, AIEC strains have also been isolated from Boxer dogs with granulomatous colitis. These *E. coli* isolates were found to have the same adherent-invasive phenotype as human AIEC strains. The Boxer dog isolates were also of the same phylotype (B2 and D) and possessed similar virulence gene profiles as LF82. In contrast to the human AIEC strains, however, the canine AIEC strains were only isolated from diseased dogs and not healthy controls. Simpson and colleagues also demonstrated that remission of colitis in Boxer dogs could be achieved by treatment with enrofloxacin, the use of which resulted in the eradication of AIEC *E. coli* [193].

2.4. The microbiota and IBD

Although no single organism has been implicated in the induction of IBD, a preponderance of studies indicates that the GI microbial community of IBD patients is different from that of healthy individuals. This imbalance is known as "dysbiosis". Characteristics of a dysbiotic community in IBD include a reduction in members of the *C. leptum* and *C. cocciodes* clusters (members of the Firmicutes phyla and major butyrate producers) and an increase in *Enterobacteriaceae* and members of the Bacteroidetes, thereby leading to a reduction in the microbial diversity of the gut. Another notable difference is the greater concentration of mucosally-associated bacteria in IBD patients as compared to healthy individuals [194-197]. Using fluorescence *in situ* hybridization (FISH), patients with CD were found to have predominantly *Enterobacteriaceae*, *γ-Proteobacteria*, or *Bacteroides/Prevotella* adherent to their mucosa and present in their submucosa [195]. Another FISH-based analysis revealed that *Bacteroides* spp. were the dominant gut bacteria, representing up to 80 % of the total mucosally-adherent bacterial population in some samples. Of interest was the authors' additional finding that treatment of IBD patients with mesalamine (an anti-inflammatory therapeutic drug) significantly reduced the numbers of mucosally-adherent bacteria [198].

Sample origin is an important factor to consider when interpreting data for microbial analyses. Although stool samples are easy to obtain, it may not accurately reflect the microbial community in the cecum and proximal colon of patients [14]. In a comparative study of the microbial populations detected in cecal versus fecal samples, more anaerobes and *Bifidobacterium* spp. were detected in fecal samples using culture-based methods [199]. Molecular probe hybridization revealed that facultative anaerobes represented by *Lactobacillus, Enterococcus,* and *E. coli* were higher in numbers in cecal contents, yet the number of strict anaerobes represented by the *Bacteroides, C. leptum,* and *C. cocciodes* groups were significantly lower in the cecal contents. Differences in microbial composition have also been observed when comparing colonic biopsies versus feces [200]. Analysis of feces from Japanese IBD patients showed that *Faecalibacterium* spp. were significantly decreased in CD patients, while *Bacteroides* spp. were significantly increased only in patients with active IBD [201]. Based on the Shannon diversity index, this study demonstrated that microbial diversity was significantly reduced in CD patients both during active disease and remission as compared to healthy individuals.

Several studies have noted a specific decrease in the numbers of *C. leptum* and *C. cocciodes* clusters present in colonic contents or feces in IBD patients [18, 197, 202-206]. A reduction in the *C. cocciodes* group in UC patients and *C. leptum* in CD patients has been shown using FISH probes on patient feces [202]. Another study employing a combination of PCR and FISH analysis of fecal samples found a reduced presence of both *C. leptum* and *C. cocciodes* clusters in IBD patients [197]. Additional analyses revealed significant decreases in the concentrations of the SCFAs (e.g., butyric and propionic acid) in the feces of IBD patients [197]. In other work, high throughput sequencing demonstrated reduced numbers of *Faecalibacterium, Ruminococcaceae, Alistipes, Collinsella,* and *Roseburia* and increased numbers of *Enterobacteriaceae* in a twin with ileal CD as compared to the healthy twin [204]. This latter study again emphasizes the

involvement of the microbiota in the development of IBD in a genetically susceptible host, as only one of the twins developed disease.

One specific member of the *C. leptum* cluster, *Faecalibacterium prausnitzii*, has recently been the subject of many published studies. This organism is a major butyrate producer that also possesses some anti-inflammatory properties [59, 207]. A study by Sokol et al. described a reduction of both *C. leptum* and *C. coccoides* clusters in the stool of patients with active IBD along with a skewed Firmicutes/Bacteroidetes ratio [203]. The authors specifically identified a reduction in *F. prausnitzii* in IBD patients, confirming results obtained using biopsy samples from twins analyzed via qPCR [205]. In other work, denaturing gradient gel electrophoresis (DGGE) analysis of biopsy samples also demonstrated a significant decrease in *Faecalibacterium* spp. along with increased levels of *E. coli* and *Clostridium* spp. in CD patients as compared to healthy individuals [208]. Using fecal cylinders and 11 different FISH probes to analyze sections GIT tissue specimens, Swidsinski and colleagues found the presence of *F. prausnitzii* to be significantly reduced in CD patients [209]. Of special note, analysis of samples from IBD patients given high-dose cortisol or infliximab to reduce inflammation revealed a dramatic increase ($>14 \times 10^9$) in the levels of *F. prausnitzii* within days of initiating treatment. This increase was short-lived, however, as levels of *F. prausnitzii* decreased when the cortisol dose was reduced or the time between infliximab infusions was increased. Together, these data indicate that inflammation plays a major role in shaping the intestinal microbiota.

Work in murine models of colitis also demonstrates that inflammation in the gut, be it chemically or bacterially induced, causes an increase in *Enterobacteriaceae* [210]. Colonization of mice with *Citrobacter rodentium* resulted in a reduction of the total number of bacteria in the colon at 7 and 14 days post-infection; this decrease coincided with the highest levels of both *C. rodentium* and intestinal inflammation [211]. Further analysis specifically determined that members of the Cytophaga-Flavobacter-Bacteroides phylum were decreased. Infection with *C. rodentium* infection is self-limiting, and the total bacterial levels returned to normal following pathogen clearance. These changes in the bacterial load were not simply associated with colonization of a new organism to the microbial community, such as *Campylobacter jejuni*, an organism that does not cause intestinal inflammation in immunocompetent CONVR mice [212], induced no detectable changes in microbial load.

The induction of GIT inflammation in mice via administration of dextran sulfate sodium (DSS) in the drinking water for seven days was associated with increased numbers of aerobic bacteria, specifically *Entercococcus faecalis* [210]. Bacteroidetes were eliminated from the community and the total number of bacteria was also decreased. A dramatic increase in the numbers of a non-pathogenic *E. coli* following DSS treatment was also observed. The authors subsequently colonized IL-10$^{-/-}$ mice, which spontaneously develop colitis [213], with this non-pathogenic *E. coli*. Upon the development of GIT inflammation, the *E. coli* was able to proliferate to the same level observed in DSS treated mice, but displacing the Firmicutes phylum instead of the Bacteroidetes. These results indicate that regardless of the source, GIT inflammation creates a niche that favors the colonization and expansion of *Enterobacteriaceae*. Collectively, these data suggest that the induction of inflammation following the colonization of the GIT by a bacterial

provocateur along with perturbations of the microbiota together have a deleterious impact on the microbiota and mucosal homeostasis.

2.5. Canine IBD

In addition to affecting humans, IBD can also occur in dogs as well [214]. As in human IBD, the interactions between genetics, the mucosal immune system, inflammation, and environmental factors (ie, diet and imbalances in the intestinal microbiome) all contribute to the pathogenesis of canine IBD (Figure 6) [215-217].
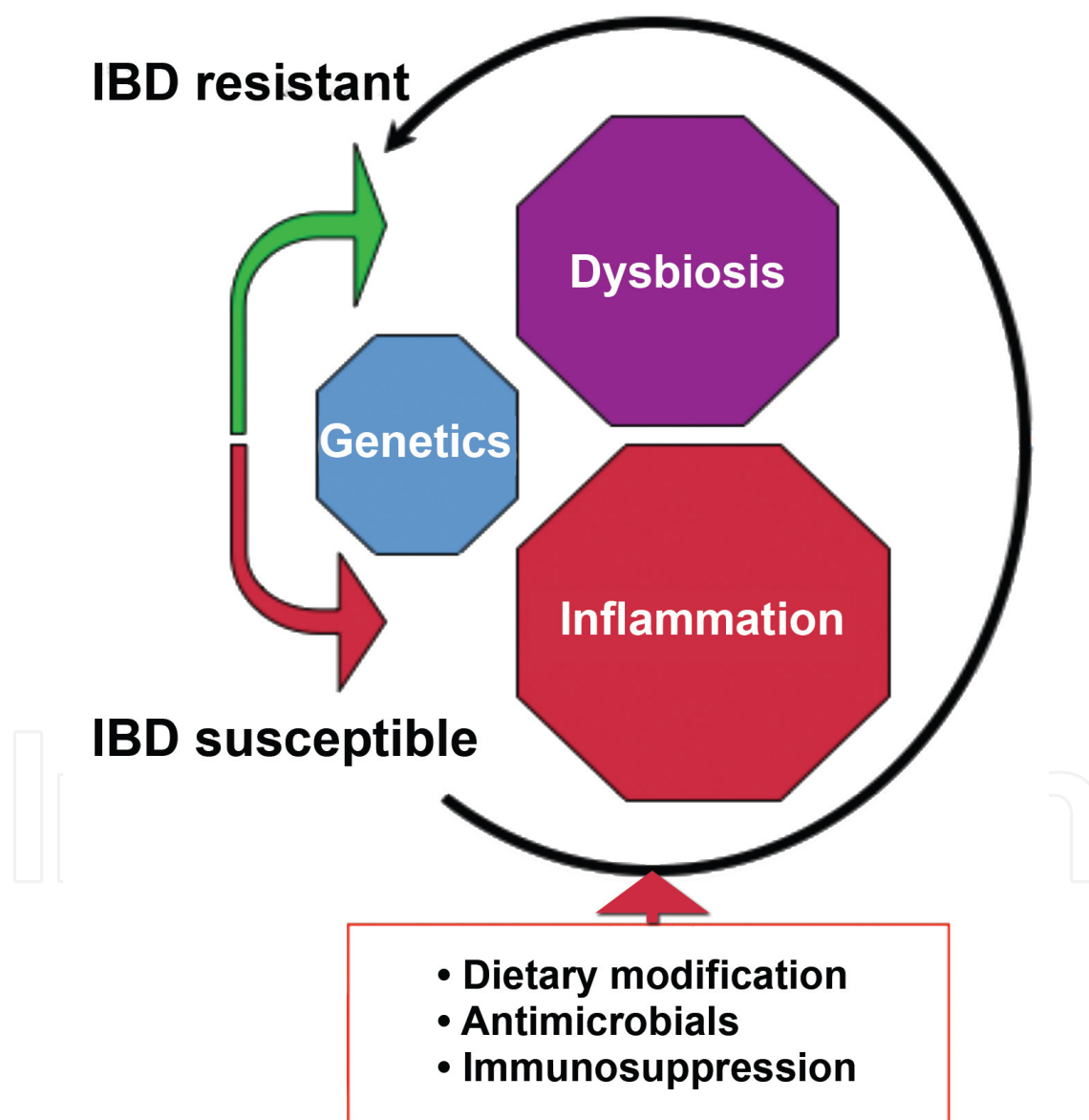

Figure 6. The etiology for canine IBD involves complex interactions between host genetics, mucosal immunity, and the enteric microbiota. Therapeutic intervention with diet, antibiotics and immunosuppressive drugs is aimed at reducing inflammation and dysbiosis.

Mutations in innate immune receptors in German shepherd dogs (TLR5, NOD2) have been linked to IBD susceptibility which in the presence of an inappropriate enteric microbiota may lead to upregulated pro-inflammatory cytokine production (e.g., IL-17, IL-22, TNF-α) and reduced bacterial clearance, thereby promoting chronic intestinal inflammation [218, 219]. Commensal bacterial antigens are likely to be important in disease pathogenesis because it has been observed that boxer dogs with granulomatous colitis (GC) show clinical remission following the eradication of mucosally associated AIEC that share a novel adherent and invasive pathotype which bears phylogenetic similarity with AIEC strains recovered from patients with ileal Crohn's disease [193, 220, 221]. Moreover, genome-wide analysis in affected boxer dogs has identified disease-associated single nucleotide polymorphisms (SNPs) in a gene (*NCF2*) involved with killing intracellular bacteria [222]. Still others have shown that CD11c⁺ cells are significantly decreased in the intestines of dogs with IBD suggesting that chronic mucosal inflammation may involve an imbalance in the intestinal dendritic cell population leading to aberrant immune activation [223]. Molecular analysis of the intestinal microbiome in different breeds of dogs with IBD have consistently shown that diseased tissues are enriched with members of the families *Enterobacteriaceae* and *Clostridiaceae* [224, 225]. These bacteria are believed to contribute to the pathogenesis of GIT inflammatory disease in dogs as in humans [187, 226]. A recent trial using high throughput 16S rDNA sequencing methods (ie, 454 pyrosequencing) on intestinal biopsies of IBD dogs revealed a dysbiosis in the mucosally-adherent microbiota with an increase in sequences belonging to Proteobacteria and a decrease in Bacteroidetes, Fusobacteria, and the Clostridiales [227]. Taken together, these studies suggest that chronic intestinal inflammation of canine IBD may be due to overly aggressive adaptive immune responses to enteric bacteria (or fungi) [225] in hosts with genetic defects that fail to properly regulate microbial killing, mucosal barrier function, or immune responses. As in human IBD, environmental factors (diet, microbiota imbalances) likely govern the onset of inflammation or reactivation and modulate genetic susceptibility to disease.

2.5.1. Clinical and diagnostic features

The clinical manifestations of IBD are diverse and are influenced by the organ(s) involved, presence of active versus inactive disease, and physiologic complications seen with enteric plasma protein loss and/or micronutrient (cobalamin) deficiency [215, 216, 228, 229]. Canine IBD is a disease that predominantly affects middle-aged animals. Vomiting and diarrhea are most commonly observed and are often accompanied by decreased appetite and weight loss. Gastric and duodenal inflammation is associated with vomiting and small bowel diarrhea while colonic involvement causes large bowel diarrhea with blood, mucus, and straining. The clinical course of IBD is generally cyclical and is characterized by spontaneous exacerbations and remissions. Importantly, the clinical signs of IBD are not disease specific and share numerous over-lapping features with other canine disorders. A diagnosis of IBD is one of exclusion and requires careful elimination of IBD mimics [230]. The possible causes for chronic intestinal inflammation may be excluded through the integration of history, physical findings, clinicopathological testing, diagnostic imaging, and histopathology of intestinal biopsies. A baseline CBC, biochemistry profile, urinalysis, and diagnostic imaging are useful in eliminating the most common systemic and metabolic disorders (e.g., renal disease, hepatopathy,

hypoadrenocorticism) causing chronic GI signs in dogs. The measure of clinical disease activity by means of quantifiable indices is well established in human IBD [231-233].

A canine IBD activity index (CIBDAI) used for assessment of inflammatory activity in dogs has been recently designed [234]. Similar to other indices, the magnitude of the numerical score is proportional to the degree of inflammatory activity. This index serves as the principal measure of response to a therapeutic regimen and may be used to tailor medical therapy for an individual patient's needs [235]. Intestinal biopsies are required to confirm histopathological inflammation and to determine the extent of mucosal disease. Diagnostic endoscopy is preferred since this technique allows for direct assessment of mucosal abnormalities and the acquisition of targeted biopsy specimens. The microscopic findings in canine IBD consist of minimal to pronounced inflammatory cell (lymphoplasmacytic) infiltration of the intestinal lamina propria accompanied by varying degrees of mucosal architectural disruption similar to that observed in tissue from human IBD patients (Figure 7).
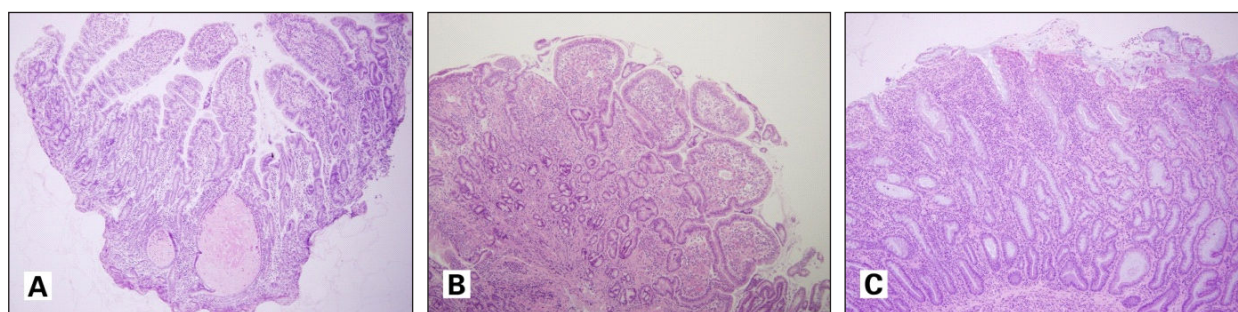

Figure 7. Histopathological lesions of (A) crypt distortion with abscessation, (B) diffuse villous atrophy, and (C) mucosal ulceration seen in duodenal biopsies of dogs with IBD.

Unfortunately, biopsy interpretation is notoriously subjective and suffers from extensive intra-observer variability and the technical constraints of procurement/processing artifacts inherent in evaluation of endoscopic specimens [236]. Although several histopathological scoring schemes have been proposed there are no uniform grading criteria that pathologists can universally agree on. One small study has resulted in development of a 'simplified model system' for defining intestinal inflammation of IBD that is presently being tested in a separate clinical trial.

2.5.2. Therapeutic approach

Treatment principles for canine IBD are empirical and consist of combination therapy using both dietary and pharmacologic interventions. As compared to clinical trials evaluating the efficacy of therapy for CD and UC, only one randomized, controlled drug trial for canine IBD has been reported [235]. There are, however, abundant evidence-based observations that feeding elimination diets and administering corticosteroids, immunosuppressive drugs, and/or select antibiotics are useful in the clinical management of canine IBD. Some clinicians prefer a sequential approach to nutritional and drug therapy for IBD. The optimal drug or drug combinations as well as duration of therapy for induc-

tion and maintenance of remission of clinical signs have not been determined for most protocols [216, 230]. In general, the administration of corticosteroids (i.e., prednisone, prednisolone or budesonide), antimicrobials (i.e., metronidazole or tylosin), and immuno-suppressive drugs (i.e., cyclosporine, chlorambucil, azathioprine) used alone or in some combination are effective in inducing clinical remission in most animals. Some dogs will require intermittent or life-long drug therapy.

The rationale for nutritional therapy of IBD is that restricting exposure to antigens (i.e., dietary proteins) known to evoke sensitivity will reduce exaggerated host responses and attenuate intestinal inflammation. Other indications for specialized nutrition include the presence of decreased appetite, impaired nutrient absorption, or enteric plasma protein loss seen with moderate-to-severe mucosal inflammation. While evidence-based observations indicate that most dogs respond favorably to dietary intervention, the superiority of one novel protein source versus another or the advantage in feeding an intact protein elimination diet versus a hydrolyzed protein elimination diet has not been shown to date. Modifying the dietary n3:n6 fatty-acid ratio may also modulate inflammatory responses by reducing production of pro-inflammatory metabolites [237]. There is relatively sparse clinical data investigating prebiotic or probiotic therapy for canine IBD (see subsequent section on probiotics).

2.5.3. Future directions in canine IBD

Canine IBD represents a common and frustrating GI disorder in veterinary medicine. More research is needed to unravel the mechanisms responsible for disease development and to translate these findings directly to human IBD. The primary features of IBD in humans and animals are remarkably similar (Table 1).

Feature	Human IBD	Canine IBD
Genetic basis	Yes	Likely
Etiology	Unknown but multifactorial	Unknown but multifactorial
Involves the microbiota	Yes	Yes
Hematochezia	Yes	Yes
Diarrhea	Yes	Yes
Definitive Diagnosis	GI biopsy	GI biopsy
Disease activity assessment	Clinical indices, biomarkers (ASCA, pANCA, CRP, calprotectin)	Clinical indices, biomarkers (pANCA, CRP, calprotectin ?)
Responsive to anti-inflammatory drugs	Yes	Yes
Responsive to antibiotics	Yes	Yes
Spontaneous GI flares	Yes	Yes

Table 1. Comparative features of IBD in humans and dogs.

Recent advances in clinical indices, histopathological standards, and the development of species-specific immunologic reagents and innovative molecular tools have made the dog an excellent 'spontaneous' animal model to study chronic immunologically-mediated intestinal inflammation. In addition, the dog has higher genomic sequence similarity to that of humans than do mice, a species traditionally used for comparative disease genetics [238]. However, clinical manifestations of complex disease in the mouse do not compare to the human form as closely as they do in the dog. Furthermore, the lifespan of the dog is much shorter than that of a human; thus, clinical trials aimed at treatment of IBD can be carried out much quicker and yield results that should have relevant application to human trials [230, 235, 239].

2.6. Treatment for human IBD

Treatments for human IBD patients typically involve the use of anti-inflammatory drugs, antibiotics, and there is a growing trend of probiotics and prebiotics being studied to determine their effects on disease activity. When patients fail to respond to treatment, the last and most drastic treatment option is surgery to resect sections of the inflamed bowel [240]. Most treatment options are primarily focused on reducing the inflammation associated with IBD. Some of these drugs also impact the microbiota and those will be discussed below.

2.6.1. Antibiotics

Given the importance of the microbiota in the pathogenesis of IBD, antibiotic therapy may seem an obvious treatment option. A meta-analysis by Khan et al. in 2011 reviewed randomized controlled trials utilizing antibiotics in the treatment of IBD [241]. Rifamycin derivatives, ciprofloxacin, and clofazamine all induced remission in CD patients. Rifaximin (a rifamycin derivative) is effective against both Gram-negative and Gram-positive anaerobes and aerobes; it is also poorly absorbed after oral administration, resulting in little to no systemic side effects [242, 243]. Analysis of the effect of the drug using an *in vitro* continuous culture method with fecal samples from CD patients revealed increases in beneficial bacteria post-rifaximin administration [244]. Significant increases in *Bifidobacterium* spp., the *Atopobium* cluster, and *F. prausnitzii* were noted, as were increases in levels of lactate, acetate, and propionate as determined by ^{1}H-NMR spectroscopy [244].

Ciprofloxacin is a fluoroquinolone with broad-spectrum antibiotic activity [245]. In addition to having anti-bacterial properties, this drug has also been shown to have immunomodulatory properties as well [246]. In a TNBS mouse model of colitis, administration of ciprofloxacin ameliorated disease as compared to mice given ceftazidime (an antibacterial with a similar spectrum of activity compared to ciprofloxacin) [245]. Clinically, mice treated with ciprofloxacin did not lose weight and had reduced histopathological inflammatory scores associated with their colons. Ciprofloxacin treated mice also had reduced expression of IL-1β, IL-8, and TNF-α as measured from colonic homogenates as well as reduced expression of NF-κB. Since ceftazidime was less effective in ameliorating GIT inflammation, there appears to be additional benefits (i.e., anti-inflammatory) to the use of ciprofloxacin in addition to its spectrum of antimicrobial activity.

Clofazamine has also been documented to significantly affect the rate of remission in CD patients [241]. This drug, used to treat leprosy, is similar to ciprofloxacin, in that it has both anti-bacterial and anti-inflammatory properties [247-249]. It is only effective against Gram-positive organisms, and its effectiveness increases in anaerobic environments [250]. Its effects on the immune system include increasing the presence of lysosomal enzymes in macrophages [251] and increasing phagocytosis by macrophages resulting in enhanced uptake and digestion of immune complexes [252]. Clofazamine has also been shown to inhibit TCR-mediated IL-2 production by T cells, thereby limiting T cell activation, a component of the pathogenesis of IBD [253].

2.6.2. Corticosteroids

The most commonly used corticosteroids used are prednisolone, methylprednisolone, and budesonide [254]. These drugs are very effective at inducing remission, however, they are not without side effects. In a study comparing prednisolone and budesonide, both were found to be effective at inducing remission [255]. However, patients on budesonide had reduced evidence of adrenal axis suppression and peripheral leucocyte counts compared to those treated with prednisolone. These results indicate that budesonide is a safer choice yet it is still as effective as prednisolone. Corticosteroids are able to prevent NFκB activation [256] as well as block infiltration of neutrophils, prevent vasodilation and enhanced vascular permeability and downregulate the production of pro-inflammatory cytokines [254]. Although it is well established that these corticosteroids have an anti-inflammatory effect, little research has been conducted on the effects they have on the gut microbiota. A study by Swidsinski et al., mentioned previously, indicated that administration of cortisol increased the population of *F. prausnitzii* in a dose dependent manner [209].

2.6.3. Immunosuppressive therapy

Drugs such as methotrexate, 6-mercaptopurine (6-MP) and azathioprine (the prodrug of 6-MP) work by inhibiting the proliferation and activation of lymphocytes and decreasing the production of pro-inflammatory cytokines [254]. 6-mercaptopurine (6-MP) and azathioprine are purine antagonists and inhibit cellular metabolism by interfering with DNA replication [257, 258]. Methotrexate is a folic acid analog that inhibits DNA synthesis and, therefore, has an anti-proliferative effect [259]. Antibacterial effects, including growth inhibition of MAP, have also been demonstrated for methotrexate and 6-MP [258].

2.6.4. 5-aminosalicyclic acid (5-ASA)

Sulfasalazine was the first 5-ASA-type drug developed and is a combination of sulfapyridine (an antimicrobial) and salicyclic acid (an anti-inflammatory) to form a pro-drug. Upon entering the colon, this pro-drug is cleaved by colonic bacteria into the two separate molecules [260, 261]. Unfortunately, the frequency of gastrointestinal side effects was quite high due to the sulfapyridine moiety [262, 263]. It was later determined that the active moiety is 5-aminosali-cyclic acid (5-ASA, mesalazine) [264]. More recently formulations of this pro-drug have eliminated the sulfapyridine moiety and replaced it with either a second salicyclic acid moiety

(disodium azodisalicylate [265]) or an inert carrier (balsalazide [266]) thereby reducing the side effects.

In addition to being an anti-inflammatory agent [267-270], 5-ASA also affects the gut microbiota. As mentioned previously, patients taking mesalamine had a reduction in mucosa-adherent bacteria [198, 271]. 5-ASA has also been shown to inhibit the growth of MAP [272] and *Bacteroides* spp. [273] and moderately inhibit the growth of *C. difficile* and *C. perfringens* in culture [273]. This drug also effects bacterial gene expression, as *Salmonella enterica* serovar Typhimurium incubated with 5-ASA had no change in growth but differentially expressed 110 genes [274]. Those genes characterized were found to be involved in invasion, metabolism, and antibiotic and stress resistance. *In vitro* assays revealed attenuation in the invasiveness of *S. enterica* serovar Typhimurium towards HeLa cells when pretreated with 5-ASA. These results indicate that in addition to inhibiting host-mediated inflammation, 5-ASA also has the potential to affect the intestinal microbiota.

2.6.5. Anti-TNF monoclonal antibodies

The induction of TNF-α is most often a downstream event following the interaction of phlogistic microbial components with toll-like receptors (TLRs) on host cells. The interest in TNF-α as a therapeutic target for IBD treatment began when the expression and secretion of this cytokine was found to be increased in IBD patients [275, 276]. For example, pediatric patients with either active UC or CD present with elevated levels of TNF-α in their stool [275]. Additionally, the incubation of GIT tissue sections in culture medium has demonstrated that significantly elevated levels of TNF-α are spontaneously secreted from inflamed tissue of both UC and CD patients when compared to the amounts produced by non-inflamed tissue and tissue from otherwise healthy individuals [276]. The predominant cell type producing the TNF-α has been shown to be the macrophage [276]. Based on the central role TNF-α appears to play in the pathogenesis of IBD, there was interest in developing a therapeutic approach to control the harmful effects of this cytokine.

Clinically, the use of monoclonal antibodies to treat IBD patients began with Infliximab, an IgG1 murine-human chimeric monoclonal antibody specific for TNF-α, which was approved for human use in 1998 for CD [277, 278]. This monoclonal antibody consists of human constant regions and murine antigen binding regions [277]. These chimeric antibodies reduce the risk of immunoreactivity that occurs when murine antibodies are used. In addition to being less immunoreactive, this chimeric antibody had improved binding and neutralization characteristics for TNF-α than that of the original murine antibody [277]. Another monoclonal anti-TNF antibody, Adalimumab, is a fully humanized IgG1 antibody that avoids the induction of anti-species IgG that neutralize the effectiveness of the anti-TNF-α reagent [278, 279]. Lastly, Certolizumab is a monoclonal antibody fragment with a polyethylene glycol moiety (PEGylated) [278]. Certolizumab lacks the crystallizable fragment (Fc) portion of the immunoglobulin molecule and is an IgG4 isotype unlike Infliximab and Adalimumab, which are IgG1 antibodies [278, 280, 281]. In addition, the PEGylation increases the half-life of the antibody thereby reducing the frequency of administration.

Anti-TNF-α therapy works via multi-factorial mechanisms. First, it neutralizes TNF-α by blocking its ability to bind to TNF receptors, thus, inhibiting the pro-inflammatory response. Second, Anti-TNF-α binds to cell surface bound TNF-α on CD4+ T cells and macrophages, resulting in both complement- and antibody-dependent cell-mediated cytotoxicity [282]. All three monoclonal antibodies bind to and neutralize both soluble and membrane forms of TNF-α [281]. Infliximab and Adalimumab both mediate complement- and antibody-dependent cell-mediated cytotoxicity; however, Certolizumab only mediates complement-dependent cellular cytotoxicity as it lacks of an Fc region. Furthermore, Infliximab and Adalimumab induce apoptosis in peripheral blood lymphocytes and monocytes, as well as cause degranulation and loss of membrane integrity of PMNs. These activities were not induced with Certolizumab. Lastly, all three monoclonal antibodies inhibit the production of IL-1β after LPS stimulation in vitro, suggesting that there is a sequential production of pro-inflammatory cytokines induced by microbial components. Infliximab and Adalimumab both inhibit T cell proliferation in mixed lymphocyte reactions in vitro, again suggesting that anti-TNF-α monoclonal antibodies ameliorate the inflammation associated with IBD via more than one mode of action [283]. The impact of these therapies on the microbiota, however, is not well studied. As previously mentioned, treatment with Infliximab resulted in increased levels of *F. prausnitzii* after administration [209]. As mentioned above, it is clear that the host inflammatory response often negatively impacts (i.e., shapes) the composition of the GIT microbiota, and that controlling mucosal inflammation benefits the health of the microbiota as well. Otherwise, there have been no specific studies performed to directly evaluate the role of anti-TNF-α therapies on the gut microbiota.

2.6.6. Complementary and alternative therapies

An estimated 70 % of IBD patients have reported using complementary and alternative medicine (CAM) products at some time to treat their symptoms In a Canadian study of IBD patients, some of the most commonly used CAM treatments included massage therapy, chiropractic visits, probiotics, herbs, and fish oils [284]. A systematic review of the literature on the use of herbal medicines reveals some anti-inflammatory benefits associated with the administration of these herbs to both animals and humans [285]. It should be stressed that prior to utilizing any herbal remedy, which are potentially biologically active, patients need to consult their physician. This is especially important because the number two reason patients using CAM gave as to why they sought these products was that "natural therapy is safe"[286]. Some of the biological properties associated with CAM products include the reduction of pro-inflammatory cytokines, increased antioxidant production, inhibition of leukotriene B4, decreased NF-κB activation, and inhibition of platelet activation [285].

Increasing evidence supports a potential therapeutic role for prebiotic and probiotic therapy in human IBD [287, 288]. If IBD in dogs is indeed driven by loss of tolerance to components of the intestinal microbiota as it is in humans, then prebiotics and probiotics may also prove beneficial as primary or adjunct therapies with diet and drugs. *Probiotics* are living microorganisms that, upon ingestion in sufficient numbers, impart health benefits beyond those of inherent basic nutrition [289]. Lactobacilli and Bifidobacterium have been the most commonly

used human probiotics, but multi-strain cocktails (e.g., VSL#3), *E. coli* Nissle 1917, and nonbacterial *Saccharomyces boulardii* have also been used as probiotics [226]. Probiotic bacteria have measurable host benefits, including the ability to improve epithelial barrier function, modulate the mucosal immune system, and alter the intestinal flora [290]. *Prebiotics* are non-digestible dietary carbohydrates, such as lactosucrose, fructo-oligosaccharides (FOS), psyllium, bran, which beneficially stimulate the growth and metabolism of endogenous enteric bacteria upon consumption [291]. Beneficial effects of prebiotics are also associated with the production of short chain fatty acids (SCFA) due to fermentation by colonic bacteria. *Synbiotics* are combinations of probiotics and prebiotics that are an emerging therapeutic modality. Increasing evidence supports a therapeutic role for probiotics, prebiotics, and synbiotics in treating gastrointestinal diseases of humans, including infectious diarrhea, *H. pylori* infection, irritable bowel syndrome, lactase deficiency, and IBD [84]. A comparison of prebiotic and probiotic preparations is outlined in Table 2.

2.6.7. Probiotics

VSL#3 is one of the most commonly used probiotic cocktails and contains a very high bacterial concentration per gram of product characterized by greater number of different bacterial species as compared to traditional probiotic preparations [292]. This commercially prepared formulation consists of 450 billion bacteria/g of viable lyophilized bacteria comprised of eight bacterial strains (*Lactobacillus casei, L plantarum, L. bulgaricus, L. acidolphilus, Bifidobacterium longum, B. breve, B. infantis* and *Streptococcus thermophiles*). While the exact mechanism of action of VSL#3 is unknown, several studies have demonstrated the effects of VSL#3 on epithelial barrier function and down regulation of cytokine secretion from immune cells. For example, Madsen [293] has shown in *in vitro* studies that epithelial barrier function could be enhanced by exposure to a soluble factor secreted by VSL#3 bacteria. Moreover, this same study demonstrated that VSL#3 did not alter the ability of the epithelial cell to activate a mucosal inflammatory response to a bacterial invasion. Studies with VSL#3 formulations have also been conducted in several animal models of colitis, inflammatory liver disease, sepsis, and irritable bowel syndrome (IBS). In models of experimentally-induced colitis, these studies demonstrated that VSL#3 normalized gut permeability and barrier function, and that VSL#3 modulated inflammatory and immune responses [226, 294]. Animal models of sepsis have also demonstrated that VSL#3 administration reduced bacterial translocation and significantly attenuated damage to the liver and intestinal mucosa [295]. The use of VSL#3 as an innovative probiotic preparation, developed specifically to balance the intestinal microbiota, is supported by studies in humans with IBD (ulcerative colitis [296], pouchitis) and in other patients with diverse gastrointestinal disorders, such as IBS [297, 298].

Studies have shown VSL#3 to induce remission of inflammation in 77 % of adult UC patients with no adverse effects [298] and 56 % of pediatric UC patients [299]. These same pediatric UC patients had a reduction in disease activity index and sigmoidoscopy scores following VSL#3 treatment. They also had reduced levels of the pro-inflammatory cytokines TNF-α and IFN-γ following therapy [299]. *In vitro* analysis of the effects of VSL#3 on Mode-K epithelial cells revealed that only one of the eight organisms, *L. casei*, inhibited TNF-induced secretion of the

pro-inflammatory chemokine IP-10 [300]. The most recent studies with VSL#3 treatment of UC show increased fecal concentrations of beneficial bacterial species, improved clinical, endoscopic and histopathological scores in most patients, and higher rates of remission compared to placebo [298]. The most recent studies with VSL#3 treatment of UC show increased fecal concentrations of beneficial bacterial species, improved clinical, endoscopic and histopathological scores in most patients, and higher rates of remission compared to placebo [298]. VSL#3 is available without prescription and can be ordered via the internet or obtained locally at the pharmacy in the U.S. [301].

	Probiotics	Prebiotics
Definition	Live microorganisms which, given in adequate amounts confer health benefits to the host	Non-digestible carbohydrate which stimulate replication of protective enteric bacteria when consumed
Examples	E. coli Nissle 1917 VSL#3 Lactobacillus species Bifidobacterium species Saccharomyces boulardii Prostora Max® Forti Flora® Proviable-DC®	Fructo-oligosaccharide (FOS) Galacto-oligosaccharide (GOS) Inulin Lactulose Psyllium Bran Beet pulp, pumpkin Resistant starch
Protective Mechanisms	Alters microbiota to suppress pathogens Improved intestinal barrier function Increased production of antimicrobial peptides Decreased expression of proinflammatory cytokines	Stimulates replication of beneficial bacteria (Bifidobacterium) Enhances production of SCFA (butyrate) Improved intestinal barrier function Decreases proinflammatory cytokines

Table 2. Basic Features of Probiotics and Prebiotics

Recent studies have also shown that dogs with IBD have distinctly different duodenal microbial communities compared to healthy dogs. Current treatments for IBD include the administration of nonspecific anti-inflammatory drugs which may confer serious side effects and do not address the underlying basis for disease, namely, altered microbial composition. The use of probiotics offers an attractive, physiologic, and non-toxic alternative to shift the balance to protective species and treat canine IBD. The authors (AEJ) have initiated a clinical trial to investigate the clinical, microbiologic, and anti-inflammatory effects of probiotic VSL#3 in the treatment of canine IBD. We hypothesize that VSL#3 used as an adjunct to standard therapy (i.e., elimination diet and prednisone) will induce a beneficial alteration of enteric

bacteria leading to induction and maintenance of remission in dogs with IBD. A randomized, controlled clinical trial of eight weeks duration will assess the efficacy of standard therapy in conjunction with VSL#3 versus standard therapy alone in the management of canine IBD. There is a need for additional data to be generated to provide proof of efficacy in probiotic therapy before these agents can be applied to widespread clinical use. These studies will also provide highly relevant insight into the anti-inflammatory effects of probiotics for treatment of human and canine IBD.

Another popular probiotic is *E. coli* Nissle 1917 also known as Mutaflor®. This probiotic has been shown to be just as effective as mesalazine in achieving and maintaining remission of inflammation in UC patients [302-304]. It has recently been shown that when *E. coli* Nissle 1917 is genetically modified to produce the quorum-sensing molecule AI-2, it affected the beneficial probiotic properties of this organism [305]. Colonization of healthy newborn infants with *E. coli* Nissle 1917 has shown that the presence of pathogenic bacteria in the gut was significantly reduced compared to non-colonized infants [306]. *In vitro* studies have shown *E. coli* Nissle 1917 is able to reduce the invasive ability of multiple pathogenic organisms [307], and also to reduce the adherent and invasive ability of AIEC [308]. *E. coli* Nissle 1917 was also shown to either inhibit the growth (49 %) or overgrowth (30 %) of 79 % of uropathogenic organisms recovered from children with urinary tract infections [309]. Unlike VSL#3, Mutaflor® cannot be purchased in the U.S. due to its reclassification by the F.D.A. from a "medical food" (which is what VSL#3 is classified as) to a "biologic" [301, 310].

There are few reports on the use of probiotic bacteria in dogs and cats. Recent *in vitro* studies have confirmed the capacity of a lyophilized probiotic cocktail (e.g., three different *Lactobacillus* spp strains) to modulate the expression of regulatory versus pro-inflammatory cytokines in dogs with chronic enteropathies [311]. However, a clinical trial using this same probiotic cocktail fed to dogs with food-responsive diarrhea failed to induce consistent patterns of regulatory (e.g., beneficial) cytokine expression in spite of obvious clinical improvement [312]. One commercially-manufactured probiotic (FortiFlora™–*Enterococcus faecium* SF68, Nestle Purina) is reported to potentially control diarrhea and enhance immune responses in dogs and cats. Several recent trials attest to the short-term efficacy of probiotics in treating acute diarrhea in dogs and cats [313]. The link between the intestinal microbiota and gastrointestinal health in companion animals is now obvious. Future developments in the pharmabiotic field must include performance of randomized clinical trials to determine the role of probiotics and prebiotics in the management of canine chronic enteropathy. One large multicenter trial investigating the efficacy of VSL#3 in reducing inflammatory activity of canine IBD is presently underway

2.6.8. Prebiotics

Prebiotics are substances that can be used to promote specific changes in the microbiota. Administration has been shown to shift the microbiota in healthy adults; for example, individuals who consume either soluble corn fiber or polydextrose had increases in *F. prausnitzii* and those who consumed the soluble corn fiber had increases in *Roseburia* spp. [314]. As mentioned previously, both of these organisms metabolically produce butyrate

and *F. prausnitzii* also has other anti-inflammatory properties. In animal models, prebiotics have been shown to be effective as well. The severity of DSS-induced colitis in rats was attenuated by administering inulin orally as evidenced by a reduction in histopathological scores and myeloperioxidase accumulation in the colons [315]. In addition, the acidity of the colonic contents increased in rats fed inulin as well as an increase in *Lactobacillus* spp. in the feces. In a multicenter trial feeding UC patients germinated barley foodstuff (GBF), patients consuming GBF had improved clinical activity index scores [316]. GBF has also been shown to be effective in maintaining remission [317] and reducing pro-inflammatory cytokine levels in UC patients [318]. In CD patients, dietary supplementation with fructo-oligosaccharide (FOS) reduced their disease activity index, increased fecal *Bifidobacterium* spp. levels as well as increased production of IL-10 and expression of TLR-2 and TLR-4 from lamina propria dendritic cells [319].

Scientific studies have also investigated the effects of dietary supplementation with prebiotics on the intestinal microbiota of healthy dogs and cats. In one study, FOS supplemented at 0.75 % dry matter produced qualitative and quantitative changes in the fecal flora of healthy cats [320]. Compared with samples from cats fed a basal diet, increased numbers of lactobacilli and *Bacteroides* spp. and decreased numbers of *E. coli* were associated with cats fed the FOS supplemented diet. However, bacteriologic examination of the duodenal juice in these same cats showed wide variation in the composition of the duodenal microbiota, across sampling periods, which was not affected by FOS supplementation [321]. Moreover, healthy Beagle dogs fed a 1 % FOS diet over a three-month trial showed inconsistent fecal excretion of *Lactobacillus* spp. and *Bifidobacterium* spp. [322]. While FOS supplementation has been shown to have health benefits, these studies demonstrate that FOS does not have an adverse affect on the microbiota and suggest that it may have positive physiological benefits as seen in humans. This observation and the lack of significant side effects associated with FOS supplementation provide evidence that FOS should be considered as an attractive alternative or adjunct therapy for IBD in dogs and cats.

3. Mouse models of IBD

Many different mouse models have been utilized in IBD research to elucidate the roles that bacteria, genetics, the immune response, and environment play in the induction and maintenance of IBD [323]. They fall into two main categories: chemically-induced and genetically-engineered models. The composition of the microbiota in the various mouse models is also discussed. Regardless of the strain of mouse employed in chemically-induced or genetically-engineered models, there are only five options for its microbiota—conventional, specific pathogen free, restricted, gnotobiotic, or germ-free. Note that the word gnotobiotic (gnostos–"known" bios –"life") indicates that all the organisms present, regardless of the numbers of species, are known and does not apply only to mice that are completely devoid of microbes (i.e., germfree).

3.1. Chemically-induced models

3.1.1. DSS

Dextran sulfate sodium (DSS) is formed by the esterification of dextran with chlorosulphonic acid [324]. Administered *ad libtum* in the drinking water, this compound causes enterocolitis in mice with disease severity being dependent on mouse strain, DSS molecular mass, and sulfur content [325-328]. Within three to seven days after the addition of DSS (1 to 10 % w/v) to the drinking water, mice exhibit loose stools, weight loss, and occult blood. Upon necropsy of mice treated with DSS, cecal atrophy and colonic shortening are noted, and histopathological examination reveals mucosal ulceration, inflammatory infiltrate, and hyperplastic epithelium in the colonic mucosa. It has also been reported that glandular dropout occurs prior to signs of inflammation. Although the exact mechanism of action for DSS-induced colitis is currently not known, it is thought to manifest epithelial toxicity [329, 330]. Other reports indicate that DSS increases mucosal permeability within three days of administration (before the appearance of inflammatory infiltrate) [331]. Studies have also shown that DSS is taken up by macrophages in the colon and mesenteric lymph node and by Kupffer cells in the liver [326, 330]. When macrophages become laden with DSS, they have a reduced ability to perform normal homeostatic functions such as tissue repair and phagocytosis of bacteria [332]. DSS was demonstrated to be cytotoxic to Caco-2 cells, binding to their nucleus, causing cell cycle arrest and reduced production of reactive oxygen species [333].

Microbial populations of the GIT are altered after DSS administration, with the microbiota of the treated mice having increased numbers of *Bacteroidaceae* and *Clostridium* spp. [326]. This result indicates that the changes to the microbiota may play a contributory role (e.g., reduction of butyrate production) in the induction of DSS-induced disease. However, DSS induces more severe colitis and increased mortality in GF mice as compared to their conventional counterparts [334, 335]. GF mice given either 5% or 1% DSS died at days three or 14, respectively, after the start of DSS administration [335]. Collectively, these studies indicate that the composition of the microbiota may influence the sensitivity of mice to DSS-induced colitis as well as support the idea that the resident microbiota affords cytoprotective benefits for the host.

3.1.2. TNBS (2,4,6,-trinitrobenzne sulfonic acid)

TNBS is a haptenating agent that causes a disease similar to CD when mixed with ethanol and administered rectally as an enema. Mice treated with TNBS develop a pan-colitis with the peak of clinical signs, such as diarrhea and rectal prolapse, occurring two to four weeks post-administration [112]. Microscopically, transmural inflammation is noted along with neutrophil infiltration, loss of goblet cells, edema, and granulomas. T cells isolated from the lamina propria secrete elevated levels of IFN-γ and IL-2 following stimulation with anti-CD3 and anti-CD28. However, administration of anti-IL-12 antibodies after induction of TNBS-induced colitis reduced the disease severity, and treated mice also showed reduced production of IFN-γ [112]. Further studies have shown that CD4+ T cells recovered from mice with TNBS-induced colitis could induce mild colitis when adoptively transferred into naive control mice; the colitic lesions were characterized by

inflammatory cell infiltrate that produced IFN-γ [336]. In that same study, researchers found that feeding mice TNBS-haptenized colonic protein caused the mice to develop oral tolerance. These mice subsequently failed to develop colitis after TNBS administration or the transfer of CD4$^+$ T cells from mice with TNBS-induced colitis. T cells from these tolerant mice secreted elevated amounts of TGF-β, IL-4, and IL-10 [336].

3.1.3. Oxazolone

Oxazolone is a haptenating agent that causes a disease in mice similar to UC when mixed with ethanol and administered rectally [337, 338]. SJL/J mice rapidly develop diarrhea and weight loss that peaks at day two post-administration with a 50 % mortality rate by day four. At day two, the distal half of the colon becomes hemorrhagic and edematous and histologically shows signs of superficial inflammation. There is epithelial cell erosion, goblet cell depletion, edema, and inflammatory cell infiltrate composed of neutrophils and eosinophils. This is similar to what is observed microscopically in the colonic tissues of human UC patients. These mice also develop elevated levels of IL-4 and IL-5, but no IFN-γ, indicating that oxazolone induces a Th2 response. Elevated levels of TGF-β are also noted and may play in a role in the induction of disease in only part of the colon. The model has also been examined for its role in determining efficacy of IBD treatments. BALB/c mice given either 5-aminosalicylic acid (5-ASA) or sodium prednisolone phosphate intra-rectally prior to and during induction of oxazolone colitis had decreased severity of disease [337]. The disease is self-limiting, and the mice that survive beyond day four show increased weight gain and are healthy by days 10-12 post-administration.

3.2. Genetically engineered models

3.2.1. IL-10$^{-/-}$

In 1993, Kuhn et al. discovered that mice deficient in the anti-inflammatory cytokine IL-10 spontaneously develop enterocolitis [213]. This model has been popular in IBD research and this genetic deficiency has been crossed onto many different genetic backgrounds of mouse [339-345]. The availability of different strains has highlighted the role that genetics plays in enterocolitis, as the severity of disease is strain dependent. The order of severity from most severe to least severe is as follows: C3Bir > 129 > BALB/c or NOD/Lt > C57BL/6 or C57BL/10. In addition to strain differences, development of entercolitis is also dependent on the microbiota, as GF IL-10$^{-/-}$ do not develop enterocolitis and disease is attenuated after administration of antibiotics to IL-10$^{-/-}$ mice harboring a conventional microbiota [346, 347]. The lack of IL-10 does not affect the development of B or T cells, but its absence does result in a lack of regulatory T cells [213, 348]. Studies to understand the development of disease in these mice have shown that B cells (while present in high numbers in the lamina propria) are not needed for the initiation of disease, but that disease is mediated by CD4$^+$ T cells [342, 349]. Transfer studies using RAG2$^{-/-}$ mice as recipients have specifically shown that naive CD4$^+$ T cells are capable of inducing colitis, and CD45RB^{low} CD4$^+$ T cells from IL-10$^{-/-}$ mice can induce disease in these RAG2$^{-/-}$ mice

[348, 349]. This latter observation implicates IL-10 as a central mediator of regulatory T cells (CD25^{+} Foxp3^{+} CD4^{+} T cells). It has also been shown that IL-12 and IFN-γ are needed for initiation but not the continuation of colitis [342, 350]. The increase in the production of IL-12 and IFN-γ along with undetectable levels of IL-4 indicates that the enterocolitis in these mice is mediated by a Th1 immune response, similar to that observed in humans with IBD [342, 346].

3.2.2. Mdr1a^{-/-}

In 1994, mice lacking the gene *mdr1a* were generated. This gene encodes a P-glycoprotein, which is a drug efflux pump, thus protecting host cells from the build-up of toxic compounds. It was noted later that these mice spontaneously develop a colitis similar to human IBD and that disease can be exacerbated by colonization with *Helicobacter bilis* [351, 352]. These *mdr1a^{-/-}* mice have reduced growth rates compared to their WT counterparts and their histological lesions begin in the proximal colon and proceed distally as disease severity progresses. *Mdr1a^{-/-}* males are more susceptible to the onset of severe disease than females [353, 354]. *Mdr1a^{-/-}* mice also have increased epithelial permeability and reduced phosphorylation of both occludin and ZO-1, tight junction proteins, compared to WT counterparts [354]. They exhibit increased bacterial translocation with bacteria detected in both the spleen and lymph nodes that correlates with disease severity. Therefore, the induction of disease is associated with a defect in the epithelial barrier function of the GI tract. In addition to the similar disease progression found in IBD patients, studying these mice are of interest because the human MDR1 gene has been mapped on a loci that that is associated with susceptibility of IBD, although this relationship is under debate [355-358].

3.2.3. TRUC

TRUC mice are both T-bet^{-/-} and RAG^{-/-} and spontaneously develop colitis by four weeks of age [359, 360]. T-bet (T-box expressed in T cells) is a transcription factor that aids in the development of a Th1 response [359]. These mice have increased permeability of their colonic epithelium that increases with age and increased rate of epithelial cell death. Microscopically, there is inflammatory cell infiltrate, goblet cell dropout, crypt loss, and the presences of ulcers. The only cytokine elevated in these mice is TNF-α and disease can be ameliorated using an anti-TNF-α antibody. The microbiota also contributes to disease in this model, as treatment with antibiotics was able to "cure" the mice of their colitis. Additionally, the TRUC colitic microbiota can be horizontally transferred to both WT and RAG^{-/-} mice, and 16S rRNA analysis of feces from TRUC mice revealed that the presence of *Klebsiella pneumoniae* and *Proteus mirabilis* correlate with colitis. Interestingly, GF TRUC mice colonized with these two organisms alone do not develop colitis unless a more complete microbiota is present [361].

3.3. CD45RB^{hi} CD4^{+} T-cell transfer model

C.B.-17 *scid* mice administered CD45RB^{hi} CD4^{+} T cells develop a wasting disease which is not seen if CD45RB^{lo} CD4^{+} T cells or CD45RB^{hi} CD8^{+} T cells are adoptively transferred [362-364]. Disease occurs three to five weeks post-administration and is limited to the

large intestine. The mucosa, submucosa, and muscularis all presented with inflammatory cell infiltrates (macrophages and CD4$^+$ T cells predominately), and there was also a loss of goblet cells, epithelial cell hyperplasia, and deep fissure ulcers. Elevated levels of IFN-γ were present in these mice, and treatment with anti-IFN-γ or anti-TNF (α and β) anti-bodies ameliorated disease in these mice. However, the protection garnered by using the antibodies was not as great as when CD45RB^{lo} CD4$^+$ T cells were transferred along with CD45RB^{hi} CD4$^+$ T cells [362]. This disease type mimics CD with respect to the type of in-flammatory response generated (Th1) and the type of intestinal inflammation present (transmural infiltrate of CD4$^+$ T cells) [365]. There is also a definite microbial component in this model of disease, as restricted microbiota mice have less severe disease as com-pared to their SPF counterparts [364].

3.4. Bacterial-induced models

3.4.1. Helicobacter spp.

Although not considered a microbial cause of IBD, the presence of *Helicobacter* spp. does adjuvant or predispose mice to the onset of colitis in some models. In our lab, we utilize a dual hit model of colitis consisting of both *Helicobacter bilis* colonization and low-dose (1.5%) DSS to elicit colitis, as shown in Figure 8 [366].
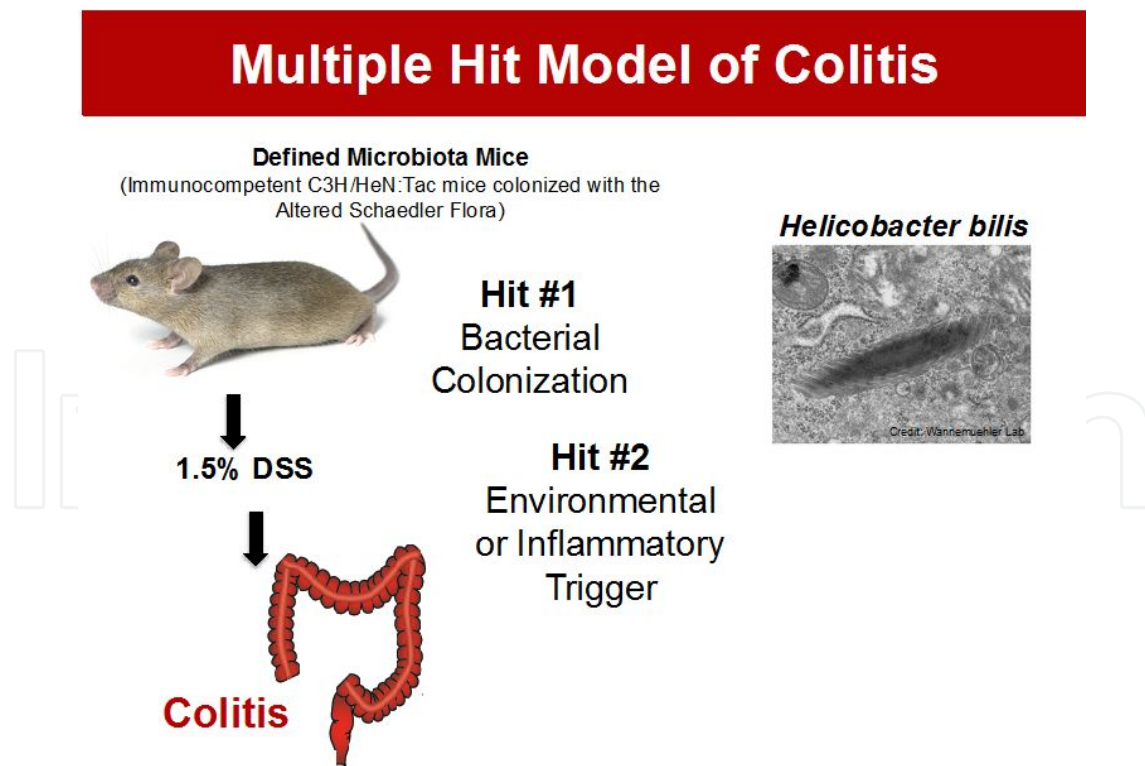

Figure 8. To increase sensitivity to a colitic insult, ASF-bearing C3H/HeN:Tac mice were colonized with a bacterial pro-vocateur, *Helicobater bilis*. An otherwise non-colitic low dose of DSS is then administered resulting in colitis.
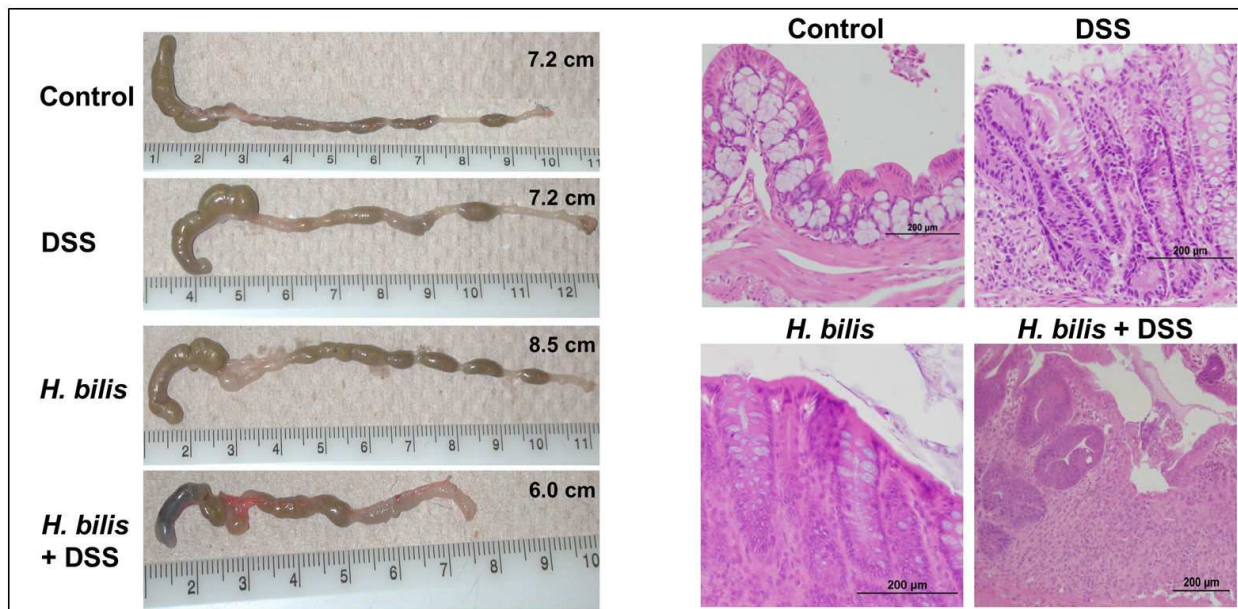
Figure 9. Mice colonized with *Helicobacter bilis* and subsequently administered a low dose of DSS exhibit colitis that is not seen with *H. bilis* or DSS alone. Disease is characterized grossly by colonic shortening, edema, cecal atrophy, enlarged lymphoid aggregates, and blood present in the contents. Microscopically, the disease presents with inflammatory cell infiltrate, crypt hyperplasia, glandular dropout, and cellular erosion

It has been noted that colonization of mice with *H. bilis* alone can change mucosal gene expression and alter the immune response in C3H/HeN mice bearing the altered Schaedler's flora (ASF) [366, 369]. Genes involved in T cell receptor signaling, the survival and activation of peripheral B cells, and chemotaxis are a few examples of the host genes that were upregulated by *H. bilis* colonization. Genes that were down regulated were involved in fatty acid metabolism and detoxification. After *H. bilis* colonization, serum antibodies directed at antigens derived from members of the ASF are induced [366, 369]. *H. hepaticus* colonization of A/JCr mice also induced changes in gene expression in the cecum, with female mice being more susceptible to the onset of disease [370, 371].

It appears that an over zealous host response to the introduction of the novel organism (i.e., provocateur) predisposes certain strains of mice for the onset of typholocoltiis following a secondary colitic insult. In a study comparing A/JCr mice (that develop mild inflammation) to C57BL/6 (who do not develop inflammation) mice, cecal gene expression profiles revealed that A/JCr mice had more genes differentially regulated (176) compared to C57BL/6 (80). Differentially expressed genes were predominantly those associated with immune response, chemotaxis, signal transduction, and antigen processing in the A/JCr mice while the genes upregulated in the C57BL/6 mice were predominately associated with immunoglobulin production.

In the *mdr1a*^{-/-} mouse, it is not simply the presence of *Helicobacter* that results in the induction of colitis but the specific species of *Helicobacter* influences the induction of a differential immune response. In *mdr1a*^{-/-} mice, *H. bilis* colonization causes colitis as early as three weeks post-infection, whereas *H. hepaticus* colonization ameliorated the severity of colitis in these

mice compared to the uninfected control *mdr1a*^{-/-} mice [352]. The disease phenotypes between spontaneous versus *Helicobacter*-induced colitis in *mdr1a*^{-/-} mice were different, with epithelial ulceration not present in the *Helicobacter*-induced colitis. When *mdr1a*^{-/-} mice were co-colonized with both *Helicobacter* species, the morbidity and mortality rate was between that of mice colonized by either *H. bilis* or *H. hepaticus* and the colitis that developed was characterized by dysplasia [372]. *H. bilis* was also able to out compete *H. hepaticus in vivo* as evidenced by recovery of higher numbers of *H. bilis*, suggesting that these two species may compete for similar niches in the GIT.

Additionally, colonization of IL-10^{-/-} C57BL/6 mice with *Helicobacter* species results in the induction of colitis in otherwise disease-free mice [373]. It was also shown that the onset and severity of colitis was species specific in relation to colonization by *H. bilis* or *H. hepaticus* [374]. With respect to C3Bir.129 IL-10^{-/-} mice, the presence of *Helicobacter* spp. is required for the spontaneous onset of disease [339]. Further studies show that colonization of RAG1^{-/-} mice with *Helicobacter* species fails to elicit clinical signs of disease after nine months of colonization and only very mild colonic inflammation was detected at necropsy [374]. Taken together, these observations indicate that disease induction can be mediated by an aberrant adaptive immune response initiated by bacterial provocateurs entering an otherwise stable host-microbe environment.

3.4.2. Brachyspira hyodysenteriae

Similar to the need for the a resident microbiota in the TRUC model of colitis, mice colonized with *Brachyspira hyodysenteriae* also require the presence of a microbiota for the induction of typhlocolitis. *B. hyodysenteriae* is an anaerobic spirochete that is the causative agent of swine dysentery [375]. While the pathogenesis of *B. hyodysenteriae* is associated with the production of a ß-hemolysin [376], disease does not develop in the absence of a resident microbiota as was demonstrated by the inoculation of germfree pigs [377-379]. The importance of the microbiota was further demonstrated when the microbiota of C3H/HeSnJ or BALB/c mice was depleted by adding a cocktail of antibiotics (rifampicin, colistin, spectinomycin, spiramycin, and vancomycin) to their drinking water. As can be seen in Figure 10, the antibiotic cocktail depleted the numbers of Gram-positive, Gram-negative, and strict anaerobes by as much as 5 to 7 $\log_{10}$ (Nibbelink and Wannemuehler, unpublished observations). On day seven, antibiotic treated and sham treated mice were inoculated with 1 x 10^{8} *B. hyodysenteirae* strain B204 and severity of disease was evaluated at 5, 10, and 15 days post-infection (DPI). As can be seen in Figure 11, the sham-treated mice developed severe typhlocolitis while the antibiotic treated mice had no lesions. At 15 DPI, the antibiotics were withdrawn from the drinking water of the remaining *B. hyodysenteriae*-infected mice to allow the microbiota to recover. On day 30 PI, the mice that had been treated with antibiotics through 15 DPI now presented with severe typhlocolitis. The presence and severity of typhlocolitis in these mice correlated with the presence of TNF-specific mRNA in the cecal tissue of the *B. hyodysenteriae*-infected mice (data not shown). Lastly, *B. hyodysenteriae*-induced typhlocolitis could also be prevented when the host's inflammatory response was inhibited [380, 381]. Collectively, these observations indicate that certain bacterial provocateurs (*K. pneumoniae, P. mirabilis,* and *B. hyodysenteriae)*

may fail to induce disease in the absence of an appropriate resident microbiota. As depicted in Figure 12, the etiology of colitis is complex and may require the presence of a microbial provocateur, the resident microbiota, and a host inflammatory response.
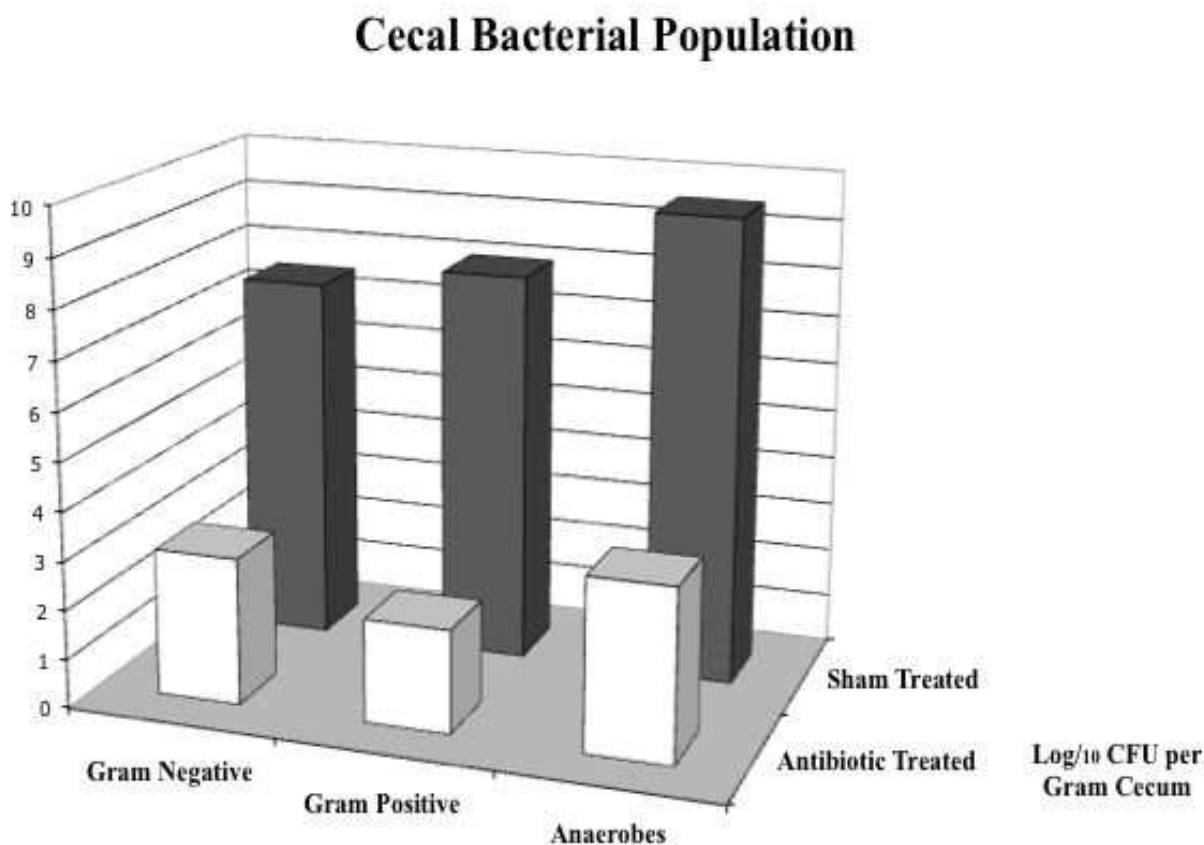

Figure 10. To assess the role of the microbiota in *Brachyspira hyodysenteriae*-induced typhlocolitis, C3H/HeSnJ mice were treated with an antibiotic cocktail to deplete the resident microbiota. Six days later, mice were infected with *B. hyodysenteriae*. The data indicate that there was a five to seven $\log_{10}$ reduction in the resident microbiota.

3.5. Conventional mice

The majority of commercially available mice harbor a "conventional" microbiota. This simply means that the composition of the community is unknown. There are different types of conventionally-reared mice. For example, Taconic Farms maintain two types of conventional microbiota mice, restricted flora™ (RF) and murine pathogen free™ (PF). RF mice are not colonized by β-hemolytic *Streptococcus* species, *K. pneumoniae, K. oxytoca, Pseudomonas aeruginosa*, or *Staphylococcus aureus*. Mice that are PF are *Helicobacter* free but can contain organisms not found in RF mice [382]. Additionally, mice on the same background purchased from different vendors can harbor different microbiota, as highlighted in work by Ivanov and colleagues demonstrating the presence of SFB in C57BL/6 mice from Taconic but not Jackson

Laboratories [54]. This lack of consistency in microbiota from mice of the same strain has even been identified at different facilities from the same vendor (Overstreet and Wannemuehler unpublished observation). Therefore, comparison of studies evaluating the microbiota of mice are difficult because there are hundreds of unknown bacterial species present in the murine microbiota and there is no standardized microbiota used by investigators. The advent of next-gen sequencing has helped to alleviate some of this challenge as all of the organisms in the gut can be identified based on 16S rRNA sequences. However, it still does not resolve the issue related to the use of disparate strains of mice with varying microbiota from different suppliers.
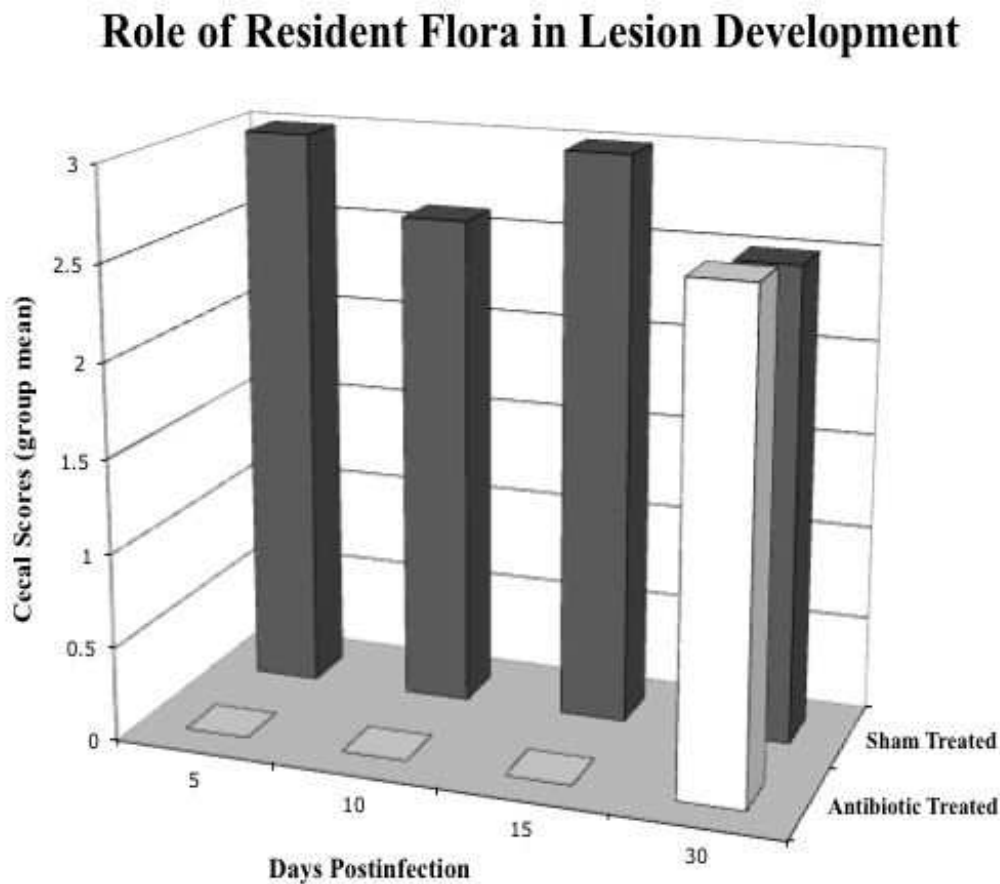

Figure 11. Assessment of *Brachyspira hyodysenteriae*-induced typhlocolitis in C3H/HeSnJ mice treated with an antibi-otic cocktail. While *B. hyodysenteriae* colonized the antibiotic-treated mice to the same extent as it did the sham-treat-ed mice, there was no evidence of typhlocolitis in the antibiotic treated mice through 15 days post-infection (DPI). However, 15 days after the antibiotics were withdrawn (30 DPI), the mice that had been treated with the antibiotics had developed severe disease once the microbiota recovered.
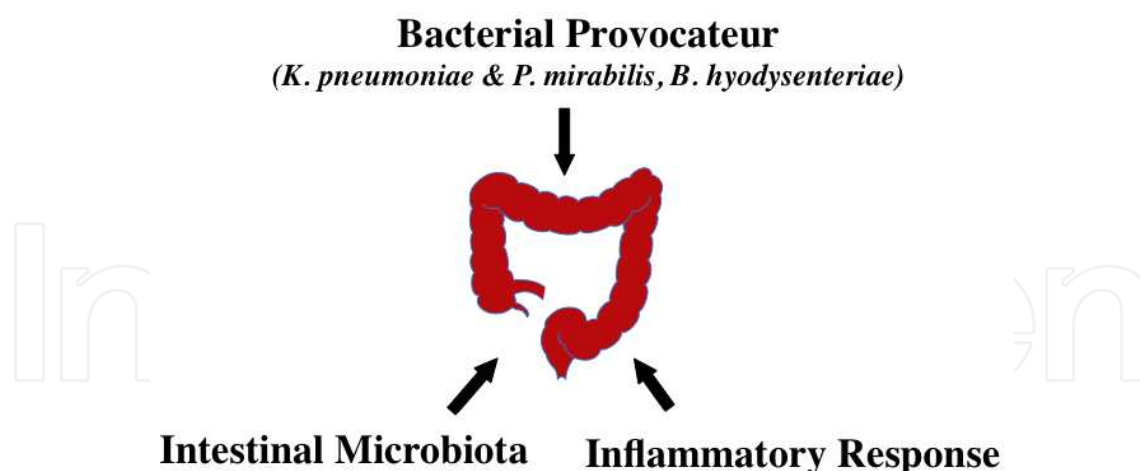
Figure 12. The pathogenesis of inflammatory bowel disease is complex. Studies from animal models indicate that the etiology of disease involves the presence of a bacterial provocateur, the resident microbiota, and the host response.

3.6. Germfree mice

GF mice are completely devoid of microbial life. As mentioned previously, this does grossly affect the anatomy of these mice, which is most evident by the enlarged cecum being the most prominent feature [34]. The discovery that many GF mouse strains that carry genetic deficiencies associated with IBD (notably the IL-10$^{-/-}$) do not develop colitis has led to the popularity of GF models to study the role of bacteria in the pathogenesis of IBD [346]. To determine if an organism is capable of initiating colitis, GF mice are monoassociated with a single bacterial species and then monitored for clinical signs of disease. Some of the bacterial strains used to date to evaluate their ability to induce disease in IL-10$^{-/-}$ mice are shown in Table 3.

Organism	Colitis Severity	Time of Disease Onset	Reference
Enterococcus faecalis	Severe	10-12 weeks p.i.	[383, 384]
Escherichia coli (from WT SPF mouse)	Moderate	3 weeks p.i.	[383]
Pseudomonas flourescens	No disease	-	[383]
Helicobacter hepaticus	No disease	-	[385]
Lactococcus lactis	No disease	-	[384]
Viridans group *Streptococcus*	No disease	-	[386]
Clostridium sordellii	No disease	-	[386]
Bacteroides vulgatus	No disease	-	[386]
Bifidobacterium animalis	Mild	23 weeks p.i.	[387]
Bifidobacterium infantis	No disease	-	[387]

Table 3. Bacterial strains used to monoassociate GF mice to examine the ability of the strain to induce colitis

Although the information gathered from these studies has been useful in analyzing the immune response to specific organisms, trying to relate the resultant disease to that characteristic of IBD is marginal at best because of the differences in the complexities of the microbiota. IBD itself is a multi-factorial disease and it has been fairly well-established that the role of bacteria in IBD is associated with a shift in community dynamics (i.e., dysbiosis) and not the presence/absence of one particular species. A perfect example of this complexity is the fact that *K. pneumonia* and *P. mirabilis* fail to induce colitis in GF TRUC mice (Figure 12) [361].

3.7. Defined microbiota mice

Also referred to as gnotobiotic, defined microbiota (DM) mice have a microbiota in which all members are known and are housed in flexible film isolators to maintain this status [388]. One of the most established DM mouse models harbors the "Altered Schaedler Flora" (ASF). Developed by Dr. Rodger Orcutt and colleagues as a request from the National Cancer Institute, these eight microbial species were originally used to standardized the microbiota of the rodents used as founders in their breeding colonies [389]. He chose to modify his mentor's (Dr. Russ Schaedler) cocktail of organisms by eliminating facultative anaerobes (*Escherichia coli* var. mutabilis and *Streptococcus fecalis*) and the anaerobic *Streptococcus* and *Clostridia* spp. that formed the original "Schaedler Flora". Dr. Orcutt added four additional species (see Table 4 below). This resulted in an anaerobic community devoid of any cocci or spore-forming blunt ended rods, which comprise the main isolator contaminants, making it easier to monitor for contamination of the gnotobiotic isolator. This new community was dubbed the "Altered Schaedler Flora" and was utilized in the breeding stock by major commercial mouse vendors in the US in that era. The ASF consists of members with different morphologies so that identification using fecal smears for microscopic evaluation was possible. The members, whose full genomes have yet to be sequenced, have had 16S rRNA sequencing performed to assist with the identification of the organisms [390].

Multiple studies have demonstrated the stability of this community both when maintained under gnotobiotic housing conditions or when part of a conventionalized microbiota [391-394]. In our own lab, all eight ASF members have been stably maintained in our breeding colony for over 12 years, indicating the remarkable stability of this model microbial community over time. PCR primers have been developed for each of the eight ASF members as well as group-specific FISH probes [391, 394]. Therefore, the effects of any perturbation of the ASF, such as with antibiotics, inflammation, or CAM treatments can be monitored by bacterial abundance as well as spatial redistribution using qPCR and FISH, respectively. All of the organisms can be cultured and whole cell sonicates produced to measure the immune response to each organism individually (something that is impossible to do with a conventional microbiota). Additionally, this community, although limited in scope, is able to synthesize all the metabolites needed by the mouse and maintains near normal cecal shape and size, something not possible in GF mice. It is important to note, however, that some of the characteristics of ASF mice are more similar to GF than conventional mice. Both ASF and GF mice have high fecal tryptic activity and possess the ability to degrade mucin and β-aspartylglycine. They also

cannot convert bilirubin to urobilinogens or cholesterol to coprostanol [395]. Interestingly, this same research team compared these parameters in CD patients versus healthy subjects and the characteristics of the microbial metabolism associated with the microbiota of CD patients were very similar to those of the ASF [396]. This study suggests that there is benefit to the use of the ASF in mouse models of IBD.

Other defined microbiota mouse studies have been published [397-400]. A study using ten bacterial species specifically chosen for their metabolic function were used to colonize GF mice [397]. Using microbial RNA-seq, the authors were able to build a model relating perturbation of the microbial community to changes in diet. By modeling the functional capacity of a gnotobiotic community under different conditions, this model and similar approaches can be used to unravel the operational dynamics of the gut microbiome with respect to nutrient utilization such that the microbiota might be manipulated to improve human and animal health [397]. Another study colonized mice with *E. rectale* and *B. thetaiotamicron*, and the authors then assessed changes in bacterial gene expression using Affymetrix GeneChips to show that the cross-talk between these two organisms affected up- and down-regulated genes in response to one another.

Strain	Identity
ASF356	Most closely related to *Clostridium propionicum* (92% max identity) [390] Member of *Clostridium* cluster XIV
ASF360	*Lactobacillus intestinalis* (99% max identity) [401]
ASF361	*Lactobacillus murinus [390]*
ASF457	*Mucispirillum schaedleri [402]*
ASF492	*Eubacterium plexicaudatum* [403] Member of *Clostridium* cluster XIV
ASF500	*Clostridium* sp. with no known related organism in GenBank Database [390]
ASF502	Most closely related to *Ruminococcus gnavus* (92% max identity) [390] Member of *Clostridium* cluster XIV
ASF519	*Parabacteroides goldsteinii* (99% max identity) [404, 405]

Table 4. Members of the altered Schaedler flora

Three human commensals, *E. coli*, *B. longum*, and *L. johnsonii*, have also been used to colonize GF mice to create a gnotobiotic community [398]. This community was used to identify the effects that the introduction of novel organisms has on the community dynamic. When colonized with a second *Lactobacillus*, *L. paracasei*, it was noted that both *Lactobacillus* spp. were able to co-habitate reaching similar fecal titers. However, that was not the same when a fifth organism was added, a second *B. longum* strain. This new strain was only maintained in the mouse at detectable levels for three days. Lastly, a second *E. coli* strain was added to the original community of three. This new *E. coli* reached the highest titer of any organism in the com-

munity. This addition caused the reduction of the original *E. coli* to drop to undetectable levels at day two post-addition. Later *L. johnsonii* also reached undetectable levels. *B. longum* decreased initially but them returned to normal titer levels. Lastly, when the original tri-associated mice were exposed to conventional mouse feces, the numbers of the original three organisms decreased.

In this chapter, we have discussed the three most common types of microbiota available for use in IBD research. Because it is clear that IBD is associated with an imbalance in the microbial community, the use of GF mice (which cannot mimic a "community" dynamic) may be less useful in unraveling the complexities of a multifactorial disease in place of defined microbiota and conventional microbiota mice. Ideally, one would want to use a simplified community (such as the ASF) where the actions of all organisms can be assessed. To understand the dynamic interactions that occur between microbes and between the microbes and the host, it will be important to start with what is known in order to begin the unraveling the enigmatic nature of gut health and disease.

Author details

Anne-Marie C. Overstreet[1], Amanda E. Ramer-Tait[3], Albert E. Jergens[2] and Michael J. Wannemuehler[1]

1 Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA

2 Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA

3 Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA

References

[1] Whitman, W.B., Coleman, D.C., and Wiebe, W.J., Prokaryotes: the unseen majority. Proceedings of the National Academy of Sciences of the United States of America, 1998. 95(12): p. 6578-83.

[2] Savage, D.C., Microbial ecology of the gastrointestinal tract. Annual Review of Microbiology, 1977. 31: p. 107-33.

[3] Zhu, B., Wang, X., and Li, L., Human gut microbiome: the second genome of human body. Protein & Cell, 2010. 1(8): p. 718-25.

[4] Structure, function and diversity of the healthy human microbiome. Nature, 2012. 486(7402): p. 207-14.

[5]   Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., et al., The human microbiome project. Nature, 2007. 449(7164): p. 804-10.

[6]   Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., et al., The NIH Human Microbiome Project. Genome research, 2009. 19(12): p. 2317-23.

[7]   Moore, S.J. and Warren, M.J., The anaerobic biosynthesis of vitamin B12. Biochemical Society Transactions, 2012. 40(3): p. 581-6.

[8]   Rossi, M., Amaretti, A., and Raimondi, S., Folate production by probiotic bacteria. Nutrients, 2011. 3(1): p. 118-34.

[9]   LeBlanc, J.G., Laino, J.E., del Valle, M.J., Vannini, V., van Sinderen, D., et al., B-group vitamin production by lactic acid bacteria--current knowledge and potential applications. Journal of Applied Microbiology, 2011. 111(6): p. 1297-309.

[10] Morishita, T., Tamura, N., Makino, T., and Kudo, S., Production of menaquinones by lactic acid bacteria. Journal of Dairy Science, 1999. 82(9): p. 1897-903.

[11] Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., et al., An obesity-associated gut microbiome with increased capacity for energy harvest. Nature, 2006. 444(7122): p. 1027-31.

[12] Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., et al., Host-gut microbiota metabolic interactions. Science, 2012. 336(6086): p. 1262-7.

[13] Bene, L., Falus, A., Baffy, N., and Fulop, A.K., Cellular and molecular mechanisms in the two major forms of inflammatory bowel disease. Pathology Oncology Research, 2011. 17(3): p. 463-72.

[14] Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., et al., Diversity of the human intestinal microbial flora. Science, 2005. 308(5728): p. 1635-8.

[15] Hold, G.L., Pryde, S.E., Russell, V.J., Furrie, E., and Flint, H.J., Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiology Ecology, 2002. 39(1): p. 33-9.

[16] O'Hara, A.M. and Shanahan, F., The gut flora as a forgotten organ. EMBO Reports, 2006. 7(7): p. 688-93.

[17] Suau, A., Bonnet, R., Sutren, M., Godon, J.J., Gibson, G.R., et al., Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Applied and Environmental Microbiology, 1999. 65(11): p. 4799-807.

[18] Frank, D.N., St Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N., et al., Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proceedings of the National Academy of Sciences of the United States of America, 2007. 104(34): p. 13780-5.

[19] Miller, T.L. and Wolin, M.J., Enumeration of *Methanobrevibacter smithii* in human feces. Archives of Microbiology, 1982. 131(1): p. 14-8.

[20] Schaedler, R.W., Dubos, R., and Costello, R., The Development of the Bacterial Flora in the Gastrointestinal Tract of Mice. The Journal of Experimental Medicine, 1965. 122: p. 59-66.

[21] Savage, D.C., Factors involved in colonization of the gut epithelial surface. The American Journal of Clinical Nutrition, 1978. 31(10 Suppl): p. S131-S135.

[22] Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., et al., Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America, 2010. 107(26): p. 11971-5.

[23] Gronlund, M.-M., Lehtonen, O.-P., Eerola, E., and Kero, P., Fecal Microflora in Healthy Infants Born by Different Methods of Delivery: Permanent Changes in Intestinal Flora After Cesarean Delivery. Journal of Pediatric Gastroenterology and Nutrition, 1999. 28(1): p. 19-25.

[24] Dominguez-Bello, M.G., Blaser, M.J., Ley, R.E., and Knight, R., Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. Gastroenterology, 2011. 140(6): p. 1713-9.

[25] Lindsay, J.A., *Clostridium perfringens* type A enterotoxin (CPE): more than just explosive diarrhea. Critical Reviews in Microbiology, 1996. 22(4): p. 257-77.

[26] Watson, J., Jones, R., and Cortes, C., Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection among Healthy newborns-Chicago and Los Angeles County, 2004. Journal of the American Medical Association, 2006. 296(1): p. 36-38.

[27] van Nimwegen, F.A., Penders, J., Stobberingh, E.E., Postma, D.S., Koppelman, G.H., et al., Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. The Journal of Allergy and Clinical Immunology, 2011. 128(5): p. 948-55 e1-3.

[28] Magnus, M.C., Haberg, S.E., Stigum, H., Nafstad, P., London, S.J., et al., Delivery by Cesarean section and early childhood respiratory symptoms and disorders: the Norwegian mother and child cohort study. American Journal of Epidemiology, 2011. 174(11): p. 1275-85.

[29] Bezirtzoglou, E., Tsiotsias, A., and Welling, G.W., Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe, 2011. 17(6): p. 478-82.

[30] Fallani, M., Amarri, S., Uusijarvi, A., Adam, R., Khanna, S., et al., Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. Microbiology, 2011. 157(Pt 5): p. 1385-92.

[31] Thompson, G.R. and Trexler, P.C., Gastrointestinal structure and function in germ-free or gnotobiotic animals. Gut, 1971. 12(3): p. 230-5.

[32] Lesher, S., Walburg, H.E., Jr., and Sacher, G.A., Jr., Generation Cycle in the Duodenal Crypt Cells of Germ-Free and Conventional Mice. Nature, 1964. 202: p. 884-6.

[33] Savage, D.C., Siegel, J.E., Snellen, J.E., and Whitt, D.D., Transit time of epithelial cells in the small intestines of germfree mice and ex-germfree mice associated with indigenous microorganisms. Applied and Environmental Microbiology, 1981. 42(6): p. 996-1001.

[34] Schaedler, R.W., Dubs, R., and Costello, R., Association of Germfree Mice with Bacteria Isolated from Normal Mice. The Journal of Experimental Medicine, 1965. 122: p. 77-82.

[35] Wostmann, B.S. and Bruckner-Kardoss, E., Oxidation-reduction potentials in cecal contents of germfree and conventional rats. Proceedings of the Society for Experimental Biology and Medicine, 1966. 121(4): p. 1111-4.

[36] Gustafsson, B.E., Daft, F.S., Mc, D.E., Smith, J.C., and Fitzgerald, R.J., Effects of vitamin K-active compounds and intestinal microorganisms in vitamin K-deficient germfree rats. The Journal of Nutrition, 1962. 78: p. 461-8.

[37] Abrams, G.D. and Bishop, J.E., Effect of the normal microbial flora on the resistance of the small intestine to infection. Journal of Bacteriology, 1966. 92(6): p. 1604-8.

[38] Coates, M.E., Gnotobiotic animals in nutrition research. The Proceedings of the Nutrition Society, 1973. 32(2): p. 53-8.

[39] Bauer, H., Horowitz, R.E., Levenson, S.M., and Popper, H., The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. The American Journal of Pathology, 1963. 42: p. 471-83.

[40] Horowitz, R.E., Bauer, H., Paronetto, F., Abrams, G.D., Watkins, K.C., et al., The Response of the Lymphatic Tissue to Bacterial Antigen. Studies in Germfree Mice. The American Journal of Pathology, 1964. 44: p. 747-61.

[41] Bauer, H., Paronetto, F., Burns, W.A., and Einheber, A., The enhancing effect of the microbial flora on macrophage function and the immune response. A study in germfree mice. The Journal of Experimental Medicine, 1966. 123(6): p. 1013-24.

[42] Johansson, M.E., Larsson, J.M., and Hansson, G.C., The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proceedings of the National Academy of Sciences of the United States of America, 2011. 108 Suppl 1: p. 4659-65.

[43] Atuma, C., Strugala, V., Allen, A., and Holm, L., The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. American Journal of Physiology, 2001. 280(5): p. G922-9.

[44] Hansson, G.C. and Johansson, M.E., The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Gut Microbes, 2010. 1(1): p. 51-54.

[45] Van der Sluis, M., De Koning, B.A., De Bruijn, A.C., Velcich, A., Meijerink, J.P., et al., Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology, 2006. 131(1): p. 117-29.

[46] Edelblum, K.L. and Turner, J.R., The tight junction in inflammatory disease: communication breakdown. Current Opinion in Pharmacology, 2009. 9(6): p. 715-20.

[47] Garcia-Lafuente, A., Antolin, M., Guarner, F., Crespo, E., Salas, A., et al., Incrimination of anaerobic bacteria in the induction of experimental colitis. The American Journal of Physiology, 1997. 272(1 Pt 1): p. G10-5.

[48] Wlodarska, M., Willing, B., Keeney, K.M., Menendez, A., Bergstrom, K.S., et al., Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated *Citrobacter rodentium*-induced colitis. Infection and Immunity, 2011. 79(4): p. 1536-45.

[49] Sturm, A. and Dignass, A.U., Epithelial restitution and wound healing in inflammatory bowel disease. World Journal of Gastroenterology 2008. 14(3): p. 348-53.

[50] Kim, Y.S. and Ho, S.B., Intestinal goblet cells and mucins in health and disease: recent insights and progress. Current Gastroenterology Reports, 2010. 12(5): p. 319-30.

[51] Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., et al., The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science, 2011. 332(6032): p. 974-7.

[52] Round, J.L. and Mazmanian, S.K., Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America, 2010. 107(27): p. 12204-9.

[53] Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., et al., Induction of colonic regulatory T cells by indigenous *Clostridium* species. Science, 2011. 331(6015): p. 337-41.

[54] Ivanov, II, Atarashi, K., Manel, N., Brodie, E.L., Shima, T., et al., Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell, 2009. 139(3): p. 485-98.

[55] Backhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., et al., The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America, 2004. 101(44): p. 15718-23.

[56] Oresic, M., Seppanen-Laakso, T., Yetukuri, L., Backhed, F., and Hanninen, V., Gut microbiota affects lens and retinal lipid composition. Experimental Eye Research, 2009. 89(5): p. 604-7.

[57] Rubinstein, R., Howard, A.V., and Wrong, O.M., *In vivo* dialysis of faeces as a method of stool analysis. IV. The organic anion component. Clinical Science, 1969. 37(2): p. 549-64.

[58] Barcenilla, A., Pryde, S.E., Martin, J.C., Duncan, S.H., Stewart, C.S., et al., Phylogenetic relationships of butyrate-producing bacteria from the human gut. Applied and Environmental Microbiology, 2000. 66(4): p. 1654-61.

[59] Hold, G.L., Schwiertz, A., Aminov, R.I., Blaut, M., and Flint, H.J., Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. Applied and Environmental Microbiology, 2003. 69(7): p. 4320-4.

[60] Suau, A., Rochet, V., Sghir, A., Gramet, G., Brewaeys, S., et al., *Fusobacterium prausnitzii* and related species represent a dominant group within the human fecal flora. Systematic and Applied Microbiology, 2001. 24(1): p. 139-45.

[61] Louis, P. and Flint, H.J., Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiology Letters, 2009. 294(1): p. 1-8.

[62] Twarog, R. and Wolfe, R.S., Role of butyryl phosphate in the energy metabolism of *Clostridium Tetanomorphum*. Journal of Bacteriology, 1963. 86: p. 112-7.

[63] Twarog, R. and Wolfe, R.S., Enzymatic phosphorylation of butyrate. The Journal of Biological Chemistry, 1962. 237: p. 2474-7.

[64] Louis, P., Duncan, S.H., McCrae, S.I., Millar, J., Jackson, M.S., et al., Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. Journal of Bacteriology, 2004. 186(7): p. 2099-106.

[65] Duncan, S.H., Barcenilla, A., Stewart, C.S., Pryde, S.E., and Flint, H.J., Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. Applied and Environmental Microbiology, 2002. 68(10): p. 5186-90.

[66] Roediger, W.E., Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut, 1980. 21(9): p. 793-8.

[67] Roediger, W.E., Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology, 1982. 83(2): p. 424-9.

[68] Fitch, M.D. and Fleming, S.E., Metabolism of short-chain fatty acids by rat colonic mucosa *in vivo*. The American Journal of Physiology, 1999. 277(1 Pt 1): p. G31-40.

[69] Clausen, M.R. and Mortensen, P.B., Kinetic studies on colonocyte metabolism of short chain fatty acids and glucose in ulcerative colitis. Gut, 1995. 37(5): p. 684-9.

[70] Donohoe, D.R., Garge, N., Zhang, X., Sun, W., O'Connell, T.M., et al., The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metabolism, 2011. 13(5): p. 517-26.

[71] Mihaylova, M.M. and Shaw, R.J., The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nature Cell biology, 2011. 13(9): p. 1016-23.

[72] Sealy, L. and Chalkley, R., The effect of sodium butyrate on histone modification. Cell, 1978. 14(1): p. 115-21.

[73] Riggs, M.G., Whittaker, R.G., Neumann, J.R., and Ingram, V.M., n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. Nature, 1977. 268(5619): p. 462-4.

[74] Kouraklis, G. and Theocharis, S., Histone deacetylase inhibitors: a novel target of anticancer therapy (review). Oncology Reports, 2006. 15(2): p. 489-94.

[75] Della Ragione, F., Criniti, V., Della Pietra, V., Borriello, A., Oliva, A., et al., Genes modulated by histone acetylation as new effectors of butyrate activity. FEBS Letters, 2001. 499(3): p. 199-204.

[76] Siavoshian, S., Segain, J.P., Kornprobst, M., Bonnet, C., Cherbut, C., et al., Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. Gut, 2000. 46(4): p. 507-14.

[77] Segain, J.P., Raingeard de la Bletiere, D., Bourreille, A., Leray, V., Gervois, N., et al., Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut, 2000. 47(3): p. 397-403.

[78] Venkatraman, A., Ramakrishna, B.S., Shaji, R.V., Kumar, N.S., Pulimood, A., et al., Amelioration of dextran sulfate colitis by butyrate: role of heat shock protein 70 and NF-kappaB. American Journal of Physiology. Gastrointestinal and Liver Physiology, 2003. 285(1): p. G177-84.

[79] Luhrs, H., Gerke, T., Muller, J.G., Melcher, R., Schauber, J., et al., Butyrate inhibits NF-kappaB activation in lamina propria macrophages of patients with ulcerative colitis. Scandinavian Journal of Gastroenterology, 2002. 37(4): p. 458-66.

[80] Tong, X., Yin, L., and Giardina, C., Butyrate suppresses Cox-2 activation in colon cancer cells through HDAC inhibition. Biochemical and Biophysical Research Communications, 2004. 317(2): p. 463-71.

[81] Tehrani, A.B., Nezami, B.G., Gewirtz, A., and Srinivasan, S., Obesity and its associated disease: a role for microbiota? Neurogastroenterology and Motility 2012. 24(4): p. 305-11.

[82] Bradley, W.D., Zwingelstein, C., and Rondinone, C.M., The emerging role of the intestine in metabolic diseases. Archives of Physiology and Biochemistry, 2011. 117(3): p. 165-76.

[83] Dorer, M.S., Talarico, S., and Salama, N.R., *Helicobacter pylori's* unconventional role in health and disease. PLoS Pathogens, 2009. 5(10): p. e1000544.

[84] Hammer, H.F., Gut microbiota and inflammatory bowel disease. Digestive Diseases, 2011. 29(6): p. 550-3.

[85] Kaur, N., Chen, C.C., Luther, J., and Kao, J.Y., Intestinal dysbiosis in inflammatory bowel disease. Gut Microbes, 2011. 2(4): p. 211-6.

[86] Vanderploeg, R., Panaccione, R., Ghosh, S., and Rioux, K., Influences of intestinal bacteria in human inflammatory bowel disease. Infectious Disease Clinics of North America, 2010. 24(4): p. 977-93, ix.

[87] Nell, S., Suerbaum, S., and Josenhans, C., The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nature Reviews: Microbiology, 2010. 8(8): p. 564-77.

[88] Meadows, R., Gut bacteria may override genetic protections against diabetes. PLoS Biology, 2011. 9(12): p. e1001215.

[89] Harris, K., Kassis, A., Major, G., and Chou, C.J., Is the gut microbiota a new factor contributing to obesity and its metabolic disorders? Journal of Obesity, 2012. 2012: p. 879151.

[90] Crohn, B.B., Ginzburg, L., and Oppenheimer, G.D., Landmark article Oct 15, 1932. Regional ileitis. A pathological and clinical entity. By Burril B. Crohn, Leon Ginzburg, and Gordon D. Oppenheimer. The Journal of the American Medical Association, 1984. 251(1): p. 73-9.

[91] Molodecky, N.A., Soon, I.S., Rabi, D.M., Ghali, W.A., Ferris, M., et al., Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology, 2012. 142(1): p. 46-54 e42; quiz e30.

[92] Lees, C.W., Barrett, J.C., Parkes, M., and Satsangi, J., New IBD genetics: common pathways with other diseases. Gut, 2011. 60(12): p. 1739-53.

[93] Franke, A., McGovern, D.P., Barrett, J.C., Wang, K., Radford-Smith, G.L., et al., Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nature Genetics, 2010. 42(12): p. 1118-25.

[94] Anderson, C.A., Boucher, G., Lees, C.W., Franke, A., D'Amato, M., et al., Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nature Genetics, 2011. 43(3): p. 246-52.

[95] Cadwell, K., Liu, J.Y., Brown, S.L., Miyoshi, H., Loh, J., et al., A key role for autophagy and the autophagy gene *Atg16l1* in mouse and human intestinal Paneth cells. Nature, 2008. 456(7219): p. 259-63.

[96] Biswas, A., Petnicki-Ocwieja, T., and Kobayashi, K.S., Nod2: a key regulator linking microbiota to intestinal mucosal immunity. Journal of Molecular Medicine, 2012. 90(1): p. 15-24.

[97] Ogura, Y., Inohara, N., Benito, A., Chen, F.F., Yamaoka, S., et al., Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. The Journal of Biological Chemistry, 2001. 276(7): p. 4812-8.

[98] Tada, H., Aiba, S., Shibata, K., Ohteki, T., and Takada, H., Synergistic effect of Nod1 and Nod2 agonists with toll-like receptor agonists on human dendritic cells to generate interleukin-12 and T helper type 1 cells. Infection and Immunity, 2005. 73(12): p. 7967-76.

[99] Hisamatsu, T., Suzuki, M., Reinecker, H.C., Nadeau, W.J., McCormick, B.A., et al., CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology, 2003. 124(4): p. 993-1000.

[100] Uehara, A., Fujimoto, Y., Fukase, K., and Takada, H., Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. Molecular Immunology, 2007. 44(12): p. 3100-11.

[101] Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P.C., et al., An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. The Journal of Biological Chemistry, 2000. 275(36): p. 27823-31.

[102] Petnicki-Ocwieja, T., Hrncir, T., Liu, Y.J., Biswas, A., Hudcovic, T., et al., Nod2 is required for the regulation of commensal microbiota in the intestine. Proceedings of the National Academy of Sciences of the United States of America, 2009. 106(37): p. 15813-8.

[103] Hampe, J., Franke, A., Rosenstiel, P., Till, A., Teuber, M., et al., A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nature Genetics, 2007. 39(2): p. 207-11.

[104] Deretic, V., Autophagy in innate and adaptive immunity. Trends in Immunology, 2005. 26(10): p. 523-8.

[105] Rioux, J.D., Xavier, R.J., Taylor, K.D., Silverberg, M.S., Goyette, P., et al., Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nature Genetics, 2007. 39(5): p. 596-604.

[106] Lapaquette, P., Bringer, M.A., and Darfeuille-Michaud, A., Defects in autophagy favour adherent-invasive *Escherichia coli* persistence within macrophages leading to increased pro-inflammatory response. Cellular Microbiology, 2012. 14(6): p. 791-807.

[107] Kuballa, P., Huett, A., Rioux, J.D., Daly, M.J., and Xavier, R.J., Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. PloS One, 2008. 3(10): p. e3391.

[108] Sartor, R.B., Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nature Clinical Practice. Gastroenterology & Hepatology, 2006. 3(7): p. 390-407.

[109] Fuss, I.J., Neurath, M., Boirivant, M., Klein, J.S., de la Motte, C., et al., Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. Journal of Immunology, 1996. 157(3): p. 1261-70.

[110] Monteleone, G., Biancone, L., Marasco, R., Morrone, G., Marasco, O., et al., Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. Gastroenterology, 1997. 112(4): p. 1169-78.

[111] Okazawa, A., Kanai, T., Watanabe, M., Yamazaki, M., Inoue, N., et al., Th1-mediated intestinal inflammation in Crohn's disease may be induced by activation of lamina propria lymphocytes through synergistic stimulation of interleukin-12 and interleukin-18 without T cell receptor engagement. The American Journal of Gastroenterology, 2002. 97(12): p. 3108-17.

[112] Neurath, M.F., Fuss, I., Kelsall, B.L., Stuber, E., and Strober, W., Antibodies to interleukin 12 abrogate established experimental colitis in mice. The Journal of Experimental Medicine, 1995. 182(5): p. 1281-90.

[113] Fuss, I.J., Heller, F., Boirivant, M., Leon, F., Yoshida, M., et al., Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. The Journal of Clinical Investigation, 2004. 113(10): p. 1490-7.

[114] Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., et al., Increased expression of interleukin 17 in inflammatory bowel disease. Gut, 2003. 52(1): p. 65-70.

[115] Kobayashi, T., Okamoto, S., Hisamatsu, T., Kamada, N., Chinen, H., et al., IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. Gut, 2008. 57(12): p. 1682-9.

[116] Olsen, T., Rismo, R., Cui, G., Goll, R., Christiansen, I., et al., TH1 and TH17 interactions in untreated inflamed mucosa of inflammatory bowel disease, and their potential to mediate the inflammation. Cytokine, 2011. 56(3): p. 633-40.

[117] Nielsen, O.H., Kirman, I., Rudiger, N., Hendel, J., and Vainer, B., Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. Scandinavian Journal of Gastroenterology, 2003. 38(2): p. 180-5.

[118] Cho, J.H. and Brant, S.R., Recent insights into the genetics of inflammatory bowel disease. Gastroenterology, 2011. 140(6): p. 1704-12.

[119] Newman, W.G., Zhang, Q., Liu, X., Amos, C.I., and Siminovitch, K.A., Genetic variants in *IL-23R* and *ATG16L1* independently predispose to increased susceptibility to Crohn's disease in a Canadian population. Journal of Clinical Gastroenterology, 2009. 43(5): p. 444-7.

[120] McGeachy, M.J. and Cua, D.J., Th17 cell differentiation: the long and winding road. Immunity, 2008. 28(4): p. 445-53.

[121] Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., et al., Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity, 2000. 13(5): p. 715-25.

[122] Parham, C., Chirica, M., Timans, J., Vaisberg, E., Travis, M., et al., A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. Journal of Immunology, 2002. 168(11): p. 5699-708.

[123] Elson, C.O., Cong, Y., Weaver, C.T., Schoeb, T.R., McClanahan, T.K., et al., Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. Gastroenterology, 2007. 132(7): p. 2359-70.

[124] Ahern, P.P., Schiering, C., Buonocore, S., McGeachy, M.J., Cua, D.J., et al., Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity, 2010. 33(2): p. 279-88.

[125] Liu, Z., Feng, B.S., Yang, S.B., Chen, X., Su, J., et al., Interleukin (IL)-23 suppresses IL-10 in inflammatory bowel disease. The Journal of Biological Chemistry, 2012. 287(5): p. 3591-7.

[126] Macpherson, A., Khoo, U.Y., Forgacs, I., Philpott-Howard, J., and Bjarnason, I., Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut, 1996. 38(3): p. 365-75.

[127] Scott, M.G., Nahm, M.H., Macke, K., Nash, G.S., Bertovich, M.J., et al., Spontaneous secretion of IgG subclasses by intestinal mononuclear cells: differences between ulcerative colitis, Crohn's disease, and controls. Clinical and Experimental Immunology, 1986. 66(1): p. 209-15.

[128] MacDermott, R.P., Nash, G.S., and Nahm, M.H., Antibody secretion by human intestinal mononuclear cells from normal controls and inflammatory bowel disease patients. Immunological Investigations, 1989. 18(1-4): p. 449-57.

[129] MacDermott, R.P., Nash, G.S., Auer, I.O., Shlien, R., Lewis, B.S., et al., Alterations in serum immunoglobulin G subclasses in patients with ulcerative colitis and Crohn's disease. Gastroenterology, 1989. 96(3): p. 764-8.

[130] Philipsen, E.K., Bondesen, S., Andersen, J., and Larsen, S., Serum immunoglobulin G subclasses in patients with ulcerative colitis and Crohn's disease of different disease activities. Scandinavian Journal of Gastroenterology, 1995. 30(1): p. 50-3.

[131] Vecchi, M., Spina, L., Cavallaro, F., and Pastorelli, L., Do Antibodies Have a Role in IBD Pathogenesis? Inflammatory Bowel Diseases, 2008. 14(S2): p. S95-S96.

[132] Orholm, M., Binder, V., Sorensen, T.I., Rasmussen, L.P., and Kyvik, K.O., Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. Scandinavian Journal of Gastroenterology, 2000. 35(10): p. 1075-81.

[133] Halfvarson, J., Bodin, L., Tysk, C., Lindberg, E., and Jarnerot, G., Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology, 2003. 124(7): p. 1767-73.

[134] Ng, S.C., Woodrow, S., Patel, N., Subhani, J., and Harbord, M., Role of genetic and environmental factors in British twins with inflammatory bowel disease. Inflammatory Bowel Diseases, 2012. 18(4): p. 725-36.

[135] Lindberg, E., Tysk, C., Andersson, K., and Jarnerot, G., Smoking and inflammatory bowel disease. A case control study. Gut, 1988. 29(3): p. 352-7.

[136] Halfvarson, J., Jess, T., Magnuson, A., Montgomery, S.M., Orholm, M., et al., Environmental factors in inflammatory bowel disease: a co-twin control study of a Swedish-Danish twin population. Inflammatory Bowel Diseases, 2006. 12(10): p. 925-33.

[137] Mahid, S.S., Minor, K.S., Soto, R.E., Hornung, C.A., and Galandiuk, S., Smoking and inflammatory bowel disease: a meta-analysis. Mayo Clinic Proceedings, 2006. 81(11): p. 1462-71.

[138] Radford-Smith, G.L., Edwards, J.E., Purdie, D.M., Pandeya, N., Watson, M., et al., Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. Gut, 2002. 51(6): p. 808-13.

[139] Cosnes, J., Carbonnel, F., Beaugerie, L., Blain, A., Reijasse, D., et al., Effects of appendicectomy on the course of ulcerative colitis. Gut, 2002. 51(6): p. 803-7.

[140] Castiglione, F., Diaferia, M., Morace, F., Labianca, O., Meucci, C., et al., Risk factors for inflammatory bowel diseases according to the "hygiene hypothesis": a case-control, multi-centre, prospective study in Southern Italy. Journal of Crohn's & Colitis, 2012. 6(3): p. 324-9.

[141] Krieglstein, C.F., Cerwinka, W.H., Laroux, F.S., Grisham, M.B., Schurmann, G., et al., Role of appendix and spleen in experimental colitis. The Journal of Surgical Research, 2001. 101(2): p. 166-75.

[142] Mizoguchi, A., Mizoguchi, E., Chiba, C., and Bhan, A.K., Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. The Journal of Experimental Medicine, 1996. 184(2): p. 707-15.

[143] Godet, P.G., May, G.R., and Sutherland, L.R., Meta-analysis of the role of oral contraceptive agents in inflammatory bowel disease. Gut, 1995. 37(5): p. 668-73.

[144] Klement, E., Cohen, R.V., Boxman, J., Joseph, A., and Reif, S., Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. The American Journal of Clinical Nutrition, 2004. 80(5): p. 1342-52.

[145] Han, D.Y., Fraser, A.G., Dryland, P., and Ferguson, L.R., Environmental factors in the development of chronic inflammation: a case-control study on risk factors for Crohn's disease within New Zealand. Mutation Research, 2010. 690(1-2): p. 116-22.

[146] Gilat, T., Hacohen, D., Lilos, P., and Langman, M.J., Childhood factors in ulcerative colitis and Crohn's disease. An international cooperative study. Scandinavian Journal of Gastroenterology, 1987. 22(8): p. 1009-24.

[147] Hviid, A., Svanstrom, H., and Frisch, M., Antibiotic use and inflammatory bowel diseases in childhood. Gut, 2011. 60(1): p. 49-54.

[148] Hildebrand, H., Malmborg, P., Askling, J., Ekbom, A., and Montgomery, S.M., Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. Scandinavian Journal of Gastroenterology, 2008. 43(8): p. 961-6.

[149] Card, T., Logan, R.F., Rodrigues, L.C., and Wheeler, J.G., Antibiotic use and the development of Crohn's disease. Gut, 2004. 53(2): p. 246-50.

[150] Shaw, S.Y., Blanchard, J.F., and Bernstein, C.N., Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. The American Journal of Gastroenterology, 2011. 106(12): p. 2133-42.

[151] Jernberg, C., Lofmark, S., Edlund, C., and Jansson, J.K., Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. The ISME journal, 2007. 1(1): p. 56-66.

[152] Sartor, R.B., Does *Mycobacterium avium* subspecies *paratuberculosis* cause Crohn's disease? Gut, 2005. 54(7): p. 896-8.

[153] Singh, A.V., Singh, S.V., Singh, P.K., and Sohal, J.S., Is *Mycobacterium avium* subsp. *paratuberculosis*, the cause of Johne's disease in animals, a good candidate for Crohn's disease in man? Indian Journal of Gastroenterology 2010. 29(2): p. 53-8.

[154] Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., Thayer, W.R., Jr., and Coutu, J.A., Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's disease. Journal of Clinical Microbiology, 1984. 20(5): p. 966-71.

[155] Thorel, M.F., Krichevsky, M., and Levy-Frebault, V.V., Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. International Journal of Systematic Bacteriology, 1990. 40(3): p. 254-60.

[156] Sanderson, J.D., Moss, M.T., Tizard, M.L., and Hermon-Taylor, J., *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Gut, 1992. 33(7): p. 890-6.

[157] Dell'Isola, B., Poyart, C., Goulet, O., Mougenot, J.F., Sadoun-Journo, E., et al., Detection of *Mycobacterium paratuberculosis* by polymerase chain reaction in children with Crohn's disease. The Journal of Infectious Diseases, 1994. 169(2): p. 449-51.

[158] Fidler, H.M., Thurrell, W., Johnson, N.M., Rook, G.A., and McFadden, J.J., Specific detection of *Mycobacterium paratuberculosis* DNA associated with granulomatous tissue in Crohn's disease. Gut, 1994. 35(4): p. 506-10.

[159] Lisby, G., Andersen, J., Engbaek, K., and Binder, V., *Mycobacterium paratuberculosis* in intestinal tissue from patients with Crohn's disease demonstrated by a nested primer polymerase chain reaction. Scandinavian Journal of Gastroenterology, 1994. 29(10): p. 923-9.

[160] Suenaga, K., Yokoyama, Y., Okazaki, K., and Yamamoto, Y., Mycobacteria in the intestine of Japanese patients with inflammatory bowel disease. The American Journal of Gastroenterology, 1995. 90(1): p. 76-80.

[161] Murray, A., Oliaro, J., Schlup, M.M., and Chadwick, V.S., *Mycobacterium paratuberculosis* and inflammatory bowel disease: frequency distribution in serial colonoscopic biopsies using the polymerase chain reaction. Microbios, 1995. 83(337): p. 217-28.

[162] Roholl, P.J., Herrewegh, A., and van Soolingen, D., Positive IS900 *in situ* hybridization signals as evidence for role of *Mycobacterium avium* subsp. *paratuberculosis* in etiology of Crohn's disease. Journal of Clinical Microbiology, 2002. 40(8): p. 3112; author reply 3112-3.

[163] Rowbotham, D.S., Mapstone, N.P., Trejdosiewicz, L.K., Howdle, P.D., and Quirke, P., *Mycobacterium paratuberculosis* DNA not detected in Crohn's disease tissue by fluorescent polymerase chain reaction. Gut, 1995. 37(5): p. 660-7.

[164] Frank, T.S. and Cook, S.M., Analysis of paraffin sections of Crohn's disease for *Mycobacterium paratuberculosis* using polymerase chain reaction. Modern Pathology 1996. 9(1): p. 32-5.

[165] Dumonceau, J.M., Van Gossum, A., Adler, M., Fonteyne, P.A., Van Vooren, J.P., et al., No *Mycobacterium paratuberculosis* found in Crohn's disease using polymerase chain reaction. Digestive Diseases and Sciences, 1996. 41(2): p. 421-6.

[166] Al-Shamali, M., Khan, I., Al-Nakib, B., Al-Hassan, F., and Mustafa, A.S., A multiplex polymerase chain reaction assay for the detection of *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Scandinavian Journal of Gastroenterology, 1997. 32(8): p. 819-23.

[167] Chiba, M., Fukushima, T., Horie, Y., Iizuka, M., and Masamune, O., No *Mycobacterium paratuberculosis* detected in intestinal tissue, including Peyer's patches and lymph follicles, of Crohn's disease. Journal of Gastroenterology, 1998. 33(4): p. 482-7.

[168] Cellier, C., De Beenhouwer, H., Berger, A., Penna, C., Carbonnel, F., et al., *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp. *silvaticum* DNA cannot be detected by PCR in Crohn's disease tissue. Gastroenterologie Clinique et Biologique, 1998. 22(8-9): p. 675-8.

[169] Kanazawa, K., Haga, Y., Funakoshi, O., Nakajima, H., Munakata, A., et al., Absence of *Mycobacterium paratuberculosis* DNA in intestinal tissues from Crohn's disease by nested polymerase chain reaction. Journal of Gastroenterology, 1999. 34(2): p. 200-6.

[170] Thayer, W.R., Jr., Coutu, J.A., Chiodini, R.J., Van Kruiningen, H.J., and Merkal, R.S., Possible role of mycobacteria in inflammatory bowel disease. II. Mycobacterial antibodies in Crohn's disease. Digestive Diseases and Sciences, 1984. 29(12): p. 1080-5.

[171] Elsaghier, A., Prantera, C., Moreno, C., and Ivanyi, J., Antibodies to *Mycobacterium paratuberculosis*-specific protein antigens in Crohn's disease. Clinical and Experimental Immunology, 1992. 90(3): p. 503-8.

[172] Vannuffel, P., Dieterich, C., Naerhuyzen, B., Gilot, P., Coene, M., et al., Occurrence, in Crohn's disease, of antibodies directed against a species-specific recombinant polypeptide of *Mycobacterium paratuberculosis*. Clinical and Diagnostic Laboratory Immunology, 1994. 1(2): p. 241-3.

[173] Kreuzpaintner, G., Das, P.K., Stronkhorst, A., Slob, A.W., and Strohmeyer, G., Effect of intestinal resection on serum antibodies to the mycobacterial 45/48 kilodalton doublet antigen in Crohn's disease. Gut, 1995. 37(3): p. 361-6.

[174] Naser, S., Shafran, I., and El-Zaatari, F., *Mycobacterium avium* subsp. *paratuberculosis* in Crohn's disease is serologically positive. Clinical and Diagnostic Laboratory Immunology, 1999. 6(2): p. 282.

[175] Suenaga, K., Yokoyama, Y., Nishimori, I., Sano, S., Morita, M., et al., Serum antibodies to *Mycobacterium paratuberculosis* in patients with Crohn's disease. Digestive Diseases and Sciences, 1999. 44(6): p. 1202-7.

[176] Naser, S.A., Hulten, K., Shafran, I., Graham, D.Y., and El-Zaatari, F.A., Specific seroreactivity of Crohn's disease patients against p35 and p36 antigens of *M. avium* subsp. *paratuberculosis*. Veterinary Microbiology, 2000. 77(3-4): p. 497-504.

[177] Cho, S.N., Brennan, P.J., Yoshimura, H.H., Korelitz, B.I., and Graham, D.Y., Mycobacterial aetiology of Crohn's disease: serologic study using common mycobacterial antigens and a species-specific glycolipid antigen from *Mycobacterium paratuberculosis*. Gut, 1986. 27(11): p. 1353-6.

[178] McFadden, J.J. and Houdayer, C., No evidence for antibodies to mycobacterial A60 antigen in Crohn's disease sera by enzyme-linked immunoabsorbent assay (ELISA). Journal of Medical Microbiology, 1988. 25(4): p. 295-8.

[179] Tanaka, K., Wilks, M., Coates, P.J., Farthing, M.J., Walker-Smith, J.A., et al., *Mycobacterium paratuberculosis* and Crohn's disease. Gut, 1991. 32(1): p. 43-5.

[180] Brunello, F., Pera, A., Martini, S., Marino, L., Astegiano, M., et al., Antibodies to *Mycobacterium paratuberculosis* in patients with Crohn's disease. Digestive Diseases and Sciences, 1991. 36(12): p. 1741-5.

[181] Stainsby, K.J., Lowes, J.R., Allan, R.N., and Ibbotson, J.P., Antibodies to *Mycobacterium paratuberculosis* and nine species of environmental mycobacteria in Crohn's disease and control subjects. Gut, 1993. 34(3): p. 371-4.

[182] Walmsley, R.S., Ibbotson, J.P., Chahal, H., and Allan, R.N., Antibodies against *Mycobacterium paratuberculosis* in Crohn's disease. QJM : Monthly Journal of the Association of Physicians, 1996. 89(3): p. 217-21.

[183] Sechi, L.A., Gazouli, M., Ikonomopoulos, J., Lukas, J.C., Scanu, A.M., et al., *Mycobacterium avium* subsp. *paratuberculosis*, genetic susceptibility to Crohn's disease, and Sardinians: the way ahead. Journal of Clinical Microbiology, 2005. 43(10): p. 5275-7.

[184] Singh, S. and Gopinath, K., *Mycobacterium avium* subspecies *Paratuberculosis* and Crohn's Regional Ileitis: How Strong is Association? Journal of Laboratory Physicians, 2011. 3(2): p. 69-74.

[185] Chiodini, R.J., Chamberlin, W.M., Sarosiek, J., and McCallum, R.W., Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association? Critical Reviews in Microbiology, 2012. 38(1): p. 52-93.

[186] Darfeuille-Michaud, A., Neut, C., Barnich, N., Lederman, E., Di Martino, P., et al., Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. Gastroenterology, 1998. 115(6): p. 1405-13.

[187] Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A.L., et al., High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology, 2004. 127(2): p. 412-21.

[188] Boudeau, J., Barnich, N., and Darfeuille-Michaud, A., Type 1 pili-mediated adherence of *Escherichia coli* strain LF82 isolated from Crohn's disease is involved in bacterial invasion of intestinal epithelial cells. Molecular Microbiology, 2001. 39(5): p. 1272-84.

[189] Barnich, N., Carvalho, F.A., Glasser, A.L., Darcha, C., Jantscheff, P., et al., CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. The Journal of Clinical Investigation, 2007. 117(6): p. 1566-74.

[190] Boudeau, J., Glasser, A.L., Masseret, E., Joly, B., and Darfeuille-Michaud, A., Invasive ability of an *Escherichia coli* strain isolated from the ileal mucosa of a patient with Crohn's disease. Infection and Immunity, 1999. 67(9): p. 4499-509.

[191] Glasser, A.L., Boudeau, J., Barnich, N., Perruchot, M.H., Colombel, J.F., et al., Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. Infection and Immunity, 2001. 69(9): p. 5529-37.

[192] Meconi, S., Vercellone, A., Levillain, F., Payre, B., Al Saati, T., et al., Adherent-invasive *Escherichia coli* isolated from Crohn's disease patients induce granulomas *in vitro*. Cellular Microbiology, 2007. 9(5): p. 1252-61.

[193] Mansfield, C.S., James, F.E., Craven, M., Davies, D.R., O'Hara, A.J., et al., Remission of histiocytic ulcerative colitis in Boxer dogs correlates with eradication of invasive

intramucosal *Escherichia coli*. Journal of Veterinary Internal Medicine, 2009. 23(5): p. 964-9.

[194] Swidsinski, A., Ladhoff, A., Pernthaler, A., Swidsinski, S., Loening-Baucke, V., et al., Mucosal flora in inflammatory bowel disease. Gastroenterology, 2002. 122(1): p. 44-54.

[195] Kleessen, B., Kroesen, A.J., Buhr, H.J., and Blaut, M., Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. Scandinavian Journal of Gastroenterology, 2002. 37(9): p. 1034-41.

[196] Schultsz, C., Van Den Berg, F.M., Ten Kate, F.W., Tytgat, G.N., and Dankert, J., The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. Gastroenterology, 1999. 117(5): p. 1089-97.

[197] Takaishi, H., Matsuki, T., Nakazawa, A., Takada, T., Kado, S., et al., Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. International Journal of Medical Microbiology 2008. 298(5-6): p. 463-72.

[198] Swidsinski, A., Weber, J., Loening-Baucke, V., Hale, L.P., and Lochs, H., Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. Journal of Clinical Microbiology, 2005. 43(7): p. 3380-9.

[199] Marteau, P., Pochart, P., Dore, J., Bera-Maillet, C., Bernalier, A., et al., Comparative study of bacterial groups within the human cecal and fecal microbiota. Applied and Environmental Microbiology, 2001. 67(10): p. 4939-42.

[200] Zoetendal, E.G., von Wright, A., Vilpponen-Salmela, T., Ben-Amor, K., Akkermans, A.D., et al., Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. Applied and Environmental Microbiology, 2002. 68(7): p. 3401-7.

[201] Andoh, A., Kuzuoka, H., Tsujikawa, T., Nakamura, S., Hirai, F., et al., Multicenter analysis of fecal microbiota profiles in Japanese patients with Crohn's disease. Journal of Gastroenterology, 2012.

[202] Sokol, H., Seksik, P., Rigottier-Gois, L., Lay, C., Lepage, P., et al., Specificities of the fecal microbiota in inflammatory bowel disease. Inflammatory Bowel Diseases, 2006. 12(2): p. 106-11.

[203] Sokol, H., Seksik, P., Furet, J.P., Firmesse, O., Nion-Larmurier, I., et al., Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. Inflammatory Bowel Diseases, 2009. 15(8): p. 1183-9.

[204] Willing, B.P., Dicksved, J., Halfvarson, J., Andersson, A.F., Lucio, M., et al., A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with in-

flammatory bowel disease phenotypes. Gastroenterology, 2010. 139(6): p. 1844-1854 e1.

[205] Willing, B., Halfvarson, J., Dicksved, J., Rosenquist, M., Jarnerot, G., et al., Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflammatory Bowel Diseases, 2009. 15(5): p. 653-60.

[206] Scanlan, P.D., Shanahan, F., O'Mahony, C., and Marchesi, J.R., Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease. Journal of Clinical Microbiology, 2006. 44(11): p. 3980-8.

[207] Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L.G., et al., *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proceedings of the National Academy of Sciences of the United States of America, 2008. 105(43): p. 16731-6.

[208] Martinez-Medina, M., Aldeguer, X., Gonzalez-Huix, F., Acero, D., and Garcia-Gil, L.J., Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflammatory Bowel Dseases, 2006. 12(12): p. 1136-45.

[209] Swidsinski, A., Loening-Baucke, V., Vaneechoutte, M., and Doerffel, Y., Active Crohn's disease and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure of the fecal flora. Inflammatory Bowel Diseases, 2008. 14(2): p. 147-61.

[210] Lupp, C., Robertson, M.L., Wickham, M.E., Sekirov, I., Champion, O.L., et al., Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*. Cell Host & Microbe, 2007. 2(2): p. 119-29.

[211] Luperchio, S.A. and Schauer, D.B., Molecular pathogenesis of *Citrobacter rodentium* and transmissible murine colonic hyperplasia. Microbes and Infection 2001. 3(4): p. 333-40.

[212] Mansfield, L.S., Bell, J.A., Wilson, D.L., Murphy, A.J., Elsheikha, H.M., et al., C57BL/6 and congenic interleukin-10-deficient mice can serve as models of *Campylobacter jejuni* colonization and enteritis. Infection and Immunity, 2007. 75(3): p. 1099-115.

[213] Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W., Interleukin-10-deficient mice develop chronic enterocolitis. Cell, 1993. 75(2): p. 263-74.

[214] Jergens, A.E. and Simpson, K.W., Inflammatory bowel disease in veterinary medicine. Frontiers in Bioscience, 2012. 4: p. 1404-19.

[215] Jergens, A.E., Moore, F.M., Haynes, J.S., and Miles, K.G., Idiopathic inflammatory bowel disease in dogs and cats: 84 cases (1987-1990). Journal of the American Veterinary Medical Association, 1992. 201(10): p. 1603-8.

[216] Jergens, A.E., Inflammatory bowel disease. Current perspectives. The Veterinary Clinics of North America. Small Animal Practice, 1999. 29(2): p. 501-21, vii.

[217] German, A.J., Hall, E.J., and Day, M.J., Immune cell populations within the duodenal mucosa of dogs with enteropathies. Journal of Veterinary Internal Medicine, 2001. 15(1): p. 14-25.

[218] Kathrani, A., House, A., Catchpole, B., Murphy, A., German, A., et al., Polymorphisms in the TLR4 and TLR5 gene are significantly associated with inflammatory bowel disease in German shepherd dogs. PloS one, 2010. 5(12): p. e15740.

[219] Allenspach, K., House, A., Smith, K., McNeill, F.M., Hendricks, A., et al., Evaluation of mucosal bacteria and histopathology, clinical disease activity and expression of Toll-like receptors in German shepherd dogs with chronic enteropathies. Veterinary Microbiology, 2010. 146(3-4): p. 326-35.

[220] Simpson, K.W., Dogan, B., Rishniw, M., Goldstein, R.E., Klaessig, S., et al., Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. Infection and Immunity, 2006. 74(8): p. 4778-92.

[221] Baumgart, M., Dogan, B., Rishniw, M., Weitzman, G., Bosworth, B., et al., Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of *Clostridiales* in Crohn's disease involving the ileum. The ISME journal, 2007. 1(5): p. 403-18.

[222] Craven, M., Mansfield, C.S., and Simpson, K.W., Granulomatous colitis of boxer dogs. The Veterinary Clinics of North America: Small Animal Practice, 2011. 41(2): p. 433-45.

[223] Kathrani, A., Schmitz, S., Priestnall, S.L., Smith, K.C., Werling, D., et al., CD11c+ cells are significantly decreased in the duodenum, ileum and colon of dogs with inflammatory bowel disease. Journal of Comparative Pathology, 2011. 145(4): p. 359-66.

[224] Xenoulis, P.G., Palculict, B., Allenspach, K., Steiner, J.M., Van House, A.M., et al., Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS microbiology ecology, 2008. 66(3): p. 579-89.

[225] Suchodolski, J.S., Xenoulis, P.G., Paddock, C.G., Steiner, J.M., and Jergens, A.E., Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Veterinary Microbiology, 2010. 142(3-4): p. 394-400.

[226] Sartor, R.B., Microbial influences in inflammatory bowel diseases. Gastroenterology, 2008. 134(2): p. 577-94.

[227] Suchodolski, J.S., Dowd, S.E., Wilke, V., Steiner, J.M., and Jergens, A.E., 16S rRNA gene pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic inflammatory bowel disease. PloS one, 2012. 7(6): p. e39333.

[228] Craven, M., Simpson, J.W., Ridyard, A.E., and Chandler, M.L., Canine inflammatory bowel disease: retrospective analysis of diagnosis and outcome in 80 cases (1995-2002). The Journal of Small Animal Practice, 2004. 45(7): p. 336-42.

[229] Allenspach, K., Wieland, B., Grone, A., and Gaschen, F., Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. Journal of Veterinary Internal Medicine 2007. 21(4): p. 700-8.

[230] Simpson, K.W. and Jergens, A.E., Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. The Veterinary Clinics of North America. Small Animal Practice, 2011. 41(2): p. 381-98.

[231] Best, W.R., Becktel, J.M., Singleton, J.W., and Kern, F., Jr., Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology, 1976. 70(3): p. 439-44.

[232] Harvey, R.F. and Bradshaw, J.M., A simple index of Crohn's-disease activity. Lancet, 1980. 1(8167): p. 514.

[233] van Hees, P.A., van Elteren, P.H., van Lier, H.J., and van Tongeren, J.H., An index of inflammatory activity in patients with Crohn's disease. Gut, 1980. 21(4): p. 279-86.

[234] Jergens, A.E., Schreiner, C.A., Frank, D.E., Niyo, Y., Ahrens, F.E., et al., A scoring index for disease activity in canine inflammatory bowel disease. Journal of Veterinary Internal Medicine, 2003. 17(3): p. 291-7.

[235] Jergens, A.E., Crandell, J., Morrison, J.A., Deitz, K., Pressel, M., et al., Comparison of oral prednisone and prednisone combined with metronidazole for induction therapy of canine inflammatory bowel disease: a randomized-controlled trial. Journal of Veterinary Internal Medicine, 2010. 24(2): p. 269-77.

[236] Washabau, R.J., Day, M.J., Willard, M.D., Hall, E.J., Jergens, A.E., et al., Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. Journal of Veterinary Internal Medicine, 2010. 24(1): p. 10-26.

[237] Trepanier, L., Idiopathic inflammatory bowel disease in cats. Rational treatment selection. Journal of Feline Medicine and Surgery, 2009. 11(1): p. 32-8.

[238] Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K., et al., The dog genome: survey sequencing and comparative analysis. Science, 2003. 301(5641): p. 1898-903.

[239] Allenspach, K., Tests to investigate gastrointestinal diseases in dogs--which markers are actually useful for the practitioner? The Journal of Small Animal Practice, 2007. 48(11): p. 607-8.

[240] Mowat, C., Cole, A., Windsor, A., Ahmad, T., Arnott, I., et al., Guidelines for the management of inflammatory bowel disease in adults. Gut, 2011. 60(5): p. 571-607.

[241] Khan, K.J., Ullman, T.A., Ford, A.C., Abreu, M.T., Abadir, A., et al., Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. The American Journal of Gastroenterology, 2011. 106(4): p. 661-73.

[242] Guslandi, M., Rifaximin in the treatment of inflammatory bowel disease. World Journal of Gastroenterology 2011. 17(42): p. 4643-6.

[243] Scarpignato, C. and Pelosini, I., Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. Chemotherapy, 2005. 51 Suppl 1: p. 36-66.

[244] Maccaferri, S., Vitali, B., Klinder, A., Kolida, S., Ndagijimana, M., et al., Rifaximin modulates the colonic microbiota of patients with Crohn's disease: an *in vitro* approach using a continuous culture colonic model system. The Journal of Antimicrobial Chemotherapy, 2010. 65(12): p. 2556-65.

[245] Lahat, G., Halperin, D., Barazovsky, E., Shalit, I., Rabau, M., et al., Immunomodulatory effects of ciprofloxacin in TNBS-induced colitis in mice. Inflammatory Bowel Diseases, 2007. 13(5): p. 557-65.

[246] Dalhoff, A., Immunomodulatory activities of fluoroquinolones. Infection, 2005. 33 Suppl 2: p. 55-70.

[247] Barry, V.C., Belton, J.G., Conalty, M.L., Denneny, J.M., Edward, D.W., et al., A new series of phenazines (rimino-compounds) with high antituberculosis activity. Nature, 1957. 179(4568): p. 1013-5.

[248] Arbiser, J.L. and Moschella, S.L., Clofazimine: a review of its medical uses and mechanisms of action. Journal of the American Academy of Dermatology, 1995. 32(2 Pt 1): p. 241-7.

[249] Cholo, M.C., Steel, H.C., Fourie, P.B., Germishuizen, W.A., and Anderson, R., Clofazimine: current status and future prospects. The Journal of Antimicrobial Chemotherapy, 2012. 67(2): p. 290-8.

[250] Van Rensburg, C.E., Joone, G.K., O'Sullivan, J.F., and Anderson, R., Antimicrobial activities of clofazimine and B669 are mediated by lysophospholipids. Antimicrobial Agents and Chemotherapy, 1992. 36(12): p. 2729-35.

[251] Sarracent, J. and Finlay, C.M., The action of Clofazimine on the level of lysosomal enzymes of cultured macrophages. Clinical and Experimental Immunology, 1982. 48(1): p. 261-7.

[252] Sarracent, J. and Finlay, C.M., Phagocytosis and intracellular degradation of 125I-labelled immune complexes by Clofazimine treated macrophage cultures. Clinical and Experimental Immunology, 1982. 48(1): p. 268-72.

[253] Ren, Y.R., Pan, F., Parvez, S., Fleig, A., Chong, C.R., et al., Clofazimine inhibits human Kv1.3 potassium channel by perturbing calcium oscillation in T lymphocytes. PloS One, 2008. 3(12): p. e4009.

[254] Triantafillidis, J.K., Merikas, E., and Georgopoulos, F., Current and emerging drugs for the treatment of inflammatory bowel disease. Drug Design, Development and Therapy, 2011. 5: p. 185-210.

[255] Campieri, M., Ferguson, A., Doe, W., Persson, T., and Nilsson, L.G., Oral budesonide is as effective as oral prednisolone in active Crohn's disease. The Global Budesonide Study Group. Gut, 1997. 41(2): p. 209-14.

[256] Ardite, E., Panes, J., Miranda, M., Salas, A., Elizalde, J.I., et al., Effects of steroid treatment on activation of nuclear factor kappaB in patients with inflammatory bowel disease. British Journal of Pharmacology, 1998. 124(3): p. 431-3.

[257] Van Scoik, K.G., Johnson, C.A., and Porter, W.R., The pharmacology and metabolism of the thiopurine drugs 6-mercaptopurine and azathioprine. Drug Metabolism Reviews, 1985. 16(1-2): p. 157-74.

[258] Greenstein, R.J., Su, L., Haroutunian, V., Shahidi, A., and Brown, S.T., On the action of methotrexate and 6-mercaptopurine on *M. avium* subspecies *paratuberculosis*. PloS One, 2007. 2(1): p. e161.

[259] Hyoun, S.C., Obican, S.G., and Scialli, A.R., Teratogen update: methotrexate. Birth Defects Research. Part A, Clinical and Molecular Teratology, 2012. 94(4): p. 187-207.

[260] Schroder, H. and Campbell, D.E., Absorption, metabolism, and excretion of salicylazosulfapyridine in man. Clinical Pharmacology and Therapeutics, 1972. 13(4): p. 539-51.

[261] Peppercorn, M.A. and Goldman, P., Distribution studies of salicylazosulfapyridine and its metabolites. Gastroenterology, 1973. 64(2): p. 240-5.

[262] Dick, A.P., Grayson, M.J., Carpenter, R.G., and Petrie, A., Controlled Trial of Sulphasalazine in the Treatment of Ulcerative Colitis. Gut, 1964. 5: p. 437-42.

[263] Dew, M.J., Hughes, P., Harries, A.D., Williams, G., Evans, B.K., et al., Maintenance of remission in ulcerative colitis with oral preparation of 5-aminosalicylic acid. British Medical Journal, 1982. 285(6347): p. 1012.

[264] Azad Khan, A.K., Piris, J., and Truelove, S.C., An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet, 1977. 2(8044): p. 892-5.

[265] Willoughby, C.P., Aronson, J.K., Agback, H., Bodin, N.O., and Truelove, S.C., Distribution and metabolism in healthy volunteers of disodium azodisalicylate, a potential therapeutic agent for ulcerative colitis. Gut, 1982. 23(12): p. 1081-7.

[266] Chan, R.P., Pope, D.J., Gilbert, A.P., Sacra, P.J., Baron, J.H., et al., Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide. Digestive Diseases and Sciences, 1983. 28(7): p. 609-15.

[267] Ahnfelt-Ronne, I., Nielsen, O.H., Christensen, A., Langholz, E., Binder, V., et al., Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. Gastroenterology, 1990. 98(5 Pt 1): p. 1162-9.

[268] Kaiser, G.C., Yan, F., and Polk, D.B., Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor kappaB activation in mouse colonocytes. Gastroenterology, 1999. 116(3): p. 602-9.

[269] Mahida, Y.R., Lamming, C.E., Gallagher, A., Hawthorne, A.B., and Hawkey, C.J., 5-Aminosalicylic acid is a potent inhibitor of interleukin 1 beta production in organ culture of colonic biopsy specimens from patients with inflammatory bowel disease. Gut, 1991. 32(1): p. 50-4.

[270] Rhodes, J.M., Bartholomew, T.C., and Jewell, D.P., Inhibition of leucocyte motility by drugs used in ulcerative colitis. Gut, 1981. 22(8): p. 642-7.

[271] Swidsinski, A., Loening-Baucke, V., Bengmark, S., Lochs, H., and Dorffel, Y., Azathioprine and mesalazine-induced effects on the mucosal flora in patients with IBD colitis. Inflammatory Bowel Diseases, 2007. 13(1): p. 51-6.

[272] Greenstein, R.J., Su, L., Shahidi, A., and Brown, S.T., On the action of 5-amino-salicylic acid and sulfapyridine on *M. avium* including subspecies *paratuberculosis*. PloS One, 2007. 2(6): p. e516.

[273] Sandberg-Gertzen, H., Kjellander, J., Sundberg-Gilla, B., and Jarnerot, G., *In vitro* effects of sulphasalazine, azodisal sodium, and their metabolites on *Clostridium difficile* and some other faecal bacteria. Scandinavian Journal of Gastroenterology, 1985. 20(5): p. 607-12.

[274] Kaufman, J., Griffiths, T.A., Surette, M.G., Ness, S., and Rioux, K.P., Effects of mesalamine (5-aminosalicylic acid) on bacterial gene expression. Inflammatory Bowel Diseases, 2009. 15(7): p. 985-96.

[275] Braegger, C.P., Nicholls, S., Murch, S.H., Stephens, S., and MacDonald, T.T., Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet, 1992. 339(8785): p. 89-91.

[276] Reinecker, H.C., Steffen, M., Witthoeft, T., Pflueger, I., Schreiber, S., et al., Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clinical and Experimental Immunology, 1993. 94(1): p. 174-81.

[277] Knight, D.M., Trinh, H., Le, J., Siegel, S., Shealy, D., et al., Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. Molecular Immunology, 1993. 30(16): p. 1443-53.

[278] Ordas, I., Mould, D.R., Feagan, B.G., and Sandborn, W.J., Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clinical Pharmacology and Therapeutics, 2012. 91(4): p. 635-46.

[279] Hanauer, S.B., Sandborn, W.J., Rutgeerts, P., Fedorak, R.N., Lukas, M., et al., Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology, 2006. 130(2): p. 323-33; quiz 591.

[280] Schreiber, S., Rutgeerts, P., Fedorak, R.N., Khaliq-Kareemi, M., Kamm, M.A., et al., A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. Gastroenterology, 2005. 129(3): p. 807-18.

[281] Nesbitt, A., Fossati, G., Bergin, M., Stephens, P., Stephens, S., et al., Mechanism of action of certolizumab pegol (CDP870): *in vitro* comparison with other anti-tumor necrosis factor alpha agents. Inflammatory Bowel Diseases, 2007. 13(11): p. 1323-32.

[282] Scallon, B.J., Moore, M.A., Trinh, H., Knight, D.M., and Ghrayeb, J., Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. Cytokine, 1995. 7(3): p. 251-9.

[283] Vos, A.C., Wildenberg, M.E., Duijvestein, M., Verhaar, A.P., van den Brink, G.R., et al., Anti-tumor necrosis factor-alpha antibodies induce regulatory macrophages in an Fc region-dependent manner. Gastroenterology, 2011. 140(1): p. 221-30.

[284] Rawsthorne, P., Clara, I., Graff, L.A., Bernstein, K.I., Carr, R., et al., The Manitoba Inflammatory Bowel Disease Cohort Study: a prospective longitudinal evaluation of the use of complementary and alternative medicine services and products. Gut, 2012. 61(4): p. 521-7.

[285] Rahimi, R., Mozaffari, S., and Abdollahi, M., On the use of herbal medicines in management of inflammatory bowel diseases: a systematic review of animal and human studies. Digestive Diseases and Sciences, 2009. 54(3): p. 471-80.

[286] Quattropani, C., Ausfeld, B., Straumann, A., Heer, P., and Seibold, F., Complementary alternative medicine in patients with inflammatory bowel disease: use and attitudes. Scandinavian Journal of Gastroenterology, 2003. 38(3): p. 277-82.

[287] Ewaschuk, J.B. and Dieleman, L.A., Probiotics and prebiotics in chronic inflammatory bowel diseases. World Journal of Gastroenterology, 2006. 12(37): p. 5941-50.

[288] O'Hara, A.M. and Shanahan, F., Gut microbiota: mining for therapeutic potential. Clinical Gastroenterology and Hepatology, 2007. 5(3): p. 274-84.

[289] Guarner, F., Prebiotics, probiotics and helminths: the 'natural' solution? Digestive diseases, 2009. 27(3): p. 412-7.

[290] Guarner, F., Casellas, F., Borruel, N., Antolin, M., Videla, S., et al., Role of microecology in chronic inflammatory bowel diseases. European Journal of Clinical Nutrition, 2002. 56 Suppl 4: p. S34-8.

[291] Guarner, F., Prebiotics in inflammatory bowel diseases. The British Journal of Nutrition, 2007. 98 Suppl 1: p. S85-9.

[292] Chapman, T.M., Plosker, G.L., and Figgitt, D.P., Spotlight on VSL#3 probiotic mixture in chronic inflammatory bowel diseases. BioDrugs, 2007. 21(1): p. 61-3.

[293] Madsen, K., The use of probiotics in gastrointestinal disease. Canadian Journal of Gastroenterology, 2001. 15(12): p. 817-22.

[294] Esposito, E., Iacono, A., Bianco, G., Autore, G., Cuzzocrea, S., et al., Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. The Journal of Nutrition, 2009. 139(5): p. 905-11.

[295] Ewaschuk, J., Endersby, R., Thiel, D., Diaz, H., Backer, J., et al., Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. Hepatology, 2007. 46(3): p. 841-50.

[296] Zwiers, A., Kraal, L., van de Pouw Kraan, T.C., Wurdinger, T., Bouma, G., et al., Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. Journal of immunology, 2012. 188(4): p. 1573-7.

[297] Gionchetti, P., Rizzello, F., Venturi, A., Brigidi, P., Matteuzzi, D., et al., Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology, 2000. 119(2): p. 305-9.

[298] Bibiloni, R., Fedorak, R.N., Tannock, G.W., Madsen, K.L., Gionchetti, P., et al., VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. The American Journal of Gastroenterology, 2005. 100(7): p. 1539-46.

[299] Huynh, H.Q., deBruyn, J., Guan, L., Diaz, H., Li, M., et al., Probiotic preparation VSL#3 induces remission in children with mild to moderate acute ulcerative colitis: a pilot study. Inflammatory Bowel Diseases, 2009. 15(5): p. 760-8.

[300] Hoermannsperger, G., Clavel, T., Hoffmann, M., Reiff, C., Kelly, D., et al., Post-translational inhibition of IP-10 secretion in IEC by probiotic bacteria: impact on chronic inflammation. PloS One, 2009. 4(2): p. e4365.

[301] VSL#3 Frequently Asked Questions. Available from: http://www.vsl3.com/discover/faq.asp.

[302] Kruis, W., Schutz, E., Fric, P., Fixa, B., Judmaier, G., et al., Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Alimentary Pharmacology & Therapeutics, 1997. 11(5): p. 853-8.

[303] Kruis, W., Fric, P., Pokrotnieks, J., Lukas, M., Fixa, B., et al., Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut, 2004. 53(11): p. 1617-23.

[304] Rembacken, B.J., Snelling, A.M., Hawkey, P.M., Chalmers, D.M., and Axon, A.T., Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet, 1999. 354(9179): p. 635-9.

[305]   Jacobi, C.A., Grundler, S., Hsieh, C.J., Frick, J.S., Adam, P., et al., Quorum sensing in the probiotic bacterium *Escherichia coli* Nissle 1917 (Mutaflor) - evidence that furanosyl borate diester (AI-2) is influencing the cytokine expression in the DSS colitis mouse model. Gut Pathogens, 2012. 4(1): p. 8.

[306]   Lodinova-Zadnikova, R. and Sonnenborn, U., Effect of preventive administration of a nonpathogenic *Escherichia coli* strain on the colonization of the intestine with microbial pathogens in newborn infants. Biology of the Neonate, 1997. 71(4): p. 224-32.

[307]   Altenhoefer, A., Oswald, S., Sonnenborn, U., Enders, C., Schulze, J., et al., The probiotic *Escherichia coli strain* Nissle 1917 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens. FEMS Immunology and Medical Microbiology, 2004. 40(3): p. 223-9.

[308]   Boudeau, J., Glasser, A.L., Julien, S., Colombel, J.F., and Darfeuille-Michaud, A., Inhibitory effect of probiotic *Escherichia coli* strain Nissle 1917 on adhesion to and invasion of intestinal epithelial cells by adherent-invasive *E. coli* strains isolated from patients with Crohn's disease. Alimentary Pharmacology & Therapeutics, 2003. 18(1): p. 45-56.

[309]   Storm, D.W., Koff, S.A., Horvath, D.J., Jr., Li, B., and Justice, S.S., *In vitro* analysis of the bactericidal activity of *Escherichia coli* Nissle 1917 against pediatric uropathogens. The Journal of Urology, 2011. 186(4 Suppl): p. 1678-83.

[310]   Why can't I buy Mutaflor in the US anymore? ; Available from: http://mutaflor.us/.

[311]   Sauter, S.N., Allenspach, K., Gaschen, F., Grone, A., Ontsouka, E., et al., Cytokine expression in an *ex vivo* culture system of duodenal samples from dogs with chronic enteropathies: modulation by probiotic bacteria. Domestic Animal Endocrinology, 2005. 29(4): p. 605-22.

[312]   Sauter, S.N., Benyacoub, J., Allenspach, K., Gaschen, F., Ontsouka, E., et al., Effects of probiotic bacteria in dogs with food responsive diarrhoea treated with an elimination diet. Journal of Animal Physiology and Animal Nutrition, 2006. 90(7-8): p. 269-77.

[313]   Bybee, S.N., Scorza, A.V., and Lappin, M.R., Effect of the probiotic *Enterococcus faecium* SF68 on presence of diarrhea in cats and dogs housed in an animal shelter. Journal of Veterinary Internal Medicine, 2011. 25(4): p. 856-60.

[314]   Hooda, S., Boler, B.M., Serao, M.C., Brulc, J.M., Staeger, M.A., et al., 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. The Journal of Nutrition, 2012. 142(7): p. 1259-65.

[315]   Videla, S., Vilaseca, J., Antolin, M., Garcia-Lafuente, A., Guarner, F., et al., Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. The American Journal of Gastroenterology, 2001. 96(5): p. 1486-93.

[316] Kanauchi, O., Suga, T., Tochihara, M., Hibi, T., Naganuma, M., et al., Treatment of ulcerative colitis by feeding with germinated barley foodstuff: first report of a multi-center open control trial. Journal of Gastroenterology, 2002. 37 Suppl 14: p. 67-72.

[317] Hanai, H., Kanauchi, O., Mitsuyama, K., Andoh, A., Takeuchi, K., et al., Germinated barley foodstuff prolongs remission in patients with ulcerative colitis. International Journal of Molecular Medicine, 2004. 13(5): p. 643-7.

[318] Faghfoori, Z., Navai, L., Shakerhosseini, R., Somi, M.H., Nikniaz, Z., et al., Effects of an oral supplementation of germinated barley foodstuff on serum tumour necrosis factor-alpha, interleukin-6 and -8 in patients with ulcerative colitis. Annals of Clinical Biochemistry, 2011. 48(Pt 3): p. 233-7.

[319] Lindsay, J.O., Whelan, K., Stagg, A.J., Gobin, P., Al-Hassi, H.O., et al., Clinical, micro-biological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. Gut, 2006. 55(3): p. 348-55.

[320] Sparkes, A.H., Papasouliotis, K., Sunvold, G., Werrett, G., Gruffydd-Jones, E.A., et al., Effect of dietary supplementation with fructo-oligosaccharides on fecal flora of healthy cats. American Journal of Veterinary Research, 1998. 59(4): p. 436-40.

[321] Sparkes, A.H., Papasouliotis, K., Sunvold, G., Werrett, G., Clarke, C., et al., Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructo-oligosaccharides. American Journal of Veterinary Research, 1998. 59(4): p. 431-5.

[322] Willard, M.D., Simpson, R.B., Cohen, N.D., and Clancy, J.S., Effects of dietary fruc-tooligosaccharide on selected bacterial populations in feces of dogs. American Journal of Veterinary Research, 2000. 61(7): p. 820-5.

[323] Mizoguchi, A., Animal models of inflammatory bowel disease. Progress in Molecular Biology and Translational Science, 2012. 105: p. 263-320.

[324] Solomon, L., Mansor, S., Mallon, P., Donnelly, E., Hoper, M., et al., The Dextran Sul-fate Sodium (DSS) Model of Colitis: An Overview. Comparative Clinical Pathology, 2010. 19: p. 235-239.

[325] Mahler, M., Bristol, I.J., Leiter, E.H., Workman, A.E., Birkenmeier, E.H., et al., Differ-ential susceptibility of inbred mouse strains to dextran sulfate sodium-induced coli-tis. The American Journal of Physiology, 1998. 274(3 Pt 1): p. G544-51.

[326] Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y., et al., A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology, 1990. 98(3): p. 694-702.

[327] Kitajima, S., Takuma, S., and Morimoto, M., Histological analysis of murine colitis in-duced by dextran sulfate sodium of different molecular weights. Experimental Ani-mals / Japanese Association for Laboratory Animal Science, 2000. 49(1): p. 9-15.

[328] Bamba, S., Andoh, A., Ban, H., Imaeda, H., Aomatsu, T., et al., The severity of dextran sodium sulfate-induced colitis can differ between dextran sodium sulfate preparations of the same molecular weight range. Digestive Diseases and Sciences, 2012. 57(2): p. 327-34.

[329] Cooper, H.S., Murthy, S.N., Shah, R.S., and Sedergran, D.J., Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Laboratory Investigation, 1993. 69(2): p. 238-49.

[330] Kitajima, S., Takuma, S., and Morimoto, M., Tissue distribution of dextran sulfate sodium (DSS) in the acute phase of murine DSS-induced colitis. The Journal of Veterinary Medical Science / The Japanese Society of Veterinary Science, 1999. 61(1): p. 67-70.

[331] Kitajima, S., Takuma, S., and Morimoto, M., Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Experimental Animals / Japanese Association for Laboratory Animal Science, 1999. 48(3): p. 137-43.

[332] Ohkusa, T., Okayasu, I., Tokoi, S., Araki, A., and Ozaki, Y., Changes in bacterial phagocytosis of macrophages in experimental ulcerative colitis. Digestion, 1995. 56(2): p. 159-64.

[333] Araki, Y., Sugihara, H., and Hattori, T., *In vitro* effects of dextran sulfate sodium on a Caco-2 cell line and plausible mechanisms for dextran sulfate sodium-induced colitis. Oncology Reports, 2006. 16(6): p. 1357-62.

[334] Bylund-Fellenius, A.C., Landstrom, E., Axelsson, L.G., and Midtvedt, T., Experimental Colitis Induced by Dextran Sulphate in Normal and Germfree Mice. Microbial Ecology in Health and Disease 1994. 7: p. 207-215.

[335] Kitajima, S., Morimoto, M., Sagara, E., Shimizu, C., and Ikeda, Y., Dextran sodium sulfate-induced colitis in germ-free IQI/Jic mice. Experimental Animals / Japanese Association for Laboratory Animal Science, 2001. 50(5): p. 387-95.

[336] Neurath, M.F., Fuss, I., Kelsall, B.L., Presky, D.H., Waegell, W., et al., Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. The Journal of Experimental Medicine, 1996. 183(6): p. 2605-16.

[337] Kojima, R., Kuroda, S., Ohkishi, T., Nakamaru, K., and Hatakeyama, S., Oxazolone-induced colitis in BALB/C mice: a new method to evaluate the efficacy of therapeutic agents for ulcerative colitis. Journal of Pharmacological Sciences, 2004. 96(3): p. 307-13.

[338] Boirivant, M., Fuss, I.J., Chu, A., and Strober, W., Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. The Journal of Experimental Medicine, 1998. 188(10): p. 1929-39.

[339] NOTES, J. New IBD Model: C3Bir.129P2(B6)-Il10tm1cgn/Lt. 2006 Summer 2006 502].

[340] Rennick, D.M. and Fort, M.M., Lessons from genetically engineered animal models. XII. IL-10-deficient (IL-10(-/-) mice and intestinal inflammation. American journal of physiology. Gastrointestinal and liver physiology, 2000. 278(6): p. G829-33.

[341] Gomes-Santos, A.C., Moreira, T.G., Castro-Junior, A.B., Horta, B.C., Lemos, L., et al., New insights into the immunological changes in IL-10-deficient mice during the course of spontaneous inflammation in the gut mucosa. Clinical & developmental immunology, 2012. 2012: p. 560817.

[342] Berg, D.J., Davidson, N., Kuhn, R., Muller, W., Menon, S., et al., Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. The Journal of clinical investigation, 1996. 98(4): p. 1010-20.

[343] Bristol, I.J., Farmer, M.A., Cong, Y., Zheng, X.X., Strom, T.B., et al., Heritable susceptibility for colitis in mice induced by IL-10 deficiency. Inflammatory bowel diseases, 2000. 6(4): p. 290-302.

[344] Mahler, M. and Leiter, E.H., Genetic and environmental context determines the course of colitis developing in IL-10-deficient mice. Inflammatory bowel diseases, 2002. 8(5): p. 347-55.

[345] Rennick, D.M., Fort, M.M., and Davidson, N.J., Studies with IL-10-/- mice: an overview. Journal of Leukocyte Biology, 1997. 61(4): p. 389-96.

[346] Sellon, R.K., Tonkonogy, S., Schultz, M., Dieleman, L.A., Grenther, W., et al., Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infection and immunity, 1998. 66(11): p. 5224-31.

[347] Madsen, K.L., Doyle, J.S., Tavernini, M.M., Jewell, L.D., Rennie, R.P., et al., Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. Gastroenterology, 2000. 118(6): p. 1094-105.

[348] Asseman, C., Mauze, S., Leach, M.W., Coffman, R.L., and Powrie, F., An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. The Journal of experimental medicine, 1999. 190(7): p. 995-1004.

[349] Davidson, N.J., Leach, M.W., Fort, M.M., Thompson-Snipes, L., Kuhn, R., et al., T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. The Journal of Experimental Medicine, 1996. 184(1): p. 241-51.

[350] Spencer, D.M., Veldman, G.M., Banerjee, S., Willis, J., and Levine, A.D., Distinct inflammatory mechanisms mediate early versus late colitis in mice. Gastroenterology, 2002. 122(1): p. 94-105.

[351] Panwala, C.M., Jones, J.C., and Viney, J.L., A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. Journal of Immunology, 1998. 161(10): p. 5733-44.

[352] Maggio-Price, L., Shows, D., Waggie, K., Burich, A., Zeng, W., et al., *Helicobacter bilis* infection accelerates and *H. hepaticus* infection delays the development of colitis in multiple drug resistance-deficient (mdr1a-/-) mice. The American Journal of Pathology, 2002. 160(2): p. 739-51.

[353] Banner, K.H., Cattaneo, C., Le Net, J.L., Popovic, A., Collins, D., et al., Macroscopic, microscopic and biochemical characterisation of spontaneous colitis in a transgenic mouse, deficient in the multiple drug resistance 1a gene. British Journal of Pharmacology, 2004. 143(5): p. 590-8.

[354] Resta-Lenert, S., Smitham, J., and Barrett, K.E., Epithelial dysfunction associated with the development of colitis in conventionally housed mdr1a-/- mice. American Journal of Physiology. Gastrointestinal and Liver Physiology, 2005. 289(1): p. G153-62.

[355] Oostenbrug, L.E., Dijkstra, G., Nolte, I.M., van Dullemen, H.M., Oosterom, E., et al., Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. Scandinavian Journal of Gastroenterology, 2006. 41(10): p. 1174-82.

[356] Ho, G.T., Soranzo, N., Nimmo, E.R., Tenesa, A., Goldstein, D.B., et al., ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach. Human Molecular Genetics, 2006. 15(5): p. 797-805.

[357] Brant, S.R., Panhuysen, C.I., Nicolae, D., Reddy, D.M., Bonen, D.K., et al., MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. American Journal of Human Genetics, 2003. 73(6): p. 1282-92.

[358] Potocnik, U., Ferkolj, I., Glavac, D., and Dean, M., Polymorphisms in multidrug resistance 1 (*MDR1*) gene are associated with refractory Crohn disease and ulcerative colitis. Genes and Immunity, 2004. 5(7): p. 530-9.

[359] Ehrhardt, R.O., Ludviksson, B.R., Gray, B., Neurath, M., and Strober, W., Induction and prevention of colonic inflammation in IL-2-deficient mice. Journal of Immunology, 1997. 158(2): p. 566-73.

[360] Garrett, W.S. and Glimcher, L.H., T-bet-/- RAG2-/- ulcerative colitis: the role of T-bet as a peacekeeper of host-commensal relationships. Cytokine, 2009. 48(1-2): p. 144-7.

[361] Garrett, W.S., Gallini, C.A., Yatsunenko, T., Michaud, M., DuBois, A., et al., *Enterobacteriaceae* act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host & Microbe, 2010. 8(3): p. 292-300.

[362] Powrie, F., Leach, M.W., Mauze, S., Menon, S., Caddle, L.B., et al., Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity, 1994. 1(7): p. 553-62.

[363] Leach, M.W., Bean, A.G., Mauze, S., Coffman, R.L., and Powrie, F., Inflammatory bowel disease in C.B-17 scid mice reconstituted with the CD45RBhigh subset of CD4+ T cells. The American Journal of Pathology, 1996. 148(5): p. 1503-15.

[364] Aranda, R., Sydora, B.C., McAllister, P.L., Binder, S.W., Yang, H.Y., et al., Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. Journal of Immunology, 1997. 158(7): p. 3464-73.

[365] Podolsky, D.K., Inflammatory bowel disease (1). The New England Journal of Medicine, 1991. 325(13): p. 928-37.

[366] Liu, Z., Ramer-Tait, A.E., Henderson, A.L., Demirkale, C.Y., Nettleton, D., et al., *Helicobacter bilis* colonization enhances susceptibility to typhlocolitis following an inflammatory trigger. Digestive Diseases and Sciences, 2011. 56(10): p. 2838-48.

[367] Jergens, A.E., Dorn, A., Wilson, J., Dingbaum, K., Henderson, A., et al., Induction of differential immune reactivity to members of the flora of gnotobiotic mice following colonization with *Helicobacter bilis* or *Brachyspira hyodysenteriae*. Microbes and Infection 2006. 8(6): p. 1602-10.

[368] Jergens, A.E., Wilson-Welder, J.H., Dorn, A., Henderson, A., Liu, Z., et al., *Helicobacter bilis* triggers persistent immune reactivity to antigens derived from the commensal bacteria in gnotobiotic C3H/HeN mice. Gut, 2007. 56(7): p. 934-40.

[369] Liu, Z., Henderson, A.L., Nettleton, D., Wilson-Welder, J.H., Hostetter, J.M., et al., Mucosal gene expression profiles following the colonization of immunocompetent defined-flora C3H mice with *Helicobacter bilis*: a prelude to typhlocolitis. Microbes and Infection 2009. 11(3): p. 374-83.

[370] Myles, M.H., Livingston, R.S., Livingston, B.A., Criley, J.M., and Franklin, C.L., Analysis of gene expression in ceca of *Helicobacter hepaticus*-infected A/JCr mice before and after development of typhlitis. Infection and Immunity, 2003. 71(7): p. 3885-93.

[371] Livingston, R.S., Myles, M.H., Livingston, B.A., Criley, J.M., and Franklin, C.L., Sex influence on chronic intestinal inflammation in *Helicobacter hepaticus*-infected A/JCr mice. Comparative Medicine, 2004. 54(3): p. 301-8.

[372] Maggio-Price, L., Bielefeldt-Ohmann, H., Treuting, P., Iritani, B.M., Zeng, W., et al., Dual infection with *Helicobacter bilis* and *Helicobacter hepaticus* in p-glycoprotein-deficient mdr1a-/- mice results in colitis that progresses to dysplasia. The American Journal of Pathology, 2005. 166(6): p. 1793-806.

[373] Kullberg, M.C., Ward, J.M., Gorelick, P.L., Caspar, P., Hieny, S., et al., *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. Infection and Immunity, 1998. 66(11): p. 5157-66.

[374] Burich, A., Hershberg, R., Waggie, K., Zeng, W., Brabb, T., et al., *Helicobacter*-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. American Journal of Physiology, 2001. 281(3): p. G764-78.

[375] Taylor, D.J. and Alexander, T.J., The production of dysentery in swine by feeding cultures containing a spirochaete. The British Veterinary Journal, 1971. 127(11): p. 58-61.

[376] Hutto, D.L. and Wannemuehler, M.J., A comparison of the morphologic effects of Serpulina hyodysenteriae or its beta-hemolysin on the murine cecal mucosa. Veterinary Pathology, 1999. 36(5): p. 412-22.

[377] Brandenburg, A.C., Miniats, O.P., Geissinger, H.D., and Ewert, E., Swine dysentery: inoculation of gnotobiotic pigs with *Treponema hyodysenteriae* and *Vibrio coli* and a *Peptostreptococcus*. Canadian Journal of Comparative Medicine, 1977. 41(3): p. 294-301.

[378] Harris, D.L., Alexander, T.J., Whipp, S.C., Robinson, I.M., Glock, R.D., et al., Swine dysentery: studies of gnotobiotic pigs inoculated with Treponema hyodysenteriae, Bacteroides vulgatus, and Fusobacterium necrophorum. Journal of the American Veterinary Medical Association, 1978. 172(4): p. 468-71.

[379] Whipp, S.C., Robinson, I.M., Harris, D.L., Glock, R.D., Matthews, P.J., et al., Pathogenic synergism between Treponema hyodysenteriae and other selected anaerobes in gnotobiotic pigs. Infection and immunity, 1979. 26(3): p. 1042-7.

[380] Sacco, R.E., Hutto, D.L., Waters, W.R., Xiasong, L., Kehrli, M.E., Jr., et al., Reduction in inflammation following blockade of CD18 or CD29 adhesive pathways during the acute phase of a spirochetal-induced colitis in mice. Microbial pathogenesis, 2000. 29(5): p. 289-99.

[381] Liu, Z., Wilson-Welder, J.H., Hostetter, J.M., Jergens, A.E., and Wannemuehler, M.J., Prophylactic treatment with *Hypoxis hemerocallidea corm* (African potato) methanolic extract ameliorates *Brachyspira hyodysenteriae*-induced murine typhlocolitis. Experimental Biology and Medicine, 2010. 235(2): p. 222-30.

[382] Taconic. Animal Health Standards. 2011.

[383] Kim, S.C., Tonkonogy, S.L., Albright, C.A., Tsang, J., Balish, E.J., et al., Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology, 2005. 128(4): p. 891-906.

[384] Balish, E. and Warner, T., *Enterococcus faecalis* induces inflammatory bowel disease in interleukin-10 knockout mice. The American Journal of Pathology, 2002. 160(6): p. 2253-7.

[385] Dieleman, L.A., Arends, A., Tonkonogy, S.L., Goerres, M.S., Craft, D.W., et al., *Helicobacter hepaticus* does not induce or potentiate colitis in interleukin-10-deficient mice. Infection and Immunity, 2000. 68(9): p. 5107-13.

[386] Sydora, B.C., Tavernini, M.M., Doyle, J.S., and Fedorak, R.N., Association with select-ed bacteria does not cause enterocolitis in IL-10 gene-deficient mice despite a system-ic immune response. Digestive Diseases and Sciences, 2005. 50(5): p. 905-13.

[387] Moran, J.P., Walter, J., Tannock, G.W., Tonkonogy, S.L., and Sartor, R.B., *Bifidobacteri-um animalis* causes extensive duodenitis and mild colonic inflammation in monoasso-ciated interleukin-10-deficient mice. Inflammatory Bowel Diseases, 2009. 15(7): p. 1022-31.

[388] Trexler, P.C. and Reynolds, L.I., Flexible film apparatus for the rearing and use of germfree animals. Applied Microbiology, 1957. 5(6): p. 406-12.

[389] Orcutt, R.P., Application of Gnotobiotic Techniques for Contamination Control, in 50 years of Laboratory Animal Science 1950-2000, W. Charles, Editor 1999, AALAS. p. 125-128.

[390] Dewhirst, F.E., Chien, C.C., Paster, B.J., Ericson, R.L., Orcutt, R.P., et al., Phylogeny of the defined murine microbiota: Altered Schaedler flora. Applied and Environmental Microbiology, 1999. 65(8): p. 3287-3292.

[391] Sarma-Rupavtarm, R.B., Ge, Z.M., Schauer, D.B., Fox, J.G., and Polz, M.F., Spatial distribution and stability of the eight microbial species of the altered Schaedler flora in the mouse gastrointestinal tract. Applied and Environmental Microbiology, 2004. 70(5): p. 2791-2800.

[392] Stehr, M., Greweling, M.C., Tischer, S., Singh, M., Blocker, H., et al., Charles River al-tered Schaedler flora (CRASF) remained stable for four years in a mouse colony housed in individually ventilated cages. Laboratoy Animals, 2009. 43(4): p. 362-70.

[393] Ge, Z., Feng, Y., Taylor, N.S., Ohtani, M., Polz, M.F., et al., Colonization dynamics of altered Schaedler flora is influenced by gender, aging, and *Helicobacter hepaticus* in-fection in the intestines of Swiss Webster mice. Applied and Environmental Microbi-ology, 2006. 72(7): p. 5100-3.

[394] Alexander, A.D., Orcutt, R.P., Henry, J.C., Baker, J., Bissahoyo, A.C., et al., Quantita-tive PCR assays for mouse enteric flora reveal strain-dependent differences in com-position that are influenced by the microenvironment. Mammalian Genome, 2006. 17(11): p. 1093-1104.

[395] Norin, E. and Midtvedt, T., Intestinal microflora functions in laboratory mice claimed to harbor a "normal" intestinal microflora. Is the SPF concept running out of date? Anaerobe, 2010. 16(3): p. 311-3.

[396] Benno, P., Bark, J., Collinder, E., Hellstrom, P.M., Midtvedt, T., et al., Major altera-tions in metabolic activity of intestinal microflora in Crohn's disease. Scandinavian Journal of Gastroenterology, 2012. 47(2): p. 251-2.

[397] Faith, J.J., McNulty, N.P., Rey, F.E., and Gordon, J.I., Predicting a human gut micro-biota's response to diet in gnotobiotic mice. Science, 2011. 333(6038): p. 101-4.

[398] Denou, E., Rezzonico, E., Panoff, J.M., Arigoni, F., and Brussow, H., A Mesocosm of *Lactobacillus johnsonii, Bifidobacterium longum, and Escherichia coli* in the mouse gut. DNA and Cell Biology, 2009. 28(8): p. 413-22.

[399] Mahowald, M.A., Rey, F.E., Seedorf, H., Turnbaugh, P.J., Fulton, R.S., et al., Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proceedings of the National Academy of Sciences of the United States of America, 2009. 106(14): p. 5859-64.

[400] Faith, J.J., Rey, F.E., O'Donnell, D., Karlsson, M., McNulty, N.P., et al., Creating and characterizing communities of human gut microbes in gnotobiotic mice. The ISME journal, 2010. 4(9): p. 1094-8.

[401] Pena, J.A., Li, S.Y., Wilson, P.H., Thibodeau, S.A., Szary, A.J., et al., Genotypic and phenotypic studies of murine intestinal lactobacilli: species differences in mice with and without colitis. Applied and Environmental Microbiology, 2004. 70(1): p. 558-68.

[402] Robertson, B.R., O'Rourke, J.L., Neilan, B.A., Vandamme, P., On, S.L., et al., *Mucispirillum schaedleri* gen. nov., sp. nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. International Journal of Systematic and Evolutionary Microbiology, 2005. 55(Pt 3): p. 1199-204.

[403] Wilkins, T.D., Fulghum, R.S., and Wilkins, J.H., *Eubacterium plexicaudatum* sp. nov., an anaerobic bacterium with a subpolar tuft of flagella, isolated from a mouse cecum. International Journal of Systemic Bacteriology, 1974. 24(4): p. 408-411.

[404] Sakamoto, M. and Benno, Y., Reclassification of *Bacteroides distasonis, Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. International Journal of Systematic and Evolutionary Microbiology, 2006. 56(Pt 7): p. 1599-605.

[405] Song, Y., Liu, C., Lee, J., Bolanos, M., Vaisanen, M.L., et al., "*Bacteroides goldsteinii* sp. nov." isolated from clinical specimens of human intestinal origin. Journal of Clinical Microbiology, 2005. 43(9): p. 4522-7.