Published in final edited form as:

Curr Opin Immunol. 2014 December ; 0: 16–23. doi:10.1016/j.coi.2014.08.004.

Diet, gut microbes, and genetics in immune function: Can we leverage our current knowledge to achieve better outcomes in inflammatory bowel diseases?

Vanessa A. Leone¹, Candace M. Cham¹, and Eugene B. Chang^{1,*}

¹University of Chicago, Department of Medicine, Gastroenterology, Hepatology and Nutrition

Abstract

Autoimmune disorders, particularly inflammatory bowel diseases (IBD), are increasing at an alarming frequency. While the exact cause remains elusive, studies have examined how the immune system is shaped in the context of genetic susceptibility, gut microbes, and environmental pressures, including dietary intake. Shifts towards a Westernized high fat, high carbohydrate diet result in changes to gut microbiota structure and function that may aid in triggering and perpetuating autoimmunity by promoting the emergence of pathobionts leading to altered immune activation. This review summarizes our current understanding of dietary-induced changes in gut microbiota on autoimmunity in the context of IBD. We provide a framework for leveraging this knowledge to develop new dietary, microbial and immune-based modulation strategies for individualized risk assessment and improving clinical outcomes.

Introduction

The development of autoimmune disorders, including inflammatory bowel diseases (IBD), rheumatoid arthritis, type I diabetes, and multiple sclerosis has been increasing in “Westernized” societies over the past half century at an alarming rate. In nearly all of these heterogeneous disorders, both human and murine studies underscore the role of genetics in both the etiology and pathophysiology of these diseases, yet genetic drift alone cannot account for the rapid increase in incidence, particularly in the development of IBD [1–7]. In IBD, environmental factors, including dietary intake and other changes in lifestyle, such as improved hygiene, alcohol consumption, smoking, and altered sleep habits, are believed to have a role in triggering and sustaining chronic disease [8]. Overconsumption (i.e., excess caloric intake) of a “Westernized” high-fat, high carbohydrate diet can have direct effects on host immunity, but it also causes dysbiosis characterized by shifts in the structure and

© 2014 Elsevier Ltd. All rights reserved.

*Corresponding author: Dr. Eugene B. Chang, M.D., Knapp Center for Biomedical Discovery, 900 E. 57th Street, Chicago, IL 60637, echang@medicine.bsd.uchicago.edu, Phone: 773.702.6458 Fax: 773.702.2283.

Conflict of interest: VAL and CMC declare no conflicts of interest. EBC has received funding support for research from Tsumura & Co. to study natural products.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

function of the gut microbiota [9–13]. The development of dysbiosis, in turn, can initiate and sustain states of inflammation through the emergence of pro-inflammatory pathobionts and decline of tolerizing commensal microbiota [14–19]. Under these circumstances, vicious cycles are created that are difficult to break despite clinical interventions. Here, we review the most recent literature relating host genetics, dietary consumption, alterations in gut microbiota, and host immune function in the development of IBD.

Genetic abnormalities of host immune factors implicate gut microbes in the etiology and pathophysiology of IBD

Inflammatory bowel diseases are chronic diseases of the gastrointestinal tract that involve a combination of host genetics and environmental triggers. Although recent literature points to the complexity of the presentation of IBD, disease falls within two phenotypes, Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease can develop along the length of the whole gastrointestinal tract from mouth to anus, however, the majority of cases exhibit regionality, particularly involving the ileum or the colon. UC only involves the colon, developing from the rectum and moving proximally as a contiguous inflammatory process. Both diseases exhibit similar symptoms including abdominal pain, bloody diarrhea, fever, and weight loss [20]. CD is characterized by discrete ulcerations that can extend through the bowel wall, whereas the inflammatory process in UC is confined to the mucosa [21,22].

The involvement of host genetics is an undeniable component to the development of IBD, but it also plays a major role in the assemblage of the gut microbiota and education of the immune system. Many of the 160 genetic variants and polymorphisms identified via genome-wide association studies (GWAS) in IBD relate to immune activation in the context of host response to microbes, including barrier function, wound healing, autophagy, immune balance, and stress responses [23–25]. Over 50% of these genes exhibit overlap between CD and UC, while less than 20% remain unique to either phenotype [23**,26]. The first of these genes to be identified, nucleotide-binding oligomerization domain-containing protein 2 (*NOD2* also called *CARD15*), provided compelling evidence that the gut microbiota might play a role in IBD development and progression, in that *NOD2* encodes an intracellular receptor protein for muramyl dipeptide (MDP), a bacterial peptidoglycan [27,28]. Pattern recognition by *NOD2* initiates the signal transduction that leads to translocation of NF κ B to the nucleus, transcription of specific genes, and activation of appropriate innate and adaptive immune responses [27]. Mutations in *NOD2* lead to decreased production of α -defensins by Paneth cells, which under normal circumstances serve vital innate immune functions that regulate gut microbiota [29]. Indeed, clinical data from surgical specimens obtained from IBD patients displaying mutations in the *NOD2* risk allele show increased Actinobacteria and Proteobacteria, while Clostridium Group XIVa and IV appear decreased compared to controls [30,31]. Furthermore, *Nod2*-deficient mice display significantly increased Bacteroides, Firmicutes, and Bacillus bacterial loads in the terminal ileum, in addition to decreased ability to clear a potentially pathogenic bacteria, *Helicobacter hepaticus* [32]. In the absence of gut microbiota (germ-free mice), *Nod2* expression levels remain low, but can be induced upon conventionalization [33]. Co-housing of wild type (WT) mice with *Nod2*-deficient mice confers increased susceptibility to chemically induced colitis as determined

by increased expression of apoptosis, necrosis, and tumorigenesis genes [33]. Thus, the NOD2 protein is important in the discrimination between commensal microbes and pathogenic bacteria. Subsequent analyses have identified several other IBD susceptibility genes, including *ATG16L1*, *CARD9*, and in some instances, *IL23R*, emphasizing the genetic role of both the innate and adaptive immune system in IBD development [25]. Data show that IL-23R, for instance, is essential for promoting an immune response against intracellular pathogens by inducing the production and maintenance of $\gamma\delta$ T cells that produce IL-17, IFN-gamma, and TNF-alpha and for induction of T-cell mediated intestinal inflammation, particularly in the presence of *H. hepaticus* infection [34–37]. These findings underscore the importance of the microbial milieu within the intestine in driving specific T cell mediated events. Clinical data supports this notion in that examination of serum and epithelial tissue from IBD patients exhibit increased levels of IL-23, suggesting heightened immune activation towards gut microbes [34].

Dysbiosis in IBD - lessons from humans and animal models

While genetic risk is a key element in the development of IBD, it is by itself not sufficient. The concordance rates amongst monozygotic twins in CD or UC, which are between 35–58% and 16–18.5%, respectively, support the notion that other factors are involved [38–40]. Moreover, many individuals that carry genetic variants identified via GWAS remain healthy [41]. Although mice hypomorphic for the *Atg16L1* mutation exhibit Paneth cell abnormalities, such as abnormal lysozyme granule packaging, humans expressing the T300A variant of the *ATG16L1* gene have only a two-fold risk for developing CD [41,42]. Further examination from the *ATG16L1* deficient mouse corroborates that a second hit, (e.g. co-infection with a specific murine norovirus) followed by dextran sodium sulfate (DSS) administration results in a CD-like intestinal pathology [42].

Perhaps even more compelling data for the involvement of gut microbes, environmental factors, and immune activation can be gleaned from other mouse models of IBD. The IL-10 deficient mouse develops spontaneous chronic enterocolitis, predominated by a T-helper 1 (Th1)-mediated IFN-gamma phenotype in most cases. However, germ-free IL-10 deficient mice never develop disease, while disease penetrance varies across genetic background and between animal facilities under specific pathogen-free conditions [43–45]. Addition of specific microbes, including *H. hepaticus*, *Bacteroides vulgatus*, or *Enterococcus faecalis* can increase the penetrance of disease to nearly 100% [44,46,47]. Further evidence that specific microbes can induce phenotypically distinct colitis can be seen in studies utilizing monoassociation of germ-free IL-10 deficient mice with commensal bacteria, including *Bifidobacterium wadsworthia* or *Escherichia coli* [45,48]. *B. wadsworthia* induces mild, low-grade Th-1 mediated colitis in the distal colon that is much more severe when *B. wadsworthia* blooms in the presence of other bacteria [45]. In contrast, *E. coli* monoassociation leads to a mild-to-moderate inflammation that is most severe in the cecum shortly after its introduction [48]. Moderate pan-colitis is induced by monoassociation with *Klebsiella*, while *Bifidobacterium animalis* monoassociation leads to distal colonic and duodenal inflammation [49,50].

Differential induction of colitis by the presence of specific gut microbes can also be seen in the CD4⁺ T-cell mediated CD45RB^{high} adoptive transfer model of colitis, which has been shown to be a predominantly Th1/Th17 inflammatory response [51,52]. Immunodeficient SCID mice with a limited community of gut microbes (devoid of anaerobes and aerobes with the exception of three commensal *Clostridium* species) develop far less severe colitis upon CD4⁺CD45RB^{high} T cell transfer compared to mice harboring a SPF community [53]. An additional study using this model examined the role of segmented filamentous bacteria (SFB) showing that only conventionalization with SPF microbiota + SFB resulted in severe colitis development, while monoassociation with SFB or co-association with specific species, including *Bacteroides distasonis* or *Fusobacterium mortiferum* did not [54]. Isolation of T cell clones that recognize specific cecal antigens has also been shown to induce colitis upon transfer to SCID mice, whereas nonspecifically activated T cells had no effect [55,56]. Further work identified that transfer of CBir1 flagellin-specific T cell clones into SCID mice could induce colitis, providing insight into antigen-driven inflammatory events within the intestine [57]. These data suggest that genetically-susceptible individuals who are in compensated immune states are at risk for shifts in gut microbes that can tip the balance towards the onset of IBD.

Despite the extensive knowledge gained through both GWAS examination of IBD patients and unique experimental models of colitis, the causative versus correlative nature of gut microbes and their interaction with host immune pathways remains incompletely understood in IBD. For instance, it is unclear what events lead to the emergence of pathobionts from the gut microbiota and/or whether the associated changes in microbial structure are actually due to the loss of symbionts from the community. Recent evidence point to the role of environmental factors, particularly diet, in shaping the assemblage of gut microbes and their associated metabolic functions leading to altered host-microbe interactions during inflammation.

Dietary intake as a driver of dysbiosis in the development of IBD

While many environmental factors have recently been associated with changes in the gut microbiome, including the exposure to environmental pollutants and xenobiotics, indiscriminant use of antibiotics, and exercise, changes in diet have to be considered as a potential driver. Human patterns of dietary intake in many societies are rapidly shifting to high-fat, high-sugar “Western” diet, which can dramatically alter gut microbial assemblage and function. Children in Burkina Faso reared on a high-fiber, plant-based diet exhibit a vastly different gut microbial community than their European counterparts who consumed more daily calories from diets richer in sugar, fat, and protein [58]. How these changes influence immune activation and the development of disease was not evident due to the nature of this study. However, through the use of animal models, a causal role for diet-induced changes of gut microbes in the development of disease has been implicated.

Using the genetically-susceptible IL-10 deficient mouse model, Devkota et al showed that feeding a Western-based diet rich in saturated milk fat elicited negative effects on intestinal health. This diet promoted a bloom of the δ -proteobacteria, *Bilophila wadsworthia*, by altering host production of the secondary bile acid, taurocholate providing an organic sulfur

source for this pathobiont to emerge, increasing the incidence and severity of Th1-mediated spontaneous colitis [45**]. While the precise antigen inducing the Th1 response has not been identified, the increased inflammation could, in part, be related to increased levels of H₂S produced by *B. wadsworthia* in the colon. Clinically, patients with UC exhibit increased representation of sulfur-reducing bacteria in their gut microbiota as well as increased production of hydrogen sulfide (H₂S) [59,60]. In non-transformed colonic epithelial cells, H₂S can be genotoxic at concentrations found in the colon as well as modulate expression of genes involved in cell cycle progression, trigger immune/inflammatory responses, and impair DNA repair [61]. Humans placed on an animal product-based diet exhibited a similar expansion of *B. wadsworthia* as well as levels of other bile-tolerant microbes, in the stool mirroring the findings of Devkota et al [62**]. In addition, decreases in Firmicutes involved in metabolism of plant polysaccharides, particularly *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii* were also reduced when compared to samples from individuals exposed to the plant-based, polysaccharide -rich alternative diet [62**].

From a functional perspective, David et al showed that the animal product-based diet reduced microbial carbohydrate fermentation products, particularly short chain fatty acids (SCFAs) due to the low dietary content of plant fiber [62**]. A study examining twin pairs discordant for CD showed that metagenomic and metaproteomic profiles were altered in afflicted twins and decreased microbial diversity was coupled with a reduction in pathways involved in SCFA production [63]. Additionally, a study using methods to predict function via 16s rRNA analyses and validated by shotgun metagenomics on a subset of samples noted a reduction in pathways involved in SCFAs in CD patients compared to healthy controls [64**]. Decreased microbial production of SCFAs correlates with decreased mucosal barrier function as well as impaired colonic T-regulatory cell (Treg) development, shifting immune homeostasis to a more pro-inflammatory state [49,65]. A group of 46 bacterial strains isolated from human stool within *Clostridia* clusters IV, XIVa, XVII induced colonic Treg differentiation, accumulation, and function as well as reduced the severity of trinitrobenzene sulphonic acid (TNBS)-induced colitis in mice [66**]. This was perhaps mediated by the increased bioavailability of SCFAs, which induced TGF- β , promoting the differentiation and expansion of Tregs [66]. The effects of SCFAs on Treg expression and expansion appear to be in part mediated by the G protein coupled receptor (GPR43), promoting epigenetic modifications that involve the inhibition of histone deacetylase [67,68]. Studies show addition of non-absorbed carbohydrates such as inulin and fructooligosaccharides (i.e., prebiotics) to the diet can enhance the growth of SCFA-producing bacteria, such as *Bifidobacterium* or *Lactobacillus* [69–71]. Further work is needed to clarify the positive effects elicited on the immune system, particularly in the context of individuals with IBD susceptibility.

Another intriguing finding by David et al is the transient passing of food-borne microbes through the gut, which reach the distal intestine that may influence the host and the endogenous commensal microbial community, particularly those coming from the animal-based diet [62]. These strains of bacteria could serve as novel probiotic-like species that produce immune modulators that have the capacity to interact with the mucosal immune system. Recently, a group identified a microbial metabolite, 1,4-dihydroxy-2-naphthoic acid

(DHNA), a precursor to Vitamin K2 made by *Propionibacterium freudenreichii* ET-3, a bacterial isolate found on cheese [72**]. Direct administration of DHNA *in vivo* activated the aryl hydrocarbon receptor (AhR), a pathway classically involved in detoxification of environmentally-derived substances with more recently described immunological functions [72**]. Several studies have described AhR's protective role in the development of IBD [73–75]. Here, delivery of DHNA prevented the induction of DSS colitis through activation of anti-microbial peptides, specifically RegIII β and γ , which reduced bacterial load within the gut [72**]. Although the immune cell type involved in DHNA's activity was not defined, based on previous findings, the authors speculated that intestinal epithelial lymphocytes (IELs) or innate lymphoid cells (ILCs) could be targets.

While novel microbial metabolites have been shown to interact directly with AhR to reduce inflammation, other studies identify tryptophan, an essential amino acid, as an important direct and indirect player in the AhR pathway. When tryptophan availability is high within the gastrointestinal tract, several species of microbes, predominantly lactobacilli, are able to utilize this amino acid, producing indole-3-aldehyde, which activates AhR-driven production of IL-22 by ILCs maintaining mucosal homeostasis [76*]. Inversely, an indirect role for host modulation of gut microbes by tryptophan, where tryptophan works through the induction of antimicrobial peptide synthesis via mTOR activation within the epithelium, and in cases where tryptophan is limiting, intestinal homeostasis is lost [77*]. However, opposing roles for tryptophan are also evident. Studies show that following AhR stimulation, tryptophan is converted to kynurenine by indoleamine 2,3-dioxygenase (IDO) in dendritic cells, suppressing their inflammatory capacity. In the absence of AhR, tryptophan conversion is reduced, leading to an aberrant pro-inflammatory T helper-17 cell response [78]. The AhR-tryptophan axis provides a unique opportunity to carefully examine host-microbe interactions and may provide a framework for how to study similar networks and pathways influenced by dietary components in the development of IBD.

Concluding remarks

Recent advances in genetics, immunology, and microbiology have allowed for a more complete understanding of the complex host-microbe interactions in the development of autoimmune disorders, particularly IBD (outlined in figure 1). Data supports the notion that genetic susceptibility of the host can prevent both immune clearance of pathobionts/pathogens, yet can also prevent development of immune tolerance to commensal microbes. How shifts to Western-based dietary intake have altered immune development/tolerance across a genetically heterogeneous population is unclear and given that experimental dietary perturbations are extreme (i.e., 60% kcal from fat when typical human dietary intake ranges from 30–35%), it remains difficult to translate the conclusions. More work is needed that examines the cross-sections between genetic susceptibility, immune development, gut microbes, and dietary intake that are relevant to the human condition. In addition, dietary manipulations and their impact on gut microbes should be examined in a variety of colitis models, since the immunological colitis outcomes can dramatically vary. Lastly, how the gut microbiota interacts with currently available drug therapies should be considered. Haiser et al have clearly shown that specific gut microbes have the ability to inactivate/modify orally-delivered drugs and understanding the microbial metabolic pathways involved can allow for

dietary interventions that prevent microbial inactivation, improving drug efficacy [79**]. This knowledge could be useful to improve host immunological outcomes in the context of currently available IBD therapies.

Despite these challenges, the next task for clinical and basic research is to leverage the information gleaned from recent insights into host-microbe interactions to reshape the gut microbiota, particularly in the context of diet. However, simply reshaping the structure of gut microbes will be insufficient in the face of inflammation, and a more concrete understanding of diet-induced microbial metagenomics and metatranscriptomics will be essential in order to therapeutically harness host-microbe interactions. As we proceed towards an age of personalized medicine, we can leverage the knowledge gained within this framework to use dietary manipulations for IBD prophylactic and therapeutic strategies.

Acknowledgments

The authors received funding from the National Institutes of Health grants P30 DK42086, R37 DK47722, R01 DK097268, T32 DK07074, the Leona M. and Harry B. Helmsley Charitable Trust, and the Gastro-intestinal Research Foundation of Chicago. We would like to thank Elizabeth Zale for assistance with manuscript formatting.

References

- Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006; 444:860–867. [PubMed: 17167474]
- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. *CA Cancer J Clin*. 2005; 55:74–108. [PubMed: 15761078]
- Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterol*. 2004; 126:1504–1517.
- Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterol*. 2012; 142:46–54.
- Tobon GJ, Youinou P, Saraux A. The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. *J Autoimmun*. 2010; 9:10–14. [PubMed: 20080387]
- Vehik K, Dabelea D. The changing epidemiology of type 1 diabetes: why is it going through the roof. *Diabetes Metab Res Rev*. 2011; 27:3–13. [PubMed: 21218503]
- Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol*. 2010; 9:520–532. [PubMed: 20398859]
- Montgomery SM, Ekbom A. Epidemiology of inflammatory bowel disease. *Curr Opin Gastroenterol*. 2002; 18:416–420. [PubMed: 17033315]
- deSouza RJ, Swain JF, Appel LJ, Sacks FM. Alternatives for the macronutrient intake and chronic disease: a comparison of the OmniHeart diets with popular diets and with dietary recommendations. *Am J Clin Nutr*. 2008; 88:1–11. [PubMed: 18614716]
- Turnbaugh P, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*. 2008; 3:213–23. [PubMed: 18407065]
- Turnbaugh P, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med*. 2008; 1:6–14.
- Hildebrandt MA, Hoffman C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen Y, Knight R, Ahima RS, Bushman R, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterol*. 2009; 137:1716–1724.
- Walter J, Ley R. The human gut microbiome; Ecology and recent evolutionary changes. *Annu Rev Microbiol*. 2011; 65:411–429. [PubMed: 21682646]

14. Kirsner JB. Historical aspects of inflammatory bowel disease. *J Clin Gastroenterol.* 1988; 10:286–297. [PubMed: 2980764]
15. Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Folsch UR, Timmis KN, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut.* 2004; 53:685–693. [PubMed: 15082587]
16. Lepage P, Seksik P, Sutren M, delaCochetiere M, Jian R, Marteau P, Dore J. Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. *Inflamm Bowel Dis.* 2006; 11:473–480. [PubMed: 15867587]
17. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux J, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut.* 2006; 55:205–211. [PubMed: 16188921]
18. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A.* 2007; 104:13780–13785. [PubMed: 17699621]
19. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immuno.* 2009; 9:313–323.
20. Podolsky DK. Inflammatory Bowel Disease. *N Engl J Med.* 2002; 347:417–429. [PubMed: 12167685]
21. Kang SS, Bloom SM, Norian LA, Geske MJ, Flavell RA, Stappenbeck TS. An antibiotic-responsive mouse model of fulminant ulcerative colitis. *PLOS Med.* 2008:e41. [PubMed: 18318596]
22. Vucelic B. Inflammatory bowel diseases: controversies in the use of diagnostic procedures. *Dig Dis.* 2009; 27:269–277. [PubMed: 19786751]
- 23*. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature.* 2012; 491:119–124. This study performs a meta-analysis of Crohn's disease and ulcerative colitis genome-wide association scans and have revealed 71 new associations and 163 inflammatory bowel disease (IBD) risk loci that contribute to these common forms of IBD. [PubMed: 23128233]
24. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet.* 2011; 43:246–252. [PubMed: 21297633]
25. Franke A, McGovern DPB, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet.* 2010; 42:1118–1125. [PubMed: 21102463]
26. Ventham NT, Kennedy NA, Nimmo ER, Satsangi J. Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics. *Gastroenterol.* 2013; 145:293–308.
27. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature.* 2001; 411:603–606. [PubMed: 11385577]
28. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morian CA, Gassull M. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001; 411:599–603. [PubMed: 11385576]
29. Wehkamp J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schroder JM. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut.* 2004; 53:1658–1664. [PubMed: 15479689]
30. Frank DN, Robertson CE, Hamm CM, Kpadeh Z, Zhang T, Chen H, Zhu W, Sartor RB, Boedeker EC, Harpaz N. Disease Phenotype and Genotype Are Associated with Shifts in Intestinal-associated Microbiota in Inflammatory Bowel Disease. *Inflamm Bowel Dis.* 2011; 17:179–184. [PubMed: 20839241]

31. Li E, Hamm CM, Gulati AS, Sartor RB, Chen H, Wu X, Zhang T, Rohlf FJ, Zhu W, Gu C. Inflammatory Bowel Diseases Phenotype, *C. difficile* and NOD2 Genotype Are Associated with Shifts in Human Ileum Associated Microbial Composition. *PLOS One*. 2012; 7:e26284. [PubMed: 22719818]
32. Petnicki-Ocwieja T, Hrcir T, Liu YJ, Biswas A, Hudcovic T, Tlaskalova-Hogenova H, Kobyashi KS. Nod2 is required for the regulation of commensal microbiota in the intestine. *Proc Natl Acad Sci U S A*. 2009; 106:15813–15818. [PubMed: 19805227]
33. Couturier-Maillard A, Secher T, Rehman A, Normand S, DeArcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest*. 2013; 123:700–711. [PubMed: 23281400]
34. Uhlig HH, McKenzie BS, Hue S, Thompson C, Joyce-Shaikh B, Stepankova R, Robinson N, Buonocore S, Tlaskalova-Hogenova H, Cua DJ. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity*. 2006; 25:309–318. [PubMed: 16919486]
35. Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ. IL-23 plays a key role in *Helicobacter hepaticus*-induced T-cell-dependent colitis. *J Exp Med*. 2006; 203:2485–2494. [PubMed: 17030948]
36. Yen D, Chueng J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest*. 2006; 116:1310–1316. [PubMed: 16670770]
37. Rio-Blanco L, Lazarevic V, Awasthi A, Mitsdoerffer M, Wilson BS, Croxford A, Waisman A, Kuchroo VK, Glimcher LH, Oukka M. IL-23 receptor regulates unconventional IL-17-producing T cells that control bacterial infections. *J Immuno*. 2010; 184:1710–1720.
38. Spehlmann ME, Begun AZ, Burghardt J, Lepage P, Raedler A, Schreiber S. Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study. *Inflamm Bowel Dis*. 2008; 14:968–976. [PubMed: 18253950]
39. Orholm M, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol*. 2000; 35:1075–1081. [PubMed: 11099061]
40. Halfvarson J, Bodin L, Tysk C, Lindberg E, Jarnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterol*. 2003; 124:1767–1773.
41. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, Bagnall R, Mirza MM, Sanderson J, Mansfield JC. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterol*. 2007; 132:1665–1671.
42. Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, Head RD, Xavier R, Stappenbeck TS, Virgin HW. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell*. 2010; 141:1135–1145. [PubMed: 20602997]
43. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennic DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10 deficient mice. *Infect Immun*. 1998; 66:5224–5231. [PubMed: 9784526]
44. Kullberg MC, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun*. 1998; 66:5157–5166. [PubMed: 9784517]
- 45*. Devkota SD, Wang Y, Musch MW, Leone VL, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in IL10^{-/-} mice. *Nature*. 2012; 487:104–108. This study found that high dietary milk fat-derived saturated fats promotes the expansion of *Bifidobacterium wadsworthia* – a sulphite-reducing pathobiont that perturbs gut immune homeostasis and significantly increases the incidence of colitis in genetically susceptible IL-10^(-/-) mice. [PubMed: 22722865]
46. Ward JM, Anver MR, Haines DC, Melhorn JM, Gorelick P, Yan L, Fox JG. Inflammatory large bowel disease in immunodeficient mice naturally infected with *Helicobacter hepaticus*. *Lab Anim Sci*. 1996; 46:15–20. [PubMed: 8699813]

47. Balish E, Warner T. Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. *Am J Pathol.* 2002; 160:2253–2257. [PubMed: 12057927]
48. Kim SC, Tonkonogy SL, Albright CA, Tsang K, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterol.* 2005; 128:891–906.
49. Sartor, RB. Microbial influences in inflammatory bowel disease: role in pathogenesis and clinical implications. In: Sartor, RB.; Sandborn, WJ., editors. *Kirsner's Inflammatory Bowel Diseases.* Philadelphia: Elsevier; 2004. p. 138-162.
50. Moran JP, Walter J, Tannock GW, Tonkonogy SL, Sartor RB. Bifidobacterium animalis causes extensive duodenitis and mild colonic inflammation in monoassociated interleukin-10-deficient mice. *Inflamm Bowel Dis.* 2009; 15:1022–1031. [PubMed: 19235917]
51. Powrie F, Leach MW, Mauze S, Baddle LB, Coffman RL. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 SCID mice. *Int Immunol.* 1993; 5:1461–1471. [PubMed: 7903159]
52. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity.* 1994; 1:553–562. [PubMed: 7600284]
53. Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. *J Immunol.* 1997; 158:3464–3473. [PubMed: 9120308]
54. Stepankova R, Powrie F, Kofronova O, Kozakova H, Hudcovic T, Hrnecir T, Uhlig H, Read S, Rehakova Z, Benada O. Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. *Inflamm Bowel Dis.* 2007; 13:1202–1211. [PubMed: 17607724]
55. Cong Y, Weaver CT, Lazenby A, Elson CO. Colitis induced by enteric bacterial antigen-specific CD4+ T cells requires CD40-CD40 ligand interactions for sustained increase in mucosal IL-12. *J Immuno.* 2000; 165:2173–2182.
56. Cong Y, Weaver CT, Lazenby A, Elson CO. Bacterial-reactive T regulatory cells inhibit pathogenic immune responses to the enteric flora. *J Immunol.* 2002; 169:6112–6119. [PubMed: 12444113]
57. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hersherberg RM. Bacterial flagellin is a dominant antigen in Crohn's disease. *J Clin Invest.* 2004; 113:1296–1306. [PubMed: 15124021]
58. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A.* 2010; 107:14691–14696. [PubMed: 20679230]
59. Roediger WE, Moore J, Babidge W. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. *Dig Dis Sci.* 1997; 42:1571–1579. [PubMed: 9286219]
60. Levine J, Ellis CJ, Furne JK, Springfield J, Levitt MD. Fecal hydrogen sulfide production in ulcerative colitis. *Am J Gastroenterol.* 1998; 93:83–87. [PubMed: 9448181]
61. Attene-Ramos MS, Nava GM, Muellner MG, Wagner ED, Plewa MJ, Gaskins HR. DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells. *Environ Mol Mutagen.* 2010; 51:304–314. [PubMed: 20120018]
- 62**. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014; 505:559–563. This study shows that the short-term consumption of diets composed entirely of animal or plant products vastly alters the human gut microbiome and eliminates significant inter-individual differences in microbial gene expression. [PubMed: 24336217]
63. Erickson AR, Cantarel BL, Lamendella R, Darzi Y, Mongodin EF, Pan C, Shah M, Halfvarson J, Tysk C, Raes J. Integrated metagenomics/metaproteomics reveals human host-microbiota signatures of Crohn's disease. *PLOS One.* 2012; 7:e49138. [PubMed: 23209564]
- 64**. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB. Dysfunction of the intestinal microbiome in inflammatory bowel

- disease and treatment. *Genome Biol.* 2012; 13:R79. This study, via 16S gene pyrosequencing and shotgun metagenomic analysis of the microbiota of 231 inflammatory bowel disease (IBD) and healthy subjects, revealed that Crohn's disease and ulcerative colitis result from alterations in intestinal microbiota that impacts the immune system. [PubMed: 23013615]
65. Vital M, Penton CR, Wang Q, Young VB, Antonopoulos DA, Sogin ML, Morrison HG, Raffals L, Chang EB, Huffnagle GB. A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. *Microbiome.* 2013; 1:8. [PubMed: 24451334]
 - 66*. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature.* 2013; 500:232–236. This study shows that 17 strains of Clostridia isolated from a healthy human faecal sample, significantly induced CD4⁺FOXP3⁺ regulatory T cells and attenuate both colitis and allergic diarrhea in mice. [PubMed: 23842501]
 - 67*. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science.* 2013; 341:569–573. Short-chain fatty acids, gut derived from bacterial fermentation, were regulate the size and function of colonic Tregs that protect against experimental colitis in mice. [PubMed: 23828891]
 68. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierror F, Yu D, Schitler HC, Rolph MS, Mackay F, Artis D. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature.* 2009; 461:1282–1286. [PubMed: 19865172]
 69. Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. *Appl Environ Microbiol.* 2005; 71:6150–6158. [PubMed: 16204533]
 70. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut.* 2013; 62:1112–1121. [PubMed: 23135760]
 71. Watson D, O'Connell Motherway M, Schoterman MH, van Neerven RJ, Nauta A, van Sinderen D. Selective carbohydrate utilization by lactobacilli and bifidobacteria. *J Appl Microbiol.* 2013; 114:1132–1146. [PubMed: 23240984]
 - 72*. Fukumoto S, Toshimitsu T, Matsuoka S, Maruyama A, Oh-Oka K, Takamura T, Nakamura Y, Ishimaru K, Fujii-Kuriyama, Ikegami S. Identification of a probiotic bacteria-derived activator of the aryl hydrocarbon receptor that inhibits colitis. *Immunol Cell Bio.* 2014; 92:460–465. This study shows that delivery of 1,4-dihydroxy-2-naphthoic acid (DHNA), a precursor of vitamin K2, that is abundantly produced by *Propionibacterium freudenreichii* ET-3, a bacterial isolate from Swiss-type cheese, modulates activity of the aryl hydrocarbon receptor (AhR) pathway and suppresses development of colitis. [PubMed: 24518984]
 73. Monteleone I, Macdonald TT, Pallone F, Monteleone G. The aryl hydrocarbon receptor in inflammatory bowel disease: linking the environment to disease pathogenesis. *Curr Opin Gastroenterol.* 2012; 28:310–313. [PubMed: 22450895]
 74. Takamura T, Harama D, Matsuoka S, Shimokawa N, Nakamura Y, Okumura K, Ogawa H, Kitamura M, Nakao A. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. *Immunol Cell Biol.* 2010; 88:685–689. [PubMed: 20231854]
 75. Monteleone I, Rizzo A, Sarra M, Sica G, Sileri P, Biancone L, MacDonald TT, Pallone F, Monteleone G. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterol.* 2011; 141:237–248.
 - 76*. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, Zecchi R, D'Angelo C, Massi-Benedetti C, Fallarino F. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity.* 2013; 39:372–385. This study shows that supplying endogenous dietary tryptophan (Trp) in mice promoted the expansion of a highly-adaptive lactobacilli strain that produced an aryl hydrocarbon receptor (AhR) ligand-indole-3-aldehyde which, in turn, resulted in a IL-22-dependent, balanced mucosal immune response. [PubMed: 23973224]

- 77*. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature*. 2012; 487:477–481. This study shows that deficiency of angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2), which is encoded from the key regulatory enzyme of the rennin-angiotensin system (RAS), results in a significantly increased susceptibility to intestinal inflammation and epithelial damage in the presence of high levels of tryptophan. [PubMed: 22837003]
78. Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci USA*. 2010; 107:19961–19966. [PubMed: 21041655]
- 79**. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science*. 2013; 341:295–298. This study identified the mechanism by which the gut microbe *Eggerthella Lenta* inactivates digoxin, a common cardiac drug, decreasing its efficacy within the host and also showed that availability of free amino acids from the diet inhibited *E.lenta*'s ability to inactivate digoxin, leading to improved host bioavailability. [PubMed: 23869020]

Figure 1.

Environmental factors, particularly Westernized dietary intake, can modulate complex host-microbe immune interactions that precipitate disease in the genetically-susceptible host (i.e., NOD2, ATG16L1, IL23R, and/or AHR). Genetic susceptibility can lead to initial alterations in microbial assemblage. Environmental influences, particularly increases in dietary saturated fat and refined carbohydrates with concomitant decreases in complex carbohydrates (fiber) result in a second hit to further modulate gut microbial structure and function by decreasing commensal microbes that produce beneficial short chain fatty acids (SCFAs) and promote the bloom of pathobionts. In addition, saturated fat can alter the profile of host bile acid production (i.e., taurocholate), which, in turn, serves as another selection pressure for the emergence of pathobionts (i.e., *Bilophila wadsworthia*) from the microbial community by acting as a source of organic sulfur for the production of H₂S. In the face of genetic susceptibility (i.e., loss of function in AhR) excess dietary components (i.e., tryptophan) can elicit a direct effect on host immune activation, increasing downstream inflammatory events. Transient exposure to food-borne microbiota and their microbial metabolites can be beneficial (i.e., DHNA delivery to the distal bowel and activation of the Aryl hydrocarbon receptor (AhR), but the quantity of metabolites delivered via probiotic-like bacteria may not be sufficient to alleviate inflammation. Ultimately, the alterations gut microbiota and their subsequent metabolites leads to increased immunogenic antigen exposure, prompting inflammatory cytokine production of both antigen-presenting cells (i.e., IL-12p40) and T cells (i.e., IFN-gamma) that increase intestinal inflammation resulting in progressive loops of immune activation and inflammation in the host that are difficult to

break (red arrows refer to potential detrimental impacts; green arrows refer to potential beneficial impacts).