Genes and Environment: How Will Our Concepts on the Pathophysiology of IBD Develop in the Future?

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Abstract

Inflammatory bowel disease (IBD) has long been known to arise from the interplay between host and environmental factors. From this, a picture is currently emerging in which IBD is likely the result of a continuum of diseases that range from mono- and oligogenically inherited familial forms at one extreme to sporadic forms at the other extreme, which are polygenic in origin and strongly influenced by environmental factors and especially those of infectious origin. The recent expansion of knowledge on the genetic underpinning of IBD has revealed several converging and inter-related functional host pathways that are central to the pathogenesis of these disorders. These include pathways such as autophagy, intracellular bacterial sensing and the unfolded protein response, which play specific roles at the interface between the host and the highly complex microbial communities within the intestines. As such they focus on the functional relationship between the intestinal epithelium

and the unique microbial and immune environments along its luminal and abluminal surfaces. Thus, the genetic and environmental factors which are relevant to IBD seem to have the common property of influencing disease by virtue of their specific impact upon the functional relationship between these microbial communities and the intestinal immune system.

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Introduction

Since its original description by Crohn and colleagues in the 1930s, it has been appreciated that inflammatory bowel disease (IBD) has a genetic and environmental basis [1]. The former was derived from observations from Crohn himself that IBD tended to occur within families. Similarly, the immediate recognition that Crohn's disease (CD) exhibited pathologic similarities with intestinal infections such as those derived from *Mycobacterium tuberculosis*, suggested a potential environmental component [2]. The latter stimulated an unsuccessful long-term

search for the identification of a pathogenic microorganism. Nonetheless, after years of intense scrutiny, the common view is that IBD represents a genetically determined and inappropriate response, not to a pathogen but rather to some component(s) of the commensal microbiota with yet-to-be defined environmental factors playing an important role in modifying this genetically defined risk [3]. This review will focus on the current concepts that underlie the interaction between genes and environment in the pathogenesis of IBD.

Epidemiologic Evidence for the Genetic Basis of IBD and the Role of the Environment

Approximately 10% of patients with IBD, either ulcerative colitis (UC) or CD will report a family history of IBD [4]. Moreover, the concordance of IBD in monozygotic twins is quite high. For example, the relative risk for concordance of CD in a monozygotic twin pair is approximately 800-fold greater than the general population [4]. Similarly, the concordance for UC in a monozygotic twin is on the order of 10–20% [4]. Although the relative risk for UC in a monozygotic twin is lower than that observed in CD this does not rule out a significant role for genetic factors in the pathogenesis of UC as well. It is well known that monozygotic twins are not completely identical genetically. For example, somatically derived copy number variations through deletion or duplication or epigenetic modifications that exist in twins may further limit the genetic relatedness of monozygotic twins [5]. It is also possible that a lack of access to relevant environmental exposures in UC may account for the decreased concordance of UC in monozygotic twins relative to that observed in monozygotic twins with CD. It is therefore clear that there is a complex relationship between genetic susceptibility and environment and that this heritability is likely to be polygenic in nature in the vast majority of patients with IBD.

However, it has also become evident that IBD can derive from the Mendelian inheritance of single genes. For example, patients who are genetically deficient in the *FOXP3* gene which controls the development of both natural (thymically-derived) or induced regulatory T cells within the intestines and which secrete inhibitory cytokines such as interleukin (IL)-10, IL-35 and TGF- β may develop a disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, x-linked) [6]. A subset, but not all, of these *FoxP3*-deficient individuals will develop enteropathy [6]. This suggests that the phe-

notypic manifestations are due to either specific genetic mutations in *FOXP3*, the contribution of other modifying genes or possibly a role for environmental modifying factors. Similarly, a subset of humans that are deficient in the Wiskott-Aldrich syndrome protein (WASP) and develop the Wiskott-Aldrich syndrome manifest a UC-like disease [7, 8]. Mouse models have suggested a complex pathogenesis that may also involve dysfunction of regulatory T cells [7, 8]. Finally, recent studies have revealed that early onset of IBD that develops in the first few years of life may be due to mutations in single genes. For example, kindreds have been described in which mutations in the IL-10 receptor α and receptor β chains that regulate the responses to IL-10 and, at least in the case of the IL-10 receptor β chain, also to IL-22 are associated with CD [9]. It is well known that IL-10 deficient mice and mice that are deficient in the IL-10 receptor β chain are susceptible to colitis and that IL-10 signaling through the IL-10 receptor involves other genetic risk factors previously described to be associated with IBD (both UC and CD) [10]. The latter involve the signaling proteins STAT3 and JAK2 [11]. Moreover, IL-22 has been shown to be associated with protection from intestinal inflammation via the production of mucus by goblet cells [12]. It is thus clear that mutations in genes that are centrally important to the maintenance of intestinal homeostasis between the immune system and the commensal bacteria are highly important to the pathogenesis of IBD. These studies also show that single genetic mutations may result in the development of IBD. However, it is also clear, as noted, that not all patients with these single genetic mutations develop intestinal inflammation, further highlighting the potential interactive relationship between genes and environment in the development of these disorders.

It might be considered therefore (fig. 1) that IBD is a syndrome of overlapping phenotypes that involves variable influences of genetic and environmental factors. In this model, the immunobiology of familial IBD (approximately 10% of IBD patients) may be different from non-familial IBD (sporadic), which constitutes the vast majority of IBD. Within this framework there may be monogenic, oligogenic and polygenic forms of IBD which require various numbers of relevant, overlapping and specific environmental factors for the disease to be expressed. At the two polar extremes of this model are the monogenic (simple mendelian) origins of disease as seen in early onset IBD or, at the other end, those in which IBD represents the expression of a yet-to-be defined infectious pathogen in which the environmental exposure is of cen-

tral importance relative to genetic factors. This model remains to be demonstrated but is an interesting potential framework within which the disease might be considered.

A recent example has been described which raises the possibility that a subset of IBD patients may be a consequence of a yet-to-be described environmental enteropathogen. In a recent study, Zhang and colleagues performed a genome wide association study of patients with multibacillary and paucibacillary leprosy. This study observed that risk alleles were associated with both forms of leprosy, especially the multibacillary form [13]. This study showed that genes associated with the regulation of autophagy (*LRRK2*), the intracellular sensing of bacteria (*NOD2* and *RIPK2*), those associated with presentation of peptides by innate immune cells to adaptive immune cells such as T lymphocytes (*HLA-DRB1*) and finally genes associated with the secretion of the TNF-like family member TL1A (TNFSF15) are involved in the regulation of the response to leprosy [13]. The genetic similarities between CD and this mycobacterial infection are quite remarkable and suggest that either a subset of IBD patients is due to an infectious pathogen or perhaps more likely that the immune response to the commensal bacteria involved in the pathogenesis of CD involves an immunologic-response pathway that is also observed during *M. leprae* infection.

A Two-Hit Hypothesis to Explain the Pathogenesis of IBD

Based upon the aforementioned comments, it would appear that IBD emerges from the effects of particular environmental factors in a genetically susceptible host on the interactions between the mucosal immune system and commensal microbiota. In this model, the major antigenic drive to the immune activation observed in IBD is that derived from the commensal microbiota; both its antigenic determinants and metabolic factors. A description of this model is shown in figure 2 and proceeds below.

Recent studies into the genetic basis of both clinical forms of IBD (UC and CD) have helped to define a number of general pathways that are involved in IBD pathogenesis. These include genes associated with the regulation of innate and adaptive immunity (e.g. *IL10*, *IL23R*, *STAT3*, *JAK2*), those that are associated with the regulation of inflammation (e.g. *CCR6*, *MST1*) and those that regulate endoplasmic reticulum (ER) stress and autophagy (*XBPI*, *ORMDL3*, *ATG16L1*, *IRGM*) [3]. It is clear that these genetically determined pathways have profound ef-

Fig. 1. The syndromic nature of IBD: a model.

Fig. 2. A 'two-hit' hypothesis for the pathogenesis of IBD. Genetic and environmental factors are risk factors for IBD as they determine the composition and function of the microbiota and the immune responsiveness of the host to microbial factors. NSAIA = Nonsteroidal anti-inflammatory agents.

fects on the manner in which the immune system is regulated and the way in which it responds to commensal bacteria. Similarly, these genetic pathways are important determinants of the composition of the commensal bacterial architecture that is contained within the intestinal milieu mainly through the regulation of inflammation per se and the function of Paneth cells that reside deep within the epithelial crypts of the small intestine [14, 15]. Paneth cells are also observed in the colon during inflammation, either idiopathic or due to enteropathogens [16]. Paneth cells are very important sources of antimicrobial peptides as will be discussed below.

In a similar manner, there is increasing evidence that the environmental factors which have been epidemiolog-

ically linked to the pathogenesis of IBD specifically affect either the regulation of the immune response within mucosal tissues or the composition and function of the commensal microbiota. For example, factors such as smoking, nonsteroidal anti-inflammatory agents or appendectomy have important effects on the regulation of the mucosal immune system [17]. Similarly, antibiotics, diet and enteropathogenic infections can have profound influences on the composition and function of the commensal microbiota [18–22]. Moreover, it is likely that genetic susceptibility is further modified by environmental factors through epigenetic changes and that genetic susceptibility can modify the response to environmental factors [23].

Thus, in a two-hit model, it can be hypothesized that necessary events for the development of IBD are an accumulation of genetic and environmental factors that both influence the composition and function of the commensal microbiota and the composition and function of the immune system associated with the intestinal tissues and its responsiveness to the commensal microbiota. In this model, disruption of the commensal microbial architecture alone without changes in the immune system's responsiveness to the commensal microbiota and vice versa will not necessarily culminate in the development of IBD. This model remains to be tested but elements of it will be discussed in some detail below.

Commensal Microbiota and IBD

The relationship between the commensal microbiota and IBD is needless to say very complex. A significant number of studies in both animal models of IBD and humans with IBD support the commensal microbiota as the major source of the antigenic drive that is responsible for the development of these disorders. This has been extensively reviewed elsewhere and will only be briefly touched upon here [24, 25]. Studies that support a role for the commensal microbiota in IBD are briefly as follows. In humans, IBD is mainly observed in the areas with the highest concentrations of microbiota (i.e. colon and distal small intestine) and antibiotics may be beneficial as a primary therapy in the treatment of CD in particular and complications of CD such as fistula [26, 27]. The study of mouse models has also strongly supported a role for the commensal microbiota as being the primary immunologic driver of the inflammation observed in these disorders such as the observations that a variety of different genetically susceptible animal models do not develop in-

testinal inflammation in the absence of an intestinal microbiota (that is, under germ-free conditions) [28].

These observations have focused significant attention of the scientific community on the composition of the microbiota. The information available has been recently extensively reviewed [24, 29]. However, these studies have clearly shown that the quantity of bacteria contained within the normal intestine are 10-fold greater than the numbers of cells in the human body and that the genetic repertoire of the microbiota is a hundred-fold greater than the expressed human genetic repertoire [30].

The human intestine contains a variety of different life forms that are predominantly bacteria but also include eukarya, viruses and archaea [29, 31]. It is estimated that there are more than 400 species of bacteria in the colonic milieu and that a significant number of these cannot be cultivated [29, 31]. This has required the development of culture-independent methodologies such as next generation, high-throughput sequencing of 16S rDNA and 'shotgun' metagenomic sequencing of bacterial DNA within the intestines. These studies have shown that the majority of bacteria within the intestines are contained within two major divisions of bacteria. These are the Bacteroidetes (predominantly Gram-negative organisms) and Firmicutes (predominantly Gram-positive organisms) [31]. In addition, other divisions of bacteria that are contained within the intestines include Proteobacteria, Actinobacteria, Fusobacteria and others [31].

The source of the microbiota and their composition are determined by a number of different factors. Most of the intestinal microbiome is inherited from the mother in early life [20]. This inherited microbiota and associated microbiome (the expressed genes) are further modified by genetic and environmental factors. The environmental factors include diet and other administered environmental factors (e.g. antibiotics). Antibiotics generate profound changes in the composition of the microbiota and consequently the immunologic activity of mucosal tissues with unknown consequences [21, 22].

A variety of genetically defined host factors also regulate the composition of microbiota. Among the many host factors that are involved in regulating composition of the microbiota are some that are worthy of note. These included the NADPH oxidase or dual oxidase system which has been shown in *Drosophila melanogaster* for example to regulate the quantity of bacteria within the intestines [32, 33]. The human neutrophil NADPH oxidase encoded by the *NCF4* gene is interestingly a genetic risk factor for the development of CD [34]. Another example of a host factor that regulates the composition of the com-

commensal microbiota is secretory immunoglobulin. Secretory IgA, in particular, has been shown to be regulated by and regulates the composition of commensal bacteria [35]. Perhaps the most important factor described to date is that which is associated with the regulation and secretion of antimicrobial peptides which are produced by epithelia. Many of these antimicrobial peptides are regulated by NF κ B, a transcription factor that is regulated by pattern recognition receptors and cytokine receptors [36]. Perhaps one of the most important types of antimicrobial peptides that are regulated in this manner are the α -defensins which are secreted by Paneth cells. It is now clear that Paneth cell expression of α -defensins is very important in the regulation of the composition of the intestinal microbiota and the ability of the intestinal microbiota to adhere to the intestinal epithelium [36–38].

Paneth Cells: An Intestinal Epithelial Cell Type at the Interface between Commensal Microbiota and Mucosal Immune System

The intestinal epithelial stem cell differentiates under the control of a variety of different transcription factors to develop into three cell types that migrate to the villus (goblet cells, enteroendocrine cells and absorptive epithelial cells) and a unique cell type that differentiates and migrates to reside within the base of the intestinal crypt. This latter cell type is the Paneth cell which is well known to be highly secretory and to produce significant quantities of antimicrobial peptides that include the α -defensins. Paneth cells provide antimicrobial protection in the small intestine and within the colon during the course of inflammation, including that associated with IBD and intestinal infections [39]. The production of α -defensins by Paneth cells is under the control of commensal bacteria through Toll receptor-like (TLR) signaling and in turn controls the composition of the bacteria [14, 36, 40]. It is not surprising therefore that environmental factors that affect the composition of the microbiota would potentially affect the activity of Paneth cells, and that Paneth cells in turn would be potentially important in antimicrobial defense along the epithelial cell surfaces. It is therefore interesting and commensurate with the bacterial hypothesis associated with IBD that a number of genetic risk factors that have been associated with risk for the development of IBD have been shown to be important in the biology of Paneth cells. These genetic risk factors include *NOD2* that is associated with intercellular bacterial sensing, *ATG16L1* that is associated with autophagy,

XBPI that is associated with regulation of ER stress and possibly *TCF4* which is involved in intestinal epithelial cell differentiation [34, 41–43]. The Paneth cell therefore is a cell type that is potentially influenced by a variety of environmental factors, is capable of influencing the composition of the commensal microbiota and is subject to regulation by a number of genetic factors that have been associated with risk for the development of both CD and UC. These pathways that affect Paneth cells will be discussed below.

Autophagy, Intracellular Bacteria Sensing and the Unfolded Protein Response: A Continuum?

Autophagy is an excellent example of a genetically mediated pathway which is abnormal in IBD and is very prone to influences by environmental forces. Autophagy is one of the most evolutionarily conserved cellular responses and is mainly initiated by changes in nutrient access. It has been extensively reviewed elsewhere [44, 45]. Autophagy represents the self-digestion of organelles and ingested extracellular bacteria and other types of pathogens via lysosomal degradation [44, 45]. Autophagy involves multiple steps of membrane fusion that are directed by groups of autophagy proteins (ATG) that drive the initial nucleation of ER-derived membranes with the formation of an autophagosome that fuses with lysosomes to create an autolysosome, which is an environment that is very important to the degradation of the internalized materials [44, 45]. A number of genes that have been linked to the pathogenesis of CD in particular, including *ATG16L1*, *IRGM* and *LRRK2* are involved in autophagy [11, 46].

It has been recently recognized that loss of *ATG16L1* function in mice and an examination of patients with the causal variant of *ATG16L1* that is associated with CD (T300A) results in abnormal Paneth cell structure and function in both mouse and human [15]. In both mouse and human, loss of *ATG16L1* function leads to abnormalities of the granular structure of Paneth cells that possess the antimicrobial peptides and a pro-inflammatory tone of the Paneth cell resulting in increased expression of transcripts for TNF, leptin, adiponectin, and serum amyloid A, an acute phase reactant [15]. It is also interesting that recent studies further suggest that the abnormalities in Paneth cell structure and function that are related to polymorphisms in *ATG16L1* are dependent upon exogenous environmental factors and in particular the presence of noroviral infection [Virgin HW and Stappenbeck T,

pers. commun.]. This is an excellent example of gene environment interactions in the pathogenesis of IBD and shows how a particular phenotype associated with a genetic risk factor may require the presence of a specific environmental factor.

Another gene of interest which is involved in intracellular bacteria sensing that is also related to Paneth cell function is *NOD2*, which encodes NOD2 or so-called caspase associated recruitment domain related protein 15 (CARD15) [47]. *NOD2* is an intracellular bacteria, mycobacteria and viral sensor. *NOD2* consists of three domains [47]. The first is a leucine rich repeat (LRR) domain that binds muramyl dipeptide derived peptidoglycan from either gram negative or gram positive bacteria, a glycolyl-derived muramyl dipeptide derived from mycobacteria or single-stranded RNA from viruses. The other two are a nucleotide oligomerization domain (NOD) that is linked to a caspase associated recruitment domain which provides the link to intracellular signaling [47]. When *NOD2* oligomerizes upon binding, its microbial-associated molecular pattern (MAMP) or pathogen-associated molecular pattern (PAMP) activates either a kinase, RICK, which leads to NF κ B activation or IRF3 that leads to interferon- β production [48–50]. In CD three major loss-of-function mutations have been described within the LRR that are associated with CD specifically. These three loss-of-function proteins cannot bind to the *NOD2*-related MAMPs or PAMPs and are derived from either one or another of the following mutations: R702W, G908R, and 1007fs [51].

A number of potential mechanisms have been described as potential mechanisms for the way in which *NOD2* is involved in the pathogenesis of CD. There are three overarching mechanisms that have been described as potentially explanatory for the pathogenesis of CD. These include a lack of mucosal tolerance to bacteria in the context of loss of function *NOD2* mutations given the observations that *NOD2* may be a negative regulator of TLR2-mediated responses and that *NOD2* may mediate tolerance to bacterial products [52–54]. Similarly, *NOD2* has been recently shown to be an important regulator of autophagy since *NOD2* can activate ATG-5, 7 and 16L1 via RIP2 to regulate the autophagic clearance of bacteria as well as the regulation of antigen presentation [55]. Finally, *NOD2*-deficient mice exhibit decreased α -defensin expression with normal Paneth cell structure [36]. Similarly, humans with CD that possess *NOD2*-related risk alleles may exhibit reduced Paneth cell α -defensins [56]. This has suggested that *NOD2* may also regulate Paneth cell function. Taken together, it is clear that

NOD2 is an important sensor of bacteria that have entered into the cytosol of cells such as intestinal epithelial cells and hematopoietic cells and is therefore an important host factor that may be affected by environmental influences.

A final example of gene-environment interactions that also interestingly involves Paneth cells is that which is associated with the unfolded protein response (UPR). The UPR is a response that occurs as a consequence of ER stress mainly due the accumulation of misfolded or unfolded proteins in the ER [57]. As such, highly secretory cells are very sensitive to ER stress and therefore require a robust UPR for the maintenance of homeostasis [57]. In the presence of ER stress, the UPR initiates a variety of adaptive mechanisms that lead to the temporary halt in translation, the induction of chaperones which enhance the secretion of proteins, chaperones that are involved in assisting in protein folding, the induction of autophagy and an enhancement in the ER associated degradative machinery that is related to protein quality control mechanisms [57]. In the presence of unabated ER stress, the UPR initiates apoptosis [41].

There are three major pathways in mammals that regulate the UPR. These have been extensively reviewed and involve either activation of the transcription factors ATF4 (activating transcription factor 4) and ATF5 secondary to the sensing of misfolded proteins in lumen of the ER by pancreatic ER kinase [57]. A second pathway involves the cleavage of the cytoplasmic tail of the ATF6p90 protein that is transcriptionally active (i.e. ATF6p50) [57]. Finally, recognition of misfolded proteins in the ER by inositol requiring enzyme 1 (IRE1) leads to the splicing of X box binding protein 1 (XBP1) mRNA that results in an alternate spliced isoform of XBP1 (XBP1s) that is transcriptionally active in inducing a program of genes involved in the UPR [57].

XBP1 has recently been linked to UC and CD as a genetic risk factor for IBD [41]. Deletion of XBP1 expression in epithelia has revealed the potential mechanism for this potential risk allele. Specifically XBP1 deficient epithelia lack Paneth cells due to programmed cell death that is associated with spontaneous enteritis and susceptibility to colitis [41]. These studies show that proper resolution of ER stress by IRE1-XBP1 function is important in the maintenance of homeostasis. In the presence of unabated ER stress or the inability to manage this stress by a proper UPR, IRE1 activation in the context of deficient XBP1 function has been shown to result in a hyperproliferation of the epithelium and increased inflammatory tone of the epithelium in response to bacterial products and cyto-

Fig. 3. The immunogenetic relationship between the processes of the UPR in response to ER stress, autophagy and intracellular (myco)bacterial and viral sensing. Genetic factors in gray type (red in the color version) are those specific for CD, those in bold type (blue in the color version) are observed in UC and CD, and those in normal-weight black type have only been determined to date in animal models.

kines through Jun-related kinase (JNK) and NFκB signaling as well as Paneth cell death [41]. This is associated with decreased mucosal barrier function and susceptibility to development of inflammation in the first instance and likely a susceptibility to perpetuation of susceptibility in the second instance.

A variety of environmental (secondary) factors may regulate the ER stress response including those that potentially associate themselves with XBP1 and other elements of the UPR. Bacterial factors (e.g. Shiga 'toxigenic' *Escherichia coli*), dietary factors (e.g. fatty acids and glucose deprivation), environmental factors (e.g. drugs such as the HIV protease inhibitor lopinavir), inflammation (e.g. hypoxia, TNF and IL-10), and even neurogenic stress (e.g. dopaminergic signaling) may lead to either increases or decreases in the UPR and may either promote or ameliorate ER stress [57]. Given this, it can be hypothesized that a host's genetic ability to manage this variety of environmental secondary factors may be an important determinant of the development of intestinal inflammation or its perpetuation. Several genetic factors whose primary role is to regulate the UPR as a consequence of ER stress have been shown to be associated with risk for developing IBD. These genetic factors include *XBP1*, anterior gradient 2 (*AGR2*) and orsomucoid like gene 3 (*ORMDL3*) [11, 41, 58–60]. Moreover, a variety of other genetic factors that result in abnormalities of protein folding, such as mutations in HLA-27 or mucin glycoproteins, may also secondarily cause ER stress [61, 62]. Therefore both primary and secondary genetic factors may regulate the ability of a host to respond to a variety of secondary environmental factors.

It is clear therefore that the genetically imposed ability of the host to regulate and be regulated by the microbiota

is an important determinant of the mucosal and systemic state of the immune response. It is also evident that microbiota can regulate innate immune functions, adaptive immune functions as well as the resolution of inflammation such as the through the binding of the metabolic products to particular host cell receptors. With regard to the latter, short chain fatty acids have been shown to bind a G protein coupled receptor 43 (GPCR43) on neutrophils to promote the resolution of inflammation in the intestines as well as extra-intestinal tissues (e.g. lung and joints) [63]. These studies suggest that the genetic composition of the host may regulate the microbiota and that environmental factors that regulate the microbiota or regulate the immune responsiveness of the host to the microbiota are important determinants of host immune tone and the susceptibility to intestinal inflammation. Thus, there is a continuum of interactions between the genetic composition of the host and the environmental factors that impinge upon these genetic susceptibilities.

IBD is thus the outcome of a continuum of environmental (microbial and metabolic) and genetic factors that sense this environment. This is most visibly played out in the genetic pathways associated with ER stress and the UPR, autophagy and intracellular (myco)bacterial and viral sensing (fig. 3).

Environmental Factors and Their Effects on the Commensal Microbiota and Immune Dysregulation

It can thus be hypothesized that the modifying role of environmental factors on genetic susceptibility is the manner in which these modifying environmental factors specifically affect microbes or the immune system. For

example, smoking protects from UC and promotes CD [64]. It is interesting therefore that T cells expressing the $\alpha 7$ nicotinic receptor respond to chronic nicotine stimulation with the production of T helper (TH) 1 cytokines (e.g. interferon- γ) [65]. TH1 pathways have been linked to CD but not to UC. Similarly, heme oxygenase 1 derived carbon monoxide promotes bacterial clearance, suppresses macrophage activity and enhances intestinal motility making it possible that the immune effects of carbon monoxide may be a protective factor in UC. In another example, appendectomy protects from UC [66]. Appendectomy prevents TH2 colitis in T cell receptor- α deficient mice by preventing the development of T cells with antibacterial specificity, raising the possibility that appendectomy inhibits the development of T cells that respond inappropriately to bacteria and thus protects from the development of UC [67, 68]. Finally, antibiotics may either protect or promote IBD based upon epidemiologic studies [69]. Broad-spectrum antibiotics have dramatic effects on the architecture of the commensal microbiota and consequently the basal tone of the mucosal immune system including effects on innate and adaptive immunity [21, 22]. Thus antibiotics can have profound effects on the microbiota and immunologic tone of the mucosal associated lymphoid tissues within the gut.

It is also interesting to consider the manner in which enteropathogens may influence the host. Enteropathogens represent important modifying environmental risk factors that are involved in the development of IBD [69]. It is clear from animal models that pathogen-induced (e.g. *Citrobacter rodentium*), chemically induced (e.g. dextran sodium sulfate induced colitis) or genetically induced (e.g. IL-10-deficient mice) inflammation disrupts the composition of the microbial architecture [18]. Specifically, all three of these examples cause similar changes in the phyla within the commensal microbiota including decreased Firmicutes and Bacteroidetes with overgrowth or relative preservation of Proteobacteria (e.g. *E. coli*) [18]. Similarly, spontaneous colitis is observed in TRUC (T-bet – RAG – UC-like) mice [70]. This colitis is interesting in that it is driven by TNF and associated with a colitogenic microbiota that is transferable to either wild type or RAG2-deficient mice which lack an adaptive immune system [70]. This suggests that inflammation can induce a pathogenic commensal microbiota.

It is also interesting that altered bacterial divisions (or phyla) have been identified in the human gut microbiota in the context of IBD [24, 30, 71]. These studies have shown that approximately one quarter of patients with

both CD and UC exhibit decreased abundance and diversity of Bacteroidetes and an altered composition of Firmicutes with a maintenance or even a bloom of Proteobacteria [24, 71]. Some of these Proteobacteria in IBD, such as adherent and invasive *E. coli*, may bind to and exhibit a pro-inflammatory behavior [24]. It is not clear whether these alterations are primarily related to IBD or secondarily related to the inflammation and as such may promote or perpetuate the inflammation. Both cases are possible. For example, in IBD there is decreased presence of a potential probiotic, *Faecalibacterium prausnitzii* [72]. Specifically, a reduction in *F. prausnitzii* has been associated with post-operative recurrence in CD and *F. prausnitzii* can protect against trinitrobenzene sulfonic acid associated colitis in mice [72].

Two final examples are worthy of note. This is the role of diet and metabolic host factors that regulate responses to metabolites in the potential development of intestinal inflammation. It is clear that a high-fat diet can determine the composition of the microbiome, in fact independently of obesity [19, 20, 73]. High fat feeding has been shown to decrease the Bacteroidetes and increase the Firmicutes as well as alter the microbiome in association with increases in the genes associated with nutrient transport, bacterial chemotaxis and flagellar assembly [19, 20, 73]. Moreover, the IBD5 locus contains several genes associated with CD susceptibility including one that is highly suggestive of being a causal variant for CD. This gene (*SLC22A5*) encodes the organic cation transporter 2 (OCTN2) protein [74]. OCTN2 is a sodium-dependent high affinity transporter for L-carnitine. L-carnitine is critical in energy metabolism and obligatory for long-chain fatty acid transport to the mitochondria for beta oxidation [75]. Interestingly, deletion of *Slc22a5* in mice leads to massively reduced gastrointestinal L-carnitine content in the mice as well as increased intestinal epithelial cell apoptosis and spontaneous ileocolitis in the *Slc22a5*-deficient mice [76]. Thus, metabolic factors may affect the composition of the microbiota and its metabolic function as revealed by changes in the microbiome, and genetic factors associated with the host may regulate the ability of the host to respond to these microbially derived changes.

Conclusion

In conclusion, there is a continuum in the interactions between the environment and the genetic composition of the host that impinges upon the commensal

microbiota and the relationship that the host has with the commensal microbiota. As such, a genetically susceptible individual in the proper environmental context is at risk for the development of IBD. Thus, interrogation of the connection between the environment and the so-called 'supraorganism' (the metagenome of the host and microbes) is important to understanding the gene-environment interactions that are important in the development of IBD.

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Disclosure Statement

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