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Host genetic susceptibility, dysbiosis and viral triggers in IBD

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Abstract

Purpose of Review—Inflammatory bowel disease (IBD) is thought to occur in genetically susceptible individuals. However, environmental factors, potentially including shifts in commensal microbiota, are also required to trigger disease. This review discusses some of the recent discoveries in host susceptibility and interaction with the microbial environment, and pinpoints key areas for advancement in our understanding of IBD pathogenesis.

Recent findings—Meta-analyses of genome wide association studies have uncovered many new exciting genes associated with susceptibility loci. In addition, improved methods to analyze the commensal microbiota path the way to better define dysbiosis and its potential role in disease. Lastly, identification of viral triggers in experimental systems of IBD suggests a potential role in IBD.

Summary—Understanding the precise microbial and immune triggers of IBD in a genetic context will hopefully lead to a better understanding of the pathogenesis of this disease and the discovery of novel therapeutic approaches including vaccines for specific viruses.

Keywords

Inflammatory bowel disease; viral triggers; dysbiosis; genetic susceptibility to disease

Introduction

Inflammatory bowel disease (IBD) is a group of chronic and debilitating conditions characterized by inflammation of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are two major types of IBD that share distinct but also overlapping clinical and pathological features. Both forms are characterized by damage to the mucosa that forms the barrier to intestinal luminal contents.

In this review, we will discuss recent studies that focus on triggers for IBD. Based on several decades of studies in both humans and mouse modes, one major thesis that has been developed is that IBD arises in a genetically susceptible background and involves an aberrant interaction at the interface between the commensal gut microbiota (including viruses) and the host. We will discuss the effects of host genetic mutations that elevate risk

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for IBD, as well as the impact of the intestinal microbiota on the host. We will identify the major gaps in our knowledge and the modes of study that need to be pursued to further test this idea.

Role of Genetics

We have known for the past half-century that host genetics play a key role in IBD pathogenesis[1–2]. More recently, genome-wide association studies (GWAS) identified ~99 single nucleotide polymorphisms (SNPs) that confer greater risk for UC and CD. The genes associated with these SNPs provide an important starting point to discover key proteins and pathways that are involved in IBD pathogenesis. The caveat is that most of these risk alleles are present at high frequency in the target populations, but confer modest increase in risk of the development of disease. This predicts that phenotypes associated with risk alleles will be triggered by environmental factors.

In the past year, extensive meta-analysis of the GWAS has increased the number of susceptibility alleles for both UC and CD to 47 and 71, respectively[3–5]. Interestingly, at least 28 of these alleles are shared between UC and CD, suggesting the intriguing possibility that different environmental factors might trigger different forms of the disease. Studies of the common group of loci will be important to understand the basic pathogenesis of IBD.

Genes in immune regulation

Dysregulation of host immune components may result in an abnormal response to normal commensal organisms and pathological inflammation. Early GWAS identified susceptibility loci associated with several genes involved in immune regulation and signaling, and gave further credence to existing theories about IBD pathogenesis. For example, the involvement of the IL- 23/IL-12 pathway was reinforced by recent GWAS. IL-23 is thought to contribute to the function of Th17 cells, which produce a variety of proinflammatory cytokines and act as a link between the innate and adaptive immune systems. Genes implicated in this pathway that are also found within IBD susceptibility loci include *IL23R*, *TYK2*, *STAT3*, *IL12*, and *JAK2*. However, the exact role of IL-23 and Th17 cells in intestinal inflammation is still undefined.

The most recent meta-analyses identified immune regulatory genes linked to IBD susceptibility loci. Genes encoding multiple cytokines and their receptors were added to the list: *IL10*, which is implicated in both CD and UC and encodes an anti-inflammatory cytokine; *RANKL*, whose protein product stimulates dendritic cells, leading to T cell proliferation and regulatory T cell induction; *IL8RA*, encoding a receptor for the neutrophil chemotactic factor IL8; *IL7R*, implicated in the regulation of the transition between effector and memory T cell development; and *IL2RA*, which encodes part of the high affinity IL-2 receptor, important in T cell activation and proliferation. Key transcription factors identified include PRDM1, the master transcriptional regulator of plasma cells; and IRF5, which regulates the transcription of Type I interferons and other cytokines. The identification of these additional genes supports the idea that alterations in immune signaling provide a basis for susceptibility for IBD.

Genes in host-microbe interactions

GWAS also linked risk loci with genes involved in the interaction between the host and commensal microorganisms. One of the first genes implicated in CD susceptibility was *NOD2*, a cytosolic pattern recognition receptor that has the genetic variants most strongly associated with CD. In addition, multiple genes involved in autophagic clearance of bacteria have also been implicated in GWAS, such as *IRGM*, *ATG16L1*, and *ULK1*[4,6–10]. The mechanisms of these interactions will be discussed later in this review. Another identified gene is *MUC19*, which encodes important components of the mucus that lines the gastrointestinal tract as a barrier defense against bacteria. A recent addition to the CD susceptibility gene list is *FUT2*, which encodes an enzyme that allows for secretion of Lewis ABO antigens on mucosal surfaces. Interestingly, alterations in this gene that prevent ABO secretion result in protection against many types of enteric norovirus infection[11–12].

Challenges in determining genetic susceptibility

Despite the ability of GWAS and their meta-analysis to identify genes with large single-effect, as much as 80% of the heritability of IBD may still be unidentified[13]. This "heritability gap" may arise from multiple factors: 1) there may be more variants with smaller effect that do not meet the stringent requirements for significance in GWA studies and thus have not yet been identified; 2) there may be rare variants that have a potentially large effect but are not included on existing genotyping arrays; 3) there are gene interactions and pathways that are not captured in current approaches that focus on discovering the most significant individual SNPs.

Although it is tempting to focus on systematically filling in the heritability gap, we must consider the end goal of identifying these disease-associated variants. The objective is ultimately to improve understanding of disease pathogenesis in order to develop better methods for prevention, diagnosis and treatment. Thus, a valuable endeavour is to try to discover gene interactions and pathways that contain gene variants that have moderate levels of significance (and are thus not identified as top SNP hits), but that are genuinely associated with the disease.

Some groups have already begun to conduct pathway analysis of GWAS, including those for CD[14–15]. One group successfully uncovered significant association between CD and the IL12/23 pathway[16], but they identified additional genes in the pathway that did not reach significance by single-marker association tests in any previous studies. The implications of this work are that there are likely other genes and pathways that have yet to be detected by conventional methods of GWAS analysis. These could then provide novel targets for therapeutic intervention or for further studies in association with manipulation of the commensal microbial environment.

Commensal microbes as potential triggers

In addition to investigating the genetic background of an individual, substantial efforts have been dedicated to examining the role of pathogenic or commensal microbiota in triggering IBD. However, our understanding of the microbial factors contributing to disease etiology

remains limited mostly because the biology of the commensal microbiota is poorly understood.

A 'balanced' commensal microbiota?

An emerging consensus hypothesis is that an intestinal dysbiosis (microbial imbalance) can be a trigger for IBD. Although this term has been extensively used since it was first proposed by Helmut Haenel more than four decades ago[17], the biological basis of this 'microbial imbalance' remains poorly defined. To begin with, what constitutes a 'balanced' commensal microbiota has not been established.

The intestinal microbiota is vast and quite diverse at the species level. Current data regarding species diversity suggest that the intestinal microbiota is host-specific[18–21]. Under these terms, classification of 'normal' microbiota is challenging because each individual possess a unique collection of microbial species. Therefore, to communicate alterations in the microbiome, one method has been to classify microbes based on 16S rRNA gene sequencing into two major groups: Firmicutes and Bacteroidetes, the two most predominant bacterial phyla inhabiting the intestinal tract[20–21]. This has utility in showing differences in microbiota composition. However, phyla represent the highest taxonomic rank in bacterial classification, and they are composed of numerous orders, classes, families and genera with diverse and broad metabolic, ecological, pathogenic or symbiotic properties. Thus, description of phylum profiles possesses only limited biological relevance to understand host-microbe interactions (remember, we as humans are classified as chordates, along with sea squirts). A challenge then is how to determine what level of taxonomic resolution will be required to test the dysbiosis hypothesis.

Thus, the description of a 'balanced' intestinal microbiota is far from accomplished. Encouragingly, some studies have tried to resolve these issues by examining predominant genetic branches. These analyses identified a 'phylogenetic core' of bacterial species found in a large proportion of human fecal samples[19]. Together, these data highlight the need for novel methodologies to improve our understanding of the biology of the intestinal microbiota.

Dysbiosis and IBD

A widespread idea is that dysbiosis in IBD patients stems from an increase in specific microbial populations while others are decreased[22–23]. However, the current data have not revealed reproducible alterations in microbial populations in IBD. The lack of consensus could be attributed to technical disparities including sample source (intestinal biopsies vs. stool samples) and the type of analysis of microbial changes (comprehensive or narrow molecular screening).

For example, evidence for dysbiosis in IBD patients has been shown in studies that analyzed stool samples[24–27], but it is well-accepted that microbial populations from stool differ from those associated with the mucosa. Mucosa-associated bacteria may have more physiological relevance in IBD as these communities are thought to be stable and in actual physical association with the host epithelium[28–30]. Thus, numerous studies have examined microbial diversity in healthy and affected intestinal biopsies. However, results from these studies are

not reproducible, and in some cases they are contradictory. As an example, some reports indicate reduced bacterial diversity, as well as a reduction in Firmicutes phylum with an enrichment in Bacteroidetes phylum, in UC and CD patients[31]. In contrast, analysis of colonic biopsies from CD patients revealed an increased prevalence of *Clostridium* spp., *Ruminococcus torques* (both members of the Firmicutes phylum) and *Escherichia coli* (Enterobacteriaceae family)[32]. Still more studies have reported no differences in microbial diversity in biopsy samples from control and IBD patients[29], or in the comparison of ulcerated and non-ulcerated mucosal samples from patients with IBD[33–35].

A promising strategy to address these inconsistencies and uncover microbial alterations linked with IBD is the use of comprehensive molecular analyses of mucosa-associated microbiota. The goal of these studies is to examine microbial changes in a large set of samples using deep sequencing. Thus far, one comprehensive study has provided notable insights into the microbial alterations occurring in IBD. Specifically, members of the Lachnospiraceae family (Firmicutes phylum) and Bacteroidales (bacterial order) were depleted in a subset of IBD samples[36]. More importantly, this study confirmed that *Faecalibacterium prausnitzii*, a member of the Lachnospiraceae family, was reduced in the mucosa of IBD patients[32].

Another interesting strategy to factor in genetic variability is to study twin cohorts. Willing *et al* analyzed the microbiota of 38 twin pairs that were either concordant or discordant for CD and UC, along with two healthy twin pairs, and identified bacterial species that were enriched and diminished in CD[34]. However, even within their healthy twin pairs, the gut microbiota was similar at the genus level but differed at the species level. Taken together, these data emphasize the importance of new methodologies for the characterization and assembling of microbial populations to first understand the biology of the intestinal microbiota, and then to reveal their contribution to IBD.

Viruses as potential triggers of IBD

It is becoming increasingly clear that enteric viruses may also play a role in IBD. Viruses may act directly on the host epithelium and immune system to induce inflammation, or may alter luminal bacterial composition that then provokes disease.

For example, a recent study by Cadwell *et al* showed intestinal abnormalities reminiscent of human CD and heightened inflammatory response to dextran sodium sulfate (DSS) in ATG16L1 hypomorphic mice, but only when in conjunction with infection by murine norovirus (MNV)[37]. This is a ubiquitous virus that does not normally cause disease in immunocompetent mice.

We may need to consider viruses, in addition to bacteria, as part of our commensal microbiome. There have been suggestions that every individual harbors approximately 8–12 chronic viral infections at any given time[38], and these may be harmful only in the limited percentage of the population that has a certain genetic predisposition. Furthermore, Reyes *et al* discovered that each individual has a unique fecal virome that is stable over a one-year period. Interestingly, while related individuals shared high similarity in fecal bacterial composition, fecal viromes varied greatly between individuals independent of relatedness,

and may act as an environmental factor in disease pathogenesis. Notably, more than 75% of the sequences did not match to any currently known viruses, an indication of how much knowledge is yet to be gained about the viral metagenome.

These chronic viral infections may have a negative impact on IBD. The involvement of cytomegalovirus (CMV) in IBD pathogenesis or exacerbation has long been a subject of debate. CMV is found in up to 70% of IBD patients[39]. After initial infection, it can remain latent and asymptomatic until reactivated under conditions of stress or immunosuppression, where it induces a colitic disease that displays some symptoms of IBD. The question is whether CMV reactivation actively worsens disease or whether re-activation is simply a bystander of inflammation. A recent report by Kim *et al* showed that colonic CMV re-activation was rare in patients with mild to moderate IBD, and lack of treatment for CMV did not adversely affect disease outcome[40]. However, the current recommendation is that patients with more severe, steroid-refractory disease should be treated for CMV reactivation if present[39].

Pathways at the intersection of host and microbe

There are variety of mechanisms by which the commensal microbes in the lumen of the intestine can interact and influence the host epithelium and immune cells. We will discuss new evidence surrounding three main pathways – Toll-like receptor (TLR) signaling, the autophagy pathway, and the induction of regulatory T cell populations.

I) Toll-like receptor signaling – the role of the negative regulator IRAK-M

TLRs are a family of innate immunity pattern recognition receptors that recognize components specific for microbes. In the intestine, ligation of TLRs on intestinal epithelial cells stimulates cellular proliferation, IgA production, and secretion of antimicrobial peptides, all contributing to the maintenance of intestinal homeostasis and an intact epithelial barrier[41]. Multiple groups have observed altered expression of TLRs on intestinal epithelial cells and lamina propria immune cells of patients with active IBD[42–44]. Because TLRs act to trigger an immune response, excessive activation of TLR signaling could be expected to give rise to inadequately controlled inflammation. In addition, mice lacking TLR5 have an altered intestinal microbiota[45], which may impact the development of IBD.

Recently, two groups demonstrated that loss of IRAK-M, a negative regulator of TLR signaling, worsens mouse models of IBD[46,47]. IRAK-M-deficient cells overproduce proinflammatory cytokines, with increased NF-κB and MAPK signaling[48]. Mice with a deficiency in both IL-10 and IRAK-M develop inflammation of the gut at a younger age than mice lacking IL-10 alone[46]. IRAK-M-deficient mice also have a greater loss in body weight and worse intestinal inflammation in response to DSS[47]. Notably, germ-free mice have diminished expression of IRAK-M in the colon, but colonization with commensal bacteria is able to induce IRAK-M expression in an age-dependent manner[46]. Thus, not only the TLRs themselves but also their regulators are impacted by luminal microbes, and this interaction affects intestinal homeostasis and damage responses.

One central question that persists is how to define the line between beneficial and detrimental TLR signaling in the intestinal epithelium. It is clear that some TLR ligation by commensal bacteria contributes to intestinal homeostasis, but whether specific bacterial or viral types or combinations are required, or how this process is dysregulated in disease, remain unanswered.

II) Autophagy – A link between NOD2 and autophagic clearance of intracellular microbes

GWA and follow-up studies have identified multiple susceptibility loci within genes involved in the autophagy pathway, including *ATG16L1*, *IRGM*, and, most recently, *ULK1*[4,7–10]. Autophagy is a cellular process by which cytosolic components are enveloped by a double membrane, which then fuses with the lysosome for degradation of its contents. Autophagy is a principal mechanism for the recycling of whole organelles and proteins, and is also important for the clearance of intracellular pathogens. Deficiencies in *ATG16L1* and *IRGM* or their murine homologs (*Atg16L1* and *Irgm1*, respectively) result in diminished ability to clear *Salmonella* and *E. coli*[10,49–50], and for *IRGM* and *Irgm1* only, mycobacteria, *Listeria monocytogenes*, and *Toxoplasma gondii*[51–53]. The functional consequences of loss of Ulk1 have not yet been demonstrated. It is conceivable that defects in the elimination of intracellular pathogens may result in dysregulated immune responses and inflammation. However, since neither mice deficient in *Irgm1* nor *Atg16L1* spontaneously develop disease, there are certainly other contributing factors, such as viral infection and toxic insult, as shown by Cadwell *et al*[54].

In addition, studies in the last year have linked *NOD2*, whose variants account for the greatest known contributions to heritability in CD, to the autophagy pathway. NOD2 is an intracellular pattern recognition receptor that binds to bacterial cell wall components and signals to produce proinflammatory cytokines and antimicrobial peptides. Studies showed that NOD2 stimulation induces autophagy, seemingly by recruiting ATG16L1 to the site of bacterial entry at the plasma membrane[55–57]. This process is required for effective clearance of intracellular *Shigella* and *Salmonella* infection in multiple cell types, including mouse embryonic fibroblasts, primary macrophages, and dendritic cells[55–57]. Interestingly, the most common variant of NOD2 associated with CD failed to recruit ATG16L1 to the plasma membrane[56], and cells with CD-linked variants of both NOD2 and ATG16L1 had less effective autophagy-mediated bacterial clearance. These findings lend further credence to the significance of altered autophagy in the pathogenesis of IBD.

Nonetheless, further research is warranted into whether autophagy actually plays a role in maintaining integrity of the intestinal barrier *in vivo*, i.e. whether commensal bacteria can enter the cell and are cleared by autophagy. It is also possible that autophagy proteins may be mediating functions outside of the classical autophagy pathway, which merit greater investigation.

III) Regulatory T cells – induction of a T cell population by specific commensal bacteria

It has been known for many years that regulatory T cells (Tregs), a T cell population with suppressive functions, are important in maintaining peripheral tolerance, especially to commensal microbes within the gastrointestinal tract. There are both natural Tregs, which

develop in the thymus, and adaptive Tregs, which develop in the periphery from existing T cells. The intestinal environment is especially conducive toward the conversion of these adaptive Tregs[58–59]. Tregs are able to ameliorate murine models of inflammatory bowel disease and infectious colitis[60–62]. They have multiple suppressive mechanisms, including production of IL-10, which has a central role in inhibiting intestinal inflammation[63].

Remarkably, two recent studies have shown the induction of Tregs and the ability to improve colitis by specific bacteria. Atarashi *et al* demonstrated the induction of colonic Tregs in germfree mice by a mixture of commensal *Clostridium* species, but not by *Bacteroides* or *Lactobacilli* strains[64]. The Tregs generated produced IL-10 and were functionally competent at suppressing effector T cells. This Treg development was not dependent on pattern recognition receptor signaling. Colonization with *Clostridium* XIVa and IV taxa was protective against experimental colitis caused by both DSS and oxazolone. Notably, these bacterial clusters are found in lower proportions in patients with IBD than in healthy individuals[36,65].

A second study by Round *et al* show that mono-association of germ-free mice with *Bacteroides fragilis* increases the percentage of IL-10-producing Foxp3+ Treg cells with effective suppressive function in the colon[66]. One important point is that this phenomenon required both the presence of bacterial polysaccharide A (PSA) and TLR2. Administration of purified PSA reduced inflammation in response to the colitis-inducing chemical TNBS, even when given days after initiation of disease.

Conclusion

These discoveries illustrate a direct method by which the intestinal microbiota is able to shape the immune response and potentially trigger IBD. A genetically predisposed individual may have alterations in their microbiota, which then disturbs the normal regulatory mechanisms at the barrier that maintain tolerance to commensal organisms. Future studies should use novel tools and technologies to focus on elucidating the details of these interactions and how dysbiosis might be initiated.

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Key points

1. In order to identify genes and pathways that elevate risk for IBD more sophisticated analysis of GWA data should be performed.
2. A better understanding and ability to classify the commensal microbiota will aid in our comprehension of microbial triggers of IBD.
3. Key pathways of interest in the development of IBD are those that are at the intersection of the host immune response and microbial stimulation.