Gene–environment interactions in inflammatory bowel disease: microbiota and genes

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Abstract

A recent research workshop gave an update on the genetics of the inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis. This mini-review summarises the updates of the gene–environmental interactions, especially those outlining the contribution of the gut microbiota to the pathogenesis of IBD.

Introduction

A recent British Society of Gastroenterology research workshop gave an update on current research into the genetics of the inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC). Genetic contributions to IBD are now well established since their early descriptions in family studies¹ of CD and UC; these showed that CD and UC are related polygenic disorders, recently confirmed in the overlap of the susceptibility loci identified by genome-wide association studies.² The incidence of IBD continues to rise, increasing in populations of the developing world as they adopt a Western lifestyle. Although the primary pathological defect is still unknown, genetics and the environment both contribute to aetio-pathogenesis. Recent research investigating gene–environmental interactions has shown that factors affecting the intestinal environment may have a profound effect on IBD pathogenesis.

Gene–environmental interactions

Gut microbiota

Gut microbiota constitute a densely populated ecosystem with $\sim 10^{14}$ bacteria in total ($\sim 10^8$ bacteria in the terminal ileum and 10^{11} in the colon); the total number of bacteria outnumbers human cells by 10:1. Taken together, bacteria act as a 'virtual organ' with an 100-fold higher number of genes than in our own genome.^{3–4}

The Human Microbiome Project (<http://commonfund.nih.gov/hmp/>) has been set up to investigate and confirm: whether the diversity of microbiota within the gut between individuals undergoes longitudinal changes; how bacterial transmission occurs following birth; whether a core microbiome exists within individuals or is highly variable; and how genetics and environment affect the composition of microbial species present.⁵ If humans are thought of as a composite of human and microbial cells with the human genetic landscape as an combination of genes from the human genome and the microbiome, and human metabolic features as a blend of human and microbial traits, the picture emerges of a 'human supra-organism'. In IBD mouse models, germ-free animals exist but they have abnormal gut function and only develop colitis when bacteria are present. Commensal bacteria are important for normal gut development, function and homeostasis; animals bred in germ-free conditions have a number of immunological defects in the gut.⁴ In addition, specific commensal bacterial species can be involved in maintaining resistance to specific pathogens.⁶

Gut microbiota have a role in CD,^{3–10} UC¹¹ and pouchitis¹² and may also affect gastrointestinal cancers, infections and obesity.¹³ Bacteria can initiate, perpetuate and modify gut inflammation¹⁴ (figure 1). CD is located in high bacterial areas and an imbalance in commensal bacterial species has been detected relating to disease location.¹⁵ In CD, faecal stream diversion can alleviate symptoms, but reintroduction of luminal contents may lead to reactivation of inflammation.¹⁶ These effects are unlikely to be due to a single organism, though adherent invasive *Escherichia coli* (AIEC) and *Mycobacterium paratuberculosis* have both been proposed.¹⁷

Figure 1 Proposed mechanisms by which bacteria and fungi induce chronic immune-mediated inflammation and injury of the intestines. (A) Pathogenic bacteria. A traditional pathogen or functional alterations in commensal bacteria, including enhanced epithelial adherence, invasion, resistance to killing by phagocytes or acquisition of virulence factors, can result in increased stimulation of innate and adaptive immune responses. (B) Abnormal microbial composition. Decreased concentrations of bacteria that produce butyrate and other short-chain fatty acids compromise epithelial barrier integrity. Meanwhile, overgrowth of aggressive commensal microbial species increases the number of adjuvants and antigens (Ag) that induce pathogenic immune responses or increase production of toxic metabolites such as hydrogen sulphide (H₂S) that block colonocyte utilisation of butyrate and increase mucosal permeability. (C) Defective host containment of commensal bacteria. Increased mucosal permeability can result in overwhelming exposure of bacterial toll-like receptor ligands and antigens that activate pathogenic innate and T cell immune responses. Defective secretion of antimicrobial peptides or secretory immunoglobulin A can lead to mucosal bacterial overgrowth. Defective killing of phagocytosed bacteria can lead to persistent intracellular bacteria and ineffective clearance of bacterial antigens. (D) Defective host immunoregulation. Antigen-presenting cells and epithelial cells overproduce cytokines due to ineffective downregulation, which results in Th1 and Th17 differentiation and inflammation. Dysfunction of regulatory T cells (T-reg) leads to decreased secretion of interleukin (IL)-10 and transforming growth factor (TGF)-β, and loss of immunological tolerance to microbial antigens (an overly aggressive T cell response). Figure reprinted from the *Journal of Internal Medicine*¹⁴ with permission of the publisher. CpG, unmethylated CpG motifs in bacterial DNA; IFN, interferon; LPS, lipopolysaccharide; PG, peptidoglycan; SCFA, short-chain fatty acids; TNF, tumour necrosis factor.

Host–bacterial interactions

Bacterial species vary widely between human individuals. Both genetic and environmental factors affect the composition of species that make up an individual's gut microbiota.¹⁸ Within the gut, a single commensal bacteria species can switch on many genes, indicating an influence on the host in a variety of physiological pathways.¹⁹ Host genotype may regulate bacterial

composition, as distinct genetic strains of germ-free mice had different numbers of Paneth cells, varying levels of antimicrobial peptides and 12 weeks after infection with the same bacterial strains they had dissimilar microbial profiles.²⁰ This is also apparent in animal models where different rat/mouse strains give varying responses to different bacterial strains: for example, AIEC strains induce variable degrees of

colitis in interleukin (IL)-10-deficient mice and none induce disease in wild-type animals, and NOD2 genotype also affects the microbial composition in both mice and humans.²¹

AIEC have been consistently isolated from patients with ileal CD.^{15,22} A direct role for AIEC in CD is supported by various lines of evidence: the number of AIEC isolates correlated with severity of mucosal inflammation;¹⁵ AIEC bind to epithelial cell proteins with increased expression in CD, for example, CEACAM6²² and Gp96²³ to enable mucosal invasion; AIEC have the ability to exploit host defects in bacterial killing and autophagy conferred by CD-related polymorphisms in ATG16L, IRGM and NOD2 to allow survival and replication;²⁴ and AIEC can also induce granulomas in vitro²⁵ and are associated with granulomatous colitis in Boxer dogs (where eradication correlates with remission).^{26,27} AIEC can invade ileal enterocytes via inflammation-induced CEACAM6 expression by ileal epithelial cells^{3,22} and have recently been shown to invade Peyer's patches,²⁸ possibly initiating the defective immune response that leads to chronic inflammation. Some AIEC strains release flagellin which bind to toll-like receptors (TLR-5), causing inflammatory responses which increase the expression of both NOD2 and CEACAM6, promoting inflammation and bacterial colonisation.²² NOD2 is important for maintenance of intestinal barrier function as epithelial cells and Peyer's patches with mutant NOD2 are less able to prevent invasion by AIEC.^{28,29} Thus, CD pathogenesis is likely to include an imbalance of antibacterial mechanisms versus invasiveness of bacteria involved.

There is some suggestion that CD results from an impaired ability of macrophages to produce cytokines and chemokines to effectively remove bacteria, leading to chronic inflammation.³⁰ Indeed, in CD patients, a number of cytokines were found to be diverted to lysosomes for degradation, rather than being released through normal secretory mechanisms.³¹ A number of genes associated with susceptibility to CD are also involved in gut homeostasis. Chronic stimulation of intestinal macrophages through NOD2 leads to tolerance by downregulation of responses via the mTOR pathway. This mechanism maintains gut homeostasis but is deficient in those carrying NOD2 mutations in CD.³² Others have suggested that inflammation or homeostasis is due to the balance of beneficial and detrimental species residing in the intestine.^{33,34} Bacteria are also known to take advantage of inflammation and to manipulate inflammatory pathways to promote their own survival.³⁵

In Swedish twin studies, microbial profiles of healthy monozygotic twins were more similar than that of twins with CD, whether discordant for disease or not. Abnormal changes in microbial composition (dysbiosis) were increased between CD twin pairs and associated with ileal involvement; bacterial diversity was higher in healthy twins than those with CD.³⁶ Reduced diversity of faecal microbial species was seen in CD,

particularly the Firmicutes and Bacterioides species.³⁷ *Faecalibacterium prausnitzii* has been found to be negatively associated with ileal CD,⁸ including in postoperative ileal recurrence.⁹ A study of CD patients from different families showed patterns of dysbiosis, not present in unaffected relatives or healthy controls. In CD patients there was a decrease in *F prausnitzii* (associated with a previous ileocolonic resection), and decreases in *Bifidobacterium adolescentis* and a *Clostridium* species *Dialister invisus*, but an increase in *Ruminococcus gnavus*. Compared with healthy subjects, unaffected relatives of CD patients had more *Ruminococcus torques* and less *Collinsella aerofaciens* and a member of the *E coli* Shigella group in their predominant microbiota.³⁸ Reduced diversity of faecal microbiota was also seen in UC, with changes in remission and clinical relapse and differences.¹¹ *F prausnitzii* was seen in UC group compared with control group, with decreased diversity of bacteria in inflamed tissue.^{39,40}

Various factors can contribute to dysbiosis: for example, host genetics, through mutations in specific genes giving abnormal immune regulation and/or uncontrolled inflammation, which, in turn, can affect microbial composition. A host could 'select' or exclude the colonisation of particular organisms, either by active interaction with specific host receptors or via the host environment being more beneficial for the growth of specific organisms. Birth in the more sterile hospital environment (or through caesarean sections) can prevent early exposure to health-promoting bacteria. Overuse of antimicrobial strategies (eg, vaccination, antibacterial therapeutics), which do not distinguish between pathogenic or symbiotic microorganisms, could adversely alter the microbiota.⁴ Thus, CD pathogenesis is via the interaction of microbiota, genetics, environmental triggers and immune response. Physiological consequences of dysbiosis could act via decreased production or utilisation of short-chain fatty acids (eg, butyrate), used as an energy source by intestinal epithelial cells.³ This, together with mucin degradation, would have profound effects on epithelial cell function.³⁸

Antibiotics have not been thought to be effective in the treatment of IBD, except in specific circumstances.^{13,17} Recent meta-analyses of antibiotic therapies in CD have shown that antibiotics may^{41,42} or may not⁴³ be effective in CD; the differences may be due to variations in the inclusion criteria and the treatment regimens of the trials included. However, antibiotics are more effective in the treatment of UC⁴¹ and pouchitis.⁴⁴ While further clarity is required for the efficacy of antibiotics in IBD treatment, this may also suggest that the aetiology of IBD is more about the dysregulation of symbiotic relationships between host and bacteria, rather than any specific microbial species.³³

Probiotics and prebiotics

Probiotics, are live organisms that, when ingested in adequate amounts, exert health benefits to the host.⁶

Figure 2 Inhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria. Schematic representation of the crosstalk between probiotic bacteria and the intestinal mucosa. Antimicrobial activities of probiotics include the (1) production of bacteriocins/defensins, (2) competitive inhibition with pathogenic bacteria, (3) inhibition of bacterial adherence or translocation and (4) reduction of luminal pH. Probiotic bacteria can also enhance intestinal barrier function by (5) increasing mucus production. Reproduced from the *Inflammatory Bowel Diseases*⁵⁰ with permission of the publisher. DC, dendritic cell.

The most commonly used probiotics are *Lactobacilli*, *Bifidobacteria* and non-pathogenic yeasts. Prebiotics are non-digestible, fermentable foods that selectively stimulate the growth and activity of health-promoting bacteria already residing in the colon.⁴⁵ The oligosaccharides in human breast milk are considered prebiotics as they facilitate the preferential growth of *Bifidobacteria* and *Lactobacilli* in the colon. Dietary fibre and lactulose used in the treatment of constipation also exert prebiotic effects.

VSL#3 is a mixture of bacteria (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis* and *Streptococcus salivarius* subspecies *thermophilus*). A Cochrane review showed that VSL#3 was more effective than placebo for both the treatment and maintenance of chronic pouchitis.⁴⁴ Early trials of VSL#3 have suggested it might have beneficial effects in UC,^{46–49} but larger trials are required.

A number of potential mechanisms through which probiotics may have their effect have been proposed⁵⁰ (figure 2). These include having direct antimicrobial activity by inducing changes in luminal pH, secretion of antimicrobial peptides and/or inhibiting bacterial adhesion and invasion of epithelial cells;¹⁷ for example, probiotics had an effect on signalling pathways in epithelial cell lines;⁵¹ they may enhance barrier function by increasing mucus production; they may also have immunomodulatory effects through actions on macrophages, dendritic cells or lymphocytes, for example, *F. prausnitzii* has been shown to have anti-inflammatory

effects through increased IL-10 production.¹⁷ VSL#3 may act via several mechanisms: induction of the anti-inflammatory cytokine, IL-10,⁵² and reduction of the pro-inflammatory cytokine, IL-12, in biopsies from UC patients (similar to effects seen with corticosteroids),⁵³ by increasing numbers of transforming growth factor β -expressing T cells⁵⁴ and/or increasing levels of the antimicrobial peptide human β -defensin-2.⁵⁵ Other potential probiotic bacteria are *Bacterioides fragilis*, which produces polysaccharide A, found to protect from inflammation by inducing anti-inflammatory cytokines and suppressing the production of pro-inflammatory cytokines induced by other bacteria.⁵⁶ Another bacterium, *E. coli* Nissle 1917, might also be useful for maintenance and/or induction remission in UC and pouchitis,⁵⁷ possibly acting via TLR-2- and TLR-4-dependent pathways.⁵⁸ Studies of probiotics and prebiotics in CD have shown these have not been effective.⁶

Future strategies for therapy

Faecal transplantation has been used as an effective treatment for fulminant and refractory *Clostridium difficile* and in a few IBD patients, without adverse effects. Faecal transplantation has some potential as an inexpensive treatment for dysbiosis and needs further trials. Standardised, controlled studies are necessary to ascertain the most effective regimen, both for donors as well as recipients, and the most acceptable method of treatment.⁵⁹

Beyond IBD, there is potential use of microbiota in other gastrointestinal disorders, atopy, diabetes, hypertension or obesity. However, various questions

and challenges need to be addressed for further successful microbiome studies. Questions include: which organism at which dose and with what concomitant treatment? What is the contribution of clinical or genetic subsets? The bacterial genome could be important for pharmacological targets for drug therapy. In addition, designer bacteria may be engineered to produce specific cytokines. To give clear answers across studies, standardisation of certain clinical, sampling and technological parameters is required with wider collaboration among clinicians, microbiologists, bio-informaticians and statisticians.

Effects of the CD susceptibility genes on bacterial responses

Three of the susceptibility genes for CD (NOD2, IRGM and ATG16L1) are involved in antibacterial responses. An association was seen between NOD2 status and dysbiosis in resected tissue from IBD patients,⁵ with a selective decrease in *Clostridia* bacteria in UC and CD, but increases in other bacterial groups. NOD2 is associated with distinct disease phenotypes (young, fibrosing, TI involvement),⁶⁰ which could be a consequence of defective killing through small-bowel Paneth cells. Studies of the mechanisms of the genetic susceptibility in animal models have shown that NOD2-deficient mice had increased concentrations of Bacterioides species and Firmicutes, with abnormal distributions of mucosally-adherent and luminal bacteria in the caecum and ileum.⁶¹ *F. prausnitzii* was decreased in NOD2-deficient mice in ileum and caecum, but with increases in *Clostridium* species. AIEC have enhanced survival in splenocytes of NOD2-deficient mice with increased IL-12/IL-23p40 and tumour necrosis factor, but were cleared in wild-type mice. IRGM and ATG16L1 are involved in the autophagy pathway. IRGM is induced by interferon- γ and mediates intracellular killing of *Mycobacterium tuberculosis*, which is defective in IRGM-deficient mice.⁶² Loss of IRGM leads to abnormal location of Paneth cells at the middle or top of crypt instead of at the bottom. The total number of cells is increased but with decreased lysosome and defensin mRNA expression. Cells had characteristics of intermediate cells containing lysosome and MUC2.⁶³ In normal responses, bacteria bind to mitochondria, inducing 'mitophagy'; IRGM-deficient mice have increased mucosal bacteria in epithelial cells with increased mitochondria but increased persistence of bacteria in macrophages.⁶⁴ Mutations of ATG16L1 also produced disruption of the Paneth cell granule exocytosis pathway. Other studies have shown that probiotics have differential specific effects in IL-10- and HLA-B27-knockout mice.⁵⁰

Defective innate immune response leads to activation of pathogenic T cell responses, that is, if macrophages and neutrophils are defective, there is no resolution of acute inflammation; lymphocyte effects then occur leading to chronic inflammation. Persistent bacterial

stimulation leads to pathogenic adaptive immune response via Th1/Th17 pathways. Colonisation with functionally altered commensal bacteria also leads to genetically determined innate defences in bacterial killing and mucosal barrier function.⁶⁵

Environmental triggers of IBD

Modification of bacteria through genetics and environment (diet, exposure, public health, infections or antibiotics) may affect the host immune response. Transient environmental triggers are important; otherwise, we would see more IBD in people carrying susceptibility genes and concordance in twins. IBD has been linked to single episodes of gastroenteritis, suggesting that enteric pathogens in those with IBD susceptibility genes can initiate abnormally regulated, early inflammatory responses resulting in chronic inflammation and injury.⁶⁵ Evidence for this has been shown in ATG16L1-deficient mice, where norovirus infection in the presence of commensal bacteria led to a CD-like disease, whereas wild-type mice remained normal.⁶⁶ Norovirus has also been isolated from patients undergoing flare-ups of their IBD during immunosuppressive therapy.⁶⁷ Exacerbation of IBD can be induced by bacterial, parasitic or viral super-infection, with *C. difficile* and cytomegalovirus being the most important.³ It is known that smoking has profound effects on susceptibility and disease course in both CD and UC;^{68, 69} more recent data suggested that smoking is associated with dysbiosis and thus a possible mechanism by which smoking has its detrimental effects in CD.⁷⁰

The complex interactions between genes and environment are apparent in IBD. The manipulation of intestinal bacteria offers strategies for further investigation and possibilities for future therapeutic interventions.

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