Host-bacteria interaction in inflammatory bowel disease

Paul Knight, Barry J. Campbell*, and Jonathan M. Rhodes
Division of Gastroenterology, School of Clinical Sciences, University of Liverpool, Crown Street, Liverpool, L69 3BX, UK

Introduction/background: Inflammatory bowel disease (IBD) results from complex interactions between: host genome, immune system, mucosa, bacteria, and environment.

Sources of data: Review of PubMed database using search terms ‘bacteria and inflammatory bowel disease’ and ‘genetics and inflammatory bowel disease’. PubMed ‘related reference’ feature and references from retrieved articles were examined.

Areas of agreement: IBD results from interaction between the microbiota of the gut and the immune system. Key gene defects associated with IBD are involved in bacterial recognition and processing. The environment at least modifies and may determine pathogenesis.

Areas of controversy: It has been disputed whether the primary defect in IBD is immunological or bacterial, and which bacteria are key.

Growing points/areas for research: ‘M cells’, the specialized epithelial cells that overlie Peyer’s patches, are a major interface between gut bacteria and the immune system. Improved understanding is needed of the bacteria involved in IBD pathogenesis, their genotypes and phenotypes, their portal of entry and their mechanism for escaping attack from the immune system. Bacterial ligands involved in bacteria–epithelial adhesion are emerging, and molecular techniques are rapidly increasing our knowledge of the human intestinal microbiota.

Keywords: Crohn’s disease/ulcerative colitis/Escherichia coli/macrophage/autophagy/M cell
Introduction

Inflammatory bowel disease (IBD) comprises mainly ulcerative colitis (UC) and Crohn’s disease (CD). Other conditions such as microscopic and collagenous colitis are not considered here. CD is characterized by deep inflammation with granulomata, and may affect any part of the gastrointestinal tract, although it most commonly affects the distal ileum and caecum. UC is largely limited to the colon, particularly the distal colon and rectum and causes more superficial ulceration. For many years, the treatment for both conditions has been corticosteroids for acute attacks and oral 5-aminosalicylic acid compounds for maintenance, with immunomodulation with azathioprine or mercaptopurine added for patients with chronic relapsing disease. Newer biological agents such as the tumour necrosis factor alpha (TNFα)-binding antibody infliximab have helped substantially but the lifetime risk for surgery remains disappointingly constant at ~25% for UC and 80% for CD. Current treatments only target the consequences of the disease and not the underlying pathogenic mechanisms. Better understanding of pathogenesis should lead to different, safer, and more effective therapies.

Genetic associations with IBD

All the genetically engineered mouse models of IBD require commensal enteric bacteria to be present in order for the development of intestinal inflammation. Studies in man show that UC and CD are polygenic disorders whose candidate predisposing genes have been clarified by a genome-wide study. Nine gene loci have been identified with high association signals for CD. The NOD2/CARD15 (nucleotide oligomerization domain 2/caspase recruitment domain 15) gene has been confirmed as an innate immune system gene polymorphism associated with ileal and stricturing phenotypic variants of CD. It probably accounts for ~15% of CD causation in Western countries although is not associated with CD in Japan. NOD receptors along with Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) for microbes. NOD proteins are cytosolic whilst TLRs are membrane bound (Fig. 1).

Other bacterial handling genes identified in the genome-wide scan, include ATG16L1 (ATG16 autophagy-related 16-like 1) gene and IRGM (immunity-related guanosine triphosphatase); a gene encoding a GTP-binding protein which induces autophagy. Autophagy is involved in killing of intracellular bacteria including Mycobacterium tuberculosis (M. tuberculosis), MST-1 (macrophage stimulating 1), also associated
with CD, encodes a protein that influences motile activity and phagocytosis by peritoneal macrophages.4

Associations with mutations in the interleukin 23 receptor, IL-23R, have also been reproducibly found.4 IL-23R encodes a subunit of the receptor for the pro-inflammatory cytokine IL-23, which is pivotal in the differentiation of T helper (Th17) cells. In animal models, the Th17 T cell subset mediates chronic and autoimmune inflammatory conditions.6

Most of these associations, apart from NOD2/CARD15, although statistically significant, have modest odds ratios for CD but give useful clues to likely pathogenic mechanisms.

The genetic contribution to UC causation is weaker with only about one in 10 identical twins of UC patients becoming affected compared with about one in two for CD. HLA-DRB1*0103 is strongly associated with extensive UC in caucasians, as is the multi-drug resistance

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**Fig. 1** NOD/TLR signalling in a mammalian host cell. Sensing of molecular moieties produced by invading microbial pathogens (so-called pathogen-associated molecular patterns or PAMPs) occurs both on the cell-surface and within the host cytoplasm, mediated by PRRs. These include TLRs triggered by bacterial moieties. MyD88 is an essential core intermediary for most TLRs leading to activation (increased nuclear localization) of transcription factor NFκB the key cell signalling molecule for inflammation. Of note, TLR5 is sited basolaterally and stimulated by bacterial flagellin. Nod-like receptors also activate NFκB via recruitment of RIP2 kinase, triggered by peptidoglycan moieties present within Gram-negative and Gram-positive bacteria cell wall, e.g. L-Ala-γ-D-Glu-meso-diaminopimelic acid (triDAP) and MDP MurNAc-L-Ala-D-isoGln. The red arrows indicate possible cross-talk between Nods and TLR signalling pathways.9

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**British Medical Bulletin 2008;88**

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1 (MDR1) gene. MDR1 expresses an apical epithelial efflux glycoprotein in distal small bowel and colonic epithelia. Mdr1 gene-knockout mice have increased susceptibility to intestinal inflammation. TNF superfamily gene members have also been associated with IBD phenotypes, with both high and low risk haplotypes of the same gene (TNFSF15) identified.

These genetic studies are providing increasing evidence to implicate defects in phagocyte function in CD and defects in barrier function in UC. The implications of some of these gene associations for interaction with the intestinal microbiota is now discussed.

**Implications of IBD gene-association for interactions with the gut microbiota**

**NOD2/CARD15**

The NOD2/CARD15 protein is expressed intracellularly in macrophages, epithelial cells and Paneth cells. The CD-related NOD2 mutations all affect the leucine-rich region which acts as the receptor for the bacterial cell wall peptidoglycan component muramyl dipeptide (MDP). There has been uncertainty about whether these are loss-of-function or gain-of-function mutations, but the consensus is moving towards regarding them as loss of function.

The NOD2 mutations produce different impairments in bacterial handling depending on the cell type involved. Defensin levels are noted to be low in the Paneth cells (present at the base of small intestinal crypts and some colonic crypts, particularly proximally) of individuals with mutant NOD2. However recent work has shown defensin deficiency to be independent of NOD2 status, and perhaps secondary to mucosal inflammation, so this remains controversial. NOD2 mutant macrophages have diminished IL-8 response to MDP and this reduced IL-8 response correlates with reduced neutrophil recruitment and generally defective acute inflammatory processing of bacteria in CD.

NOD2 mutant mice are particularly susceptible to challenge with oral *Listeria monocytogenes*, have decreased β defensin secretion and increased nuclear factor kappa B (NFκB) pro-inflammatory signalling in response to MDP. It seems possible that a point is reached in IBD where NFκB signalling becomes pathological rather than homeostatic, and the NOD2 mutation could lower the threshold at which this point is reached.

In addition, there is recent evidence that co-operation between Nod-like receptor (NLR) and TLR signalling pathways ultimately lead to NFκB activation (Fig. 1): Cross-talk is proposed to occur between
the Nod-like receptor pathway [Nod proteins and receptor-interacting protein 2 (RIP2) kinase] with the TGFβ-activated kinase, TAK1, activated following TLR interaction with adaptor protein myeloid differentiation factor 88 (MyD88).9

Defensins

The strong expression of NOD2/CARD15 in Paneth cells, the main source of bactericidal defensins, has led to great interest in the role of defective defensin production/function in CD pathogenesis.10 Paneth cells are particularly plentiful in the distal small intestine and the typically low number of bacteria in the small intestine may be partly due to the high abundance of α-defensins (cryptidins). Defensins can also act as a chemoattractant for cells expressing chemokine receptor CCR6, such as dendritic cells (a specialized antigen-presenting cell) and help to integrate host innate and adaptive immune response.10

There is increased mucosal expression of human β-defensin 2 in UC, which may reflect the type of epithelial interaction involved in the pathogenesis. Mucosal peptides extracted from patients with CD, UC and healthy individuals showed different abilities to kill bacteria, as assessed by flow cytometry.10 There is a decreased anti-microbial effect in CD, when compared with UC, independent of inflammation status or concurrent steroid treatment, and notably a decreased anti-microbial effect on Escherichia coli (E. coli)10 – a key pathogenic suspect. In ileal CD, there is reduced expression of ‘wingless-type’ (Wnt) signalling, a regulatory pathway directing cell–cell interactions and transcription factors. This leads to reduced Paneth cell differentiation and reduced defensin release and might be a primary problem in ileal CD.10

Defective phagocytic clearance

There are marked similarities between CD and the intestinal lesions found in patients with inherited phagocyte disorders such as chronic granulomatous disease and glycogen storage disease type 1b in humans. Further support for the defective phagocyte hypothesis comes from the identification of autophagy gene mutations in association with CD.

The neutrophil is the ‘professional killer’ of bacteria and it has been suggested that defective neutrophil recruitment as a consequence of reduced IL-8 expression by mononuclear cells, e.g. as a result of NOD2/CARD15 mutation, may result in macrophages being left to clear bacteria which they are less competent to kill.11 Repeat mucosal
biopsy, hours apart, in the same sites of the ileum and rectum in CD, UC and Control patients, resulted in fewer neutrophils and IL-8 producing cells being recruited into the biopsy site in CD intestinal tissue. Neutrophil migration into skin blisters in CD patients was also reduced.

There is also good evidence that macrophages in CD tissue contain bacteria. Immunocytochemical searches for bacterial antigens in mesenteric and lymph node specimens from patients with CD show that macrophages and giant cells contain *Listeria* spp., *E. coli* and Streptococcal antigens. These were present near ulcers, fissures, abscesses and in granulomata, as well as within germinal centres of mesenteric lymph nodes. *E. coli* DNA has also been identified by laser capture dissection of CD tissue granulomas, and was found in 12/15 CD granulomas compared with 1/10 from Controls.

An adherent invasive *E. coli* (AIEC), LF82, isolated from a patient with ileal CD, has been shown to be endocytosed normally by macrophage endomembrane organelles and then to replicate within mature phagolysosomes without triggering host cell lysis. There is also evidence that AIEC induce macrophages to form granulomata.

Bacteria can also suppress macrophage responses. A mannose-containing glycoconjugate that is expressed by *Mycobacterium paratuberculosis* as well as by *Candida albicans* and other yeasts (but not *E. coli*), is able to inhibit the killing of *E. coli* by macrophages. This effect is dependent on the MyD88 regulatory pathway and TLR4. A circulating anti-mannan antibody is commonly found in CD, the anti-*Saccharomyces cerevisiae* antibody (ASCA). Thus, the pathogenesis of CD may be contributed to by primarily impaired phagocyte function, secondary suppression of macrophage function by bacteria, or both (Fig. 2).

**Mucosal barrier**

The integrity of the mucosal barrier in the colon relies upon the mucus layer (and associated secreted proteins), the underlying glycocalyx and the epithelial cell membranes and tight junctions. The mucus layer is continuous in the colon but discontinuous in the small intestine. The mucosal barrier has been shown to be compromised in IBD, and in unaffected relatives of CD patients where it may precede lesion development.

The mucosal barrier weakness may be genetic or acquired. Absence of the apical efflux glycoprotein gene MDR1, and also possibly abnormalities of the trans-membrane mucin MUC3 are associated with UC. Acquired barrier defects can also occur, although may be at least
in part secondary to inflammation. *In vitro* studies have demonstrated that pro-inflammatory cytokines such as interferon-gamma and TNFα, which are increased in the intestinal mucosa of patients with IBD as a result of bacterial NOD/TLR stimulation or through hypersensitivity, can induce mucosal permeability, mediated by internalization of junctional proteins.\(^{19}\)

The activation of NFκB in epithelial cells is an early event in IBD that can be found in the surface epithelium of unaffected identical twins of IBD patients, even in the absence of histological inflammation. This seems likely to be a consequence of bacterial–epithelial interaction and may again reflect a barrier defect.\(^{20}\) Weakness of the mucosal barrier allowing flagellin to pass through tight epithelial junctions and thus have access to basolateral TLR5 with subsequent triggering of IL-8 release has been described in mouse models,\(^{21}\) and represents a plausible model for UC (Fig. 3), perhaps with inflammation perpetuated by autoimmunity.\(^{22}\)
Role of the intestinal microbiota

The gastrointestinal tract is colonized by 500–1000 bacterial species with 80% of these yet to be cultured. The bacterial population increases distally along the small bowel with Gram negative aerobes and some obligate anaerobes present, whilst the colon is heavily populated with anaerobes, \( \sim 10^{14} \) per gram of luminal content. The use of culture-independent techniques such as real time polymerase chain reaction (PCR), FISH, ribosomal intergenic spacer analysis (RISA) and 16S ribosomal DNA sequencing can allow analysis of microbes involved in CD by their expressed genes and demonstrate their cellular location. It can provide additional insight into the microbiota changes in CD, particularly when a combination of techniques is used.

A major problem is discerning underlying changes in the microbiota from those that may be secondary to inflammation. \textit{E. coli} and \textit{Bacteroides} detected by PCR of faeces were particularly noted to be increased in number in parallel with increased levels of intestinal inflammation, particularly in CD, although other studies have shown...
negative results. Rodent studies also show that bacterial composition can change with colonic inflammation.

**Loss of diversity in IBD**

Automated ribosomal intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphisms (T-RFLP) and denaturing gradient gel electrophoresis have all shown reduced diversity in both faeces and mucosa-associated microbiota in IBD. In one study, species richness increased from Control to non-inflamed UC and CD tissue but then declined in more inflamed tissue. Firmicutes and Bacteroidetes diversity are particularly affected, though an ARISA study demonstrated an increase in diversity of less well characterized Firmicutes.

**Specific faecal bacteria**

There is clear evidence that relapses of UC may be triggered by infection with conventional pathogens such as *Salmonella* spp. and *Campylobacter* spp. but with this notable exception there is more evidence to support specific pathogens in the pathogenesis of CD. The faecal microbiota do not reflect the mucosa-associated bacteria, which may be of much greater significance in IBD. FISH probes can be used to identify 11 bacterial groups in healthy patients and those with IBD and demonstrate distinct differences between faecal samples. Depletion of *Faecalibacterium prausnitzii* (*fprau*) bacteria in CD, and conversely high *fprau* in UC, enabled recognition of active UC or CD with good sensitivity and specificity. Differences in bacteria numbers and species in the colonic bacteria in the mucus layer have also been noted. Other changes noted in IBD faecal microbiota included depletion of *Clostridium coccoides* in UC and *Clostridium leptum* in CD. *Bacteroides fragilis* has been shown to account for 60% of the biofilm (mucosa-associated bacteria within the mucus layer) mass of IBD patients and *Eubacterium rectale-C. coccoides* in 15% of the same biofilm. Culture-independent analysis of ileal mucosa has also demonstrated relative depletion of Clostridiales but an increase in AIEC in ileal CD. *Listeria* and Streptococcal antigens have also been found in CD tissue, but further attempts to identify *Listeria* spp. by PCR have been negative.

Emerging evidence also suggests that genetics and/or environmental exposure during childhood, in part, may determine the gut microbial composition. Using T-RFLP molecular fingerprinting, Dicksved et al. recently demonstrated not only that the faecal microbiota was more similar between healthy identical twins than between twins with CD,
but that there were significant differences between identical twins concordant and discordant for CD.

**Growing evidence for a pathogenic role of E. coli in CD**

The colon, unlike the small intestine, has a near continuous mucus coat, and the bacteria adherent to this may differ in type (e.g. anaerobic) and number to those under the mucus (a microaerophilic niche).\(^{34}\) Aerobic culture of colonoscopic biopsies after removal of mucus is often sterile in Control colons, but in CD contains increased bacterial numbers. More than half of these are *E. coli*,\(^{35}\) even though they represent <1% of the faecal microbiota. A similar result has also been seen in ileal mucosa in CD.\(^{36}\) AIEC have been found in 21.7% of CD cases versus 6.2% in Controls. At least eight groups have identified independently an increase in mucosa-associated *E. coli* in CD,\(^{22}\) and culture-independent molecular techniques have confirmed this.\(^{24,26}\) Two of these studies also demonstrated increased numbers of *E. coli* in UC.

*E. coli* DNA has also been identified by laser dissection of CD tissue granulomas\(^{14}\) and was found in 12/15 CD granulomas compared with 1/10 from ‘Controls’. RISA has been used to identify DNA segments more commonly present in CD and UC mucosal biopsies than Controls.\(^{26}\) Five segments specific for CD mucosa have been sequenced and all found to contain *E. coli* DNA. Poor correlation was noted between site of inflammation and *E. coli*, compatible with *E. coli* being a causative organism rather than simply colonizing inflamed mucosa. The *E. coli* isolates identified by RISA were more likely to be from phylogenetic groups B2 and D,\(^{26}\) characteristically more likely to be pathogenic at extraintestinal sites and adherent to intestinal cells. Group B2 *E. coli* have virulence factors conferring strong colonizing capacity,\(^{37}\) and this provides an explanation for their increased prevalence in CD mucosa and also for the tendency for abscess and fistula formation in CD. *E. coli* isolated from ileal biopsies in patients with CD have been shown to be adherent and invasive in epithelial cell lines, and able to survive and replicate within macrophages\(^{15}\) without causing cell death but inducing secretion of the proinflammatory cytokine TNFα. A broad spectrum of faecal microbiota lacking these virulence factors may invade after a mucosal breach.

**E. coli virulence factors**

CD *E. coli* do not possess any known invasive determinants seen in other groups of *E. coli* but interact with epithelial cells via type 1 pili,
flagellae, outer membrane vesicles, and outer membrane porin C (OmpC).\textsuperscript{38} Mucinases are also produced by CD \textit{E. coli} which more commonly possess serine protease autotransporters,\textsuperscript{26} perhaps explaining why they have been found within and beneath the mucus layer. Bacteria in the B2 phylogenetic group characteristic of CD patients\textsuperscript{26} have enhanced persistence in the colon which is dependent on P fimbriae (facilitating adherence) and aerobactin, an iron trapping compound.\textsuperscript{37} Group B2 seem to have accumulated virulence factor genes and it can be speculated that improved hygiene has possibly reduced expression of other \textit{E. coli} groups in the West.\textsuperscript{37}

Type 1 pili of \textit{E. coli} mediate bacterial adherence to ileal epithelia, and mannose has been shown to diminish this adherence.\textsuperscript{39} The carcino-embryonic antigen cell adhesion molecules CEACAM5 (otherwise known as CEA) and CEACAM6 have been shown to be overexpressed in ileal epithelial cells in CD and \textit{E. coli} adherence is prevented \textit{in vitro} by blockade of CEACAM6.\textsuperscript{39} \textit{E. coli} induces expression of CEACAM6 on cultured intestinal cells directly, and also indirectly by induction of macrophages to secrete TNF\textsubscript{a} which increases CEACAM6 levels.\textsuperscript{39}

\textit{Mycobacterium avium} subspecies \textit{paratuberculosis}

\textit{Mycobacterium avium} subspecies \textit{paratuberculosis} (MAP) has been a putative causative organism for CD for almost a hundred years and is a continuing source of controversy.\textsuperscript{40} Johne’s disease in cattle, caused by \textit{paratuberculosis} and CD have similarities. Reported detection rates for \textit{paratuberculosis} DNA in CD tissue range between 0 and 100\%.\textsuperscript{3} Laser dissection of CD tissue has identified MAP DNA within granulomas but not within distant non-granulomatous tissue which might explain some of the discrepancies.\textsuperscript{14} MAP has been shown to release mannose-containing glycoconjugates that inhibit bacterial killing by macrophages \textit{in vitro}\textsuperscript{17} and an indirect role for MAP in CD pathogenesis is plausible.

The link between MAP and IBD, assessed by presence of IS900 (a MAP DNA insertion element) in Crohn’s and Control tissue PCR, and enzyme-linked immunosorbent assay (ELISA) of serum immunoglobulin reaction to MAP, have been examined in a recent meta-analysis. This produced a pooled odds ratio of 7.01, for PCR and 1.72 for ELISA when comparing CD and Controls,\textsuperscript{41} but proof of a causal role needs further work. A recent controlled trial of anti-MAP therapy in CD has been disappointingly negative. Moreover anti-TNF therapy can cause reactivation of \textit{M. tuberculosis} and its efficacy in CD is surprising if MAP is a significant pathogen.
Immune responses to bacterial antigens

An immune response to gut bacteria is seen in CD more than UC. Antibodies include those directed against *E. coli* OmpC, the *Pseudomonas fluorescens*-associated protein I2, perinuclear anti-neutrophil cytoplasmic autoantigen (pANCA) and ASCA mannan. Reported seroprevalence is 55% for anti-OmpC, and 50% for anti-I2 in CD. ASCA has been noted in 39–61% of CD and 5–15% of patients with UC. In contrast, pANCA is present in 50–73% of UC patients and 4–24% of those with CD.

Sera from CD patients also display increased immune response to CBir1, a Clostridial flagellin protein. Anti-CBir1 response is independently associated with small bowel and fibrostenosing CD. Good clinical response to faecal (bacterial) diversion in CD, e.g. by stoma formation, can be predicted by I2 antibody positivity.

Bacterial antibodies thus associate with disease phenotype in IBD but their role in pathogenesis remains unclear.

M cells as a probable portal of entry in CD

There is evidence that M cells represent the portal of entry for most gut pathogens. It has been shown that at least 14 different species of pathogenic and non-pathogenic bacteria selectively adhere to M cells. Overtly invasive pathogens such as *Shigella* and *Salmonella* are unable to invade via normal colon cells and enter via M cells. If unequivocal pathogens need M cells as a portal, it seems likely that AIEC found in CD, although lacking conventional pathogenicity genes, will also have to enter via them (Fig. 4).

M cells (membranous/microfold cells) are an epithelial cell phenotype of the follicle-associated epithelium (FAE) that translocate ‘foreign’ material from the gut lumen to lymphoid tissue within the intestinal mucosa. They account for ~1–3% of the cells in the dome epithelium that overlies Peyer’s patches in the distal small intestine and the lymphoid follicles in the colon. Since the initial lesions in CD occur at Peyer’s patches and lymphoid follicles knowledge of the interaction of M cells with bacteria is particularly important. The apical microfold membranes of M cells facilitate adherence and uptake of microorganisms and microbial antigens for presentation to lymphoid or antigen-presenting cells in the sub-epithelial tissue. The M cells have distinct features: a poorly organized brush border, marked endocytic activity, and a distinctive lymphocyte ‘pocket’. The lack of mucus (there are no goblet cells in the dome epithelium) and also a lack of the ‘fuzzy’ glycocalyx that overlies other intestinal epithelial cells facilitates contact between
bacteria and the M cell-surface. In addition, invaginations of the basolateral membrane of M cells may amplify the cell-surface microfold architecture so as to minimize the distance that endocytic vesicles translocate across the cell.43,44 This also provides a basolateral location for lymphocytes which interact with the dense network of antigen-processing cells including macrophages sited beneath the epithelium. Overt pathogens such as Shigella, Salmonella, and Yersinia have more than one mode of interaction with M cells,45 and outcome often depends on their mechanisms for surviving macrophage engulfment. Non-invasive Salmonella do not accumulate in Peyer’s patches whereas invasive Salmonella do.47 Both strains induce an IgG response, which suggests that non-invasive Salmonella may be taken up from the lumen by dendritic cell extensions and transported from there to the mesenteric lymph nodes and spleen. Dendritic cells are known to populate the entire gastrointestinal tract, with distinct sub-types having specific function.48 They are phagocytic cells, which may depend on cytokine expression and TLR signalling47 and are involved in uptake of luminal bacteria regardless of their invasiveness.49 It is likely that both M cells and dendritic cells have key roles in bacterial and immune system ‘cross-talk’ and that specific bacteria involved in CD interact with both
M cells and dendritic cells to generate immune responses and disease. Characterization of these interactions and the ligands involved may be pivotal in understanding IBD.

In UC, there is no good evidence for mucosal invasion by bacteria and the typically continuous inflammation seen does not suggest any specific focus around lymphoid follicles.

**Antibiotics in IBD**

If CD results from inadequate clearance of mucosal bacteria then use of antibiotics would be predicted to have beneficial effects but, results have been variable. Choice and combination of antibiotics is key and should target the suspected bacterial pathogens in CD. Many studies are non-blinded and not placebo controlled, and can involve other treatments making comparisons difficult. Rifabutin, clarithromycin and clofazimine (targeting *M. paratuberculosis*) have shown improvement over placebo at 16 weeks, but this was not sustained. A meta-analysis of broad spectrum antibiotics, involving six randomized and blinded trials, in patients with active CD demonstrated an advantage for antimicrobial therapy, particularly when ciprofloxacin was used.

The susceptibility of CD *E. coli* within macrophages to antibiotics has been evaluated *in vitro*. Ciprofloxacin, rifampicin, tetracycline, sulphamethoxazole, clarithromycin and trimethoprim were all associated with kill rates >50% at $C_{\text{max}}$, the published peak serum concentration of antibiotic achievable in patients taking a conventional dosing regimen. A combination of ciprofloxacin, tetracycline, and trimethoprim resulted in a 97% kill rate versus 86% for ciprofloxacin alone when assessed at 10% $C_{\text{max}}$. Further studies are required, with appropriately targeted antibiotic therapy, particularly in combination.

**Prebiotics, probiotics and soluble fibre**

Prebiotics are ‘food ingredients fermented by intestinal bacteria that selectively promote changes in the gut ecosystem’. Inulin and oligofructose stimulate saccharolysis in the colon and enhance growth of *Lactobacilli* and *Bifidobacteria*. This effect has been associated with reduced mucosal inflammation in several animal models of IBD. Preliminary clinical trials have evaluated inulin in pouchitis (a condition in which the ileal pouch becomes inflamed after colectomy and reconstructive ileal pouch-anal anastomosis for UC) and oligofructose in UC and CD, generally involving patients with mild to moderate disease, and inulin improved endoscopic and histological appearance.
in pouchitis. The other trials suffered from low numbers or absence of a Control arm (in the CD study) and were inconclusive.

Probiotics have been assessed in animal models and some clinical trials. However, there is an absence of large randomized double blind clinical trials. The probiotic VSL#3 reduced pouchitis relapse rate by 85% in one study and there is some suggestion that it can have an effect in maintaining UC in remission. It may be that probiotics and prebiotics can be used as co-therapies (synbiotics) in genetically/bacteriologically susceptible patients if further evidence accumulates.

Currently, there are more studies showing beneficial effects of probiotics in prevention of inflammation rather than treatment of inflammation and it may be that the contrasting effects of basolateral versus luminal stimulation of TLR9 and the potential for flagellin released from probiotics to access basolateral TLR5 in inflamed mucosa might explain this difference.

We have been investigating the hypothesis that bacteria–epithelial interactions, many of which may be lectin-mediated, might be inhibited by soluble plant fibres. A number of fibres, particularly, soluble plantain fibre, have been shown to inhibit adhesion of E. coli to the intestinal mucosa. This has obvious potential therapeutic benefit in arresting the events that induce mucosal inflammation in CD (Fig. 2). In areas of the world where plantain is consumed in large amounts (Africa, India and the West Indies), the prevalence of CD is rare. It is possible that other foodstuffs or fibres could inhibit or promote (Western diet) bacterial adherence, and this merits further evaluation.

Conclusions

Areas of agreement

It is generally agreed that IBD is caused by a dysregulated immune response to luminal bacteria and that relevant components include defensins, macrophage function, M cells, dendritic cells, neutrophils and the adaptive immune response. The mucosa-associated microbiota in IBD is clearly different to that in health. The genetic associations with IBD are increasingly defined and include genes that are relevant to bacterial detection and killing.

Areas of controversy

There is ongoing debate about whether IBD is caused by specific pathogens or whether it reflects an altered immune response to commensals.
The species of bacteria involved may be different depending on host factors. There is an apparent paradox that immunosuppressive treatments like the anti-TNF antibody Infliximab are successful in a condition where bacteria are apparently involved in pathogenesis.

**Growing points**

Interaction between multiple scientific disciplines will be essential to answer the questions posed by IBD. Knowledge about changes in microbiota composition should guide diagnostics and help research into pre-, pro- and synbiotics. Further antibiotic studies will be important and may be the final proof of principle as per *Helicobacter pylori* and duodenal ulcers. The characterization of bacterial and host factors by molecular techniques is progressing rapidly, although the task of fully characterizing the human microbiome is vast compared with the human genome programme. Identification of ligands involved in *E. coli* M cell immune interfacing will be key, and may provide novel therapeutic targets. Dietary modifications that inhibit *E. coli* translocation may provide simple effective treatment but need further evaluation.

The bacteria involved in IBD and their modes of interaction with the host are slowly being elucidated. Full appreciation of genetic, immune, microbiological and dietary/environmental factors is emerging slowly, and advances in understanding will present us with new targets. Future patient management is likely to reflect this.

**Funding**

Work in the authors laboratory is supported by grants from the National Association for Colitis and Crohn’s Disease (NACC), National Institute of Health Research (NIHR), Wellcome Trust, Medical Research Council and Provexis Plc. PK is a clinical research fellow within the NIHR Specialist Biomedical Research Centre in Microbial Disease based at the Royal Liverpool & Broadgreen University Hospitals NHS Trust and University of Liverpool.

**Conflict of interest:** BJC and JMR are currently conducting research sponsored by Provexis Plc examining the use of a soluble fibre preparation as maintenance therapy for Crohn’s disease. JMR is a past/present member of advisory boards for Procter and Gamble, Schering-Plough, Chiesi, Falk, and Celltech/UCB, and, with the University of Liverpool and Provexis Plc, holds a patent for use of a soluble fibre preparation as maintenance therapy for Crohn’s disease. PK has no conflicts of interest to disclose.
References