Bacteria in the pathogenesis of inflammatory bowel disease

Paul Flanagan, Barry J. Campbell and Jonathan M. Rhodes
Department of Gastroenterology, Institute of Translational Medicine, University of Liverpool, Duncan Building, Daulby Street, Liverpool L69 3GA, U.K.

Abstract
Twin studies have demonstrated the importance of environmental factors in the pathogenesis of inflammatory bowel disease, but progress has been relatively slow in identifying these, with the exception of smoking, which is positively associated with Crohn’s disease and negatively associated with ulcerative colitis. Genetic studies have identified risk alleles which are involved in host-bacterial interactions and the mucosal barrier, and evidence is building for a likely pathogenic role for changes in the gut microbiome, with respect to both faecal and mucosa-associated microbiota. Some of these changes may be secondary to inflammation, nevertheless promising new therapeutic targets are beginning to emerge.

Introduction
UC (ulcerative colitis) and Crohn’s disease are the two predominant phenotypes of IBD (inflammatory bowel disease). Crohn’s disease can cause transmural inflammation, affect any part of the gastrointestinal tract and is typified histologically by the formation of granulomas (coalescence of macrophages), stricturing and fistulae. In contrast, UC is typified by mucosal inflammation and is limited to the colon [1]. There is significant overlap between isolated colonic Crohn’s disease (which accounts for about one-third of Crohn’s disease cases) and UC, as both are negative for the NOD2 (nucleotide-binding oligomerization domain-containing 2) gene association, negative for the ASCA (anti-Saccharomyces cerevisiae antibody), commonly positive for the pANCA (perinuclear anti-neutrophil cytoplasmic antibody) and both are strongly associated with the HLA DRB1*0103 haplotype [2–4]. This raises the possibility that colonic Crohn’s disease and UC are at different ends of the same disease spectrum, with ileal Crohn’s disease as a distinct disease entity.

IBD has a prevalence in the U.K. of 122 per 100 000 (UC) and 214 per 100 000 (Crohn’s disease), but there are significant variations between and within countries, with the highest rates seen in northern latitudes [5,6]. In developed countries, Crohn’s disease is the more prevalent disease, with UC more common in developing countries. The mainstays of treatment are 5-aminosalicylates, steroids, thiopurines and anti-TNF (tumour necrosis factor) agents, but, despite this, 10–33% of UC and 50–80% of Crohn’s disease patients will require surgery at some point [7].

Key words: autophagy, Crohn’s disease, dysbiosis, microbiota, ulcerative colitis.

Abbreviations used: AIEC, adherent and invasive Escherichia coli; ATG16L1, autophagy-related 16-like 1; ASCA, anti-Saccharomyces cerevisiae antibody; IBD, inflammatory bowel disease; IL, interleukin; IRGM, immunity-related GTPase; MAP, M. avium subsp. paratuberculosis; M cel, muralid cell; MDP, muramyl dipeptide; NOD2, nucleotide-binding oligomerization domain-containing 2; TNF, tumour necrosis factor; UC, ulcerative colitis.

Genetics of IBD: links to innate immunity, regulation of the acquired immune response and the mucosal barrier
Gene associations with IBD are nearing 100, although many of these have modest odds ratios. The genes identified can be broadly grouped into three categories: innate immune response including autophagy [e.g. NOD2/CARD15, ATG16L1 (ATG16 autophagy-related 16-like 1)], regulation of acquired immune response {IL23R (IL (interleukin)-23 receptor) and mucosal barrier [ECM1 (extracellular matrix protein 1), CDH1 (cadherin type 1), HNF4A (hepatocyte nuclear factor 4α) and LAMB1 (laminin β1)]. NOD2, which is expressed in Paneth cells, macrophages and epithelial cells, was identified as a susceptibility gene for ileal Crohn’s disease (but not UC) in 2001 [4]. A large GWAS (genome-wide association study) then identified additional associations between two autophagy genes and Crohn’s disease: ATG16L1 and IRGM (immunity-related GTPase) [8]. The NOD2 protein acts as an intracellular receptor for MDP (muramyl dipeptide), a component of Gram-negative and Gram-positive bacterial cell wall peptidoglycan, leading to production of Paneth cell defensins, which have a potent antimicrobial effect [9,10]. Autophagy, a process by which phagosomes and lysosomes fuse to form double-membraned vacuoles within macrophages, is an important cellular mechanism for clearing intracellular bacteria. MDP-engaged NOD2 plays an important role in autophagosome generation, recruiting ATG16L1 to the cell-surface membrane where bacterial engulfment occurs. Crohn’s disease-related mutations in NOD2 have complex effects, including impaired autophagy (via a failure to recruit ATG16L1) and bacterial killing by macrophages, a reduced mononuclear cell IL-8 response to bacteria and reduced defensin production by Paneth cells [10]. Loss of ATG16L1 and IRGM function has also been shown to alter Paneth cell granule morphology [11], to increase IL-1β production and allow enhanced intracellular bacterial survival [12].
However, genetics can only explain part of the IBD pathogenesis. Only between 6 and 33% of IBD patients have another affected family member [13] and concordance in monozygotic twins is low (27% in Crohn’s disease, 15% in UC) [14]. It has been estimated that the 71 locus polymorphisms associated with Crohn’s disease and the 47 associated with UC only explain 23.2% and 16% of disease hereditability respectively [15,16]. However, it is possible that this may be an underestimate, and environmental factors alone might be sufficient to trigger disease, for example, by inducing a phenotypic change in innate immune function similar to that caused by the IBD gene polymorphisms. Crohn’s disease patients have been shown to have decreased neutrophil recruitment to sites of injury, possibly as a consequence of disordered macrophage cytokine response, and a reduced ability of macrophages to clear bacteria [17,18]. Smoking has been shown to impair the ability of lung macrophages to kill intracellular bacteria [19] and vitamin D deficiency has been shown to impair dendritic cell/macrophage function and to reduce NOD2 expression, and vitamin D supplementation has been shown to reduce the risk of Crohn’s disease relapse [20–22].

The human gut microbiome
The total human gut microbiome consists of approximately 1150 bacterial species, with each individual host having approximately 160 species [23]. Gut colonization is established within the first 2 weeks of life, with further changes on weaning, and then usually remains remarkably stable over time [24]. It is important to note that the mucosa and faecal microbiomes consist of distinct bacterial populations, and mucosa-associated bacteria may be more important in IBD [25]. Although it is only possible to culture 20–30% of the gut microbiota, modern molecular techniques have illuminated changes in the microbiome in IBD.

Faecal dysbiosis in IBD: unstable and reduced diversity
At least seven independent groups have now consistently found differences in the faecal microbiome in patients with IBD compared with healthy controls. First, there is a significantly reduced biodiversity in both Crohn’s disease and UC with changes in the dominant organisms. Secondly, whereas the microbiota in healthy individuals remains stable over time, significant temporal variations are seen in IBD. Furthermore, significant inter-individual differences are seen in IBD, but not in health [26–37].

In health, the Firmicutes and Bacteroidetes phyla predominate and contribute to the production of epithelial metabolic substrates including butyrate [38]. In contrast, in Crohn’s disease, changes are observed in the microbiota characterized by a relative lack of Firmicutes (specifically a reduction in Clostridium leptum) and Bacteroidetes with an over-representation of enterobacteria (including Escherichia coli), which may conceivably alter epithelial homeostasis. In UC, the dominant organisms are less clear, but a reduction in Clostridium spp. and increase in E. coli have been reported [30,36,37]. In both Crohn’s disease and UC, relapses have been commonly reported in association with pathogenic infections such as Campylobacter and Salmonella gastroenteritis [39].

It is conceivable that some of these observed variations arise as a result of environmental and dietary influences, but family and twin studies suggest that they are disease-specific. In families with at least three members affected by Crohn’s disease, there were still significant differences in the faecal microbiota of patients compared with unaffected family members [28]. These findings are supported by a study in which monozygotic twins discordant for Crohn’s disease were shown to have significantly different faecal microbiota with a reduction in biodiversity seen in Crohn’s disease, a change most marked in patients with ileal disease [29].

At present, it is not possible to say with any certainty whether these observed differences represent a primary or secondary phenomenon. Changes in faecal microbiota are also seen in infectious colitis and in models of inflammation such as DSS (dextran sodium sulfate)-induced colitis [40], so, although the pattern is different between UC and Crohn’s disease, the faecal dysbiosis may arise at least in part as a consequence of inflammation rather than as a primary event [27]. Against this, the temporal instability of the microbiota, disease-specific microbiota patterns and the consistent finding of changes in both active and quiescent disease (in Crohn’s disease) argues for a primary role.

Faecalibacterium prauznitzii: a potent anti-inflammatory commensal organism that is negatively associated with ileal Crohn’s disease
In ileal Crohn’s disease, both mucosal and faecal bacterial studies have demonstrated reduced levels of the Firmicute F. prauznitzii, a member of the C. leptum group. In patients with ileal Crohn’s disease, a strong association is seen with depleted F. prauznitzii in biopsies taken from the colon and ileum, and this is not the case for colonic disease. This reduction in F. prauznitzii appears to be associated with a concomitant increase in ileal and colonic E. coli [41] (Figure 1). This may be clinically relevant, as low levels of mucosa-associated F. prauznitzii are associated with a much greater risk of recurrent Crohn’s disease following surgery and the bacteria and its supernatant are able to reduce severity of chemical [TNBS (2,4,6-trinitrobenzenesulfonic acid)]-induced colitis in murine models [42].

Mucosa-associated bacteria
In the healthy colon, there is a continuous mucus coating consisting of two layers, the outer of which is loosely adherent and has a favourable environment for bacterial growth, but the inner tightly adherent layer is normally sterile. In the small intestine, the adherent layer is much thinner and probably discontinuous. In IBD, particularly Crohn’s disease, there
Molecular Biology of Inflammatory Bowel Disease

**Figure 1** Mucosa-associated *F. prauznitzii* (‘good’) and *E. coli* (‘bad’) in ileal and colonic biopsy samples from identical twins discordant for IBD

CCD, colonic Crohn’s disease; HC, healthy controls; ICD, ileal Crohn’s disease. For each numbered individual, biopsies have been sampled (left to right) from ileum, ascending, transverse and descending colon, and rectum. Ileal Crohn’s disease is shown to be associated with a reduction in *F. prauznitzii* and an increase in mucosa-associated *E. coli*. In colonic Crohn’s disease, there is a more modest increase in *E. coli* and no change in *F. prauznitzii*. Reprinted from [41] with permission from Wiley.

is a marked increase in bacteria associated with the colonic adherent mucus layer [43–45].

In Crohn’s disease, consistent increases in mucosa-associated Proteobacteria (including *E. coli*) and reductions in Firmicutes are reported, but results for Bacteroidetes vary between studies [44,46,47]. There is strong evidence for an increase in mucosa-associated *E. coli* in both the ileum and colon, and their presence within Crohn’s disease granulomas argues for a primary pathogenic role [48]. Crohn’s disease *E. coli* isolates tend to have an adherent and invasive (AIEC) phenotype typified by invasion of epithelial cells and replication within macrophages [49]. Moreover, AIEC have been shown to induce granuloma formation in vitro, and *E. coli* with a similar AIEC phenotype induce granulomatous colitis in Boxer dogs [50,51]. The pattern in UC is less clear, with some studies reporting no difference from healthy controls and others reporting an increase in mucosa-associated *E. coli* [43,52]. The effect of mucosal inflammation is also unclear, as, in both UC and Crohn’s disease, there have been differing reports, with some seeing a marked reduction in bacterial diversity in inflamed tissue and others observing little or no difference [52–54].

**M (microfold) cells as a portal of entry**

M cells are specialized epithelial cells which account for approximately 5–10% of the dome epithelium overlying Peyer’s patches in the distal ileum and lymphoid follicles in the colon. They differentiate as a result of an incompletely understood interaction between surface epithelial cells and factor(s) released by the underlying B-lymphocytes. They represent the major portal of entry for invasive gut pathogens, and it is likely that they are also the initial portal of entry by which AIEC are able to invade (Figure 2). In support of this, the aphthoid ulcers that are the earliest lesions of Crohn’s disease are commonly seen to overlay Peyer’s patches and lymphoid follicles, moreover there is a striking correlation between the age-related incidence of Crohn’s disease and
the number of Peyer’s patches in the small bowel, the latter peaking in late adolescence and then falling away [55].

**MAP (Mycobacterium avium subsp. paratuberculosis)**

MAP has long been postulated as a potential pathogenic agent in Crohn’s disease, even by Dalziel who reported the condition in 1913 [56], before the classic paper by Crohn et al. [57]. MAP DNA has been identified within Crohn’s granulomas and NOD2 polymorphisms are associated with an impaired innate immune response to *Mycobacterium leprae* [58,59]. Additionally, ASCAs are detectable in 68% of Crohn’s disease patients, and the mannan (mannose α-1,3-mannose) epitope for this antibody is shared by MAP [2]. However, the improvement seen with anti-TNF agents and the lack of response to anti-mycobacterial treatment argues against a primary role for MAP [60]. It might still have a pathogenic role as a co-factor and it has been shown to locally release a mannose-containing glycoconjugate which reduces killing of *E. coli* by macrophages [61].

**Antibiotics as a treatment**

In view of the observed bacterial changes in IBD, a clinical response to antibiotics might be expected, but the responses are variable. In active UC, there have been several trials of different antibiotics, but, overall, there is relatively little evidence to support their use [62]. In Crohn’s disease, antibiotics are effective at reducing post-operative recurrence of Crohn’s disease (nitroimidazoles), at treating peri-anal disease (ciprofloxacin and metronidazole) and treating active colonic (but not ileal) disease [63,64]. Several antibiotics have been shown to have efficacy in killing intra-macrophage bacteria (for example, *E. coli* in *vivo*; however, in clinical use, antibiotics have generally been chosen on empiric grounds [65]. There is now a need for specific clinical trials of antibiotics which kill bacteria within macrophages. Targeted therapy against MAP has not been shown to have lasting benefit, although it has been argued that the treatment regimen used was suboptimal [66].

**The role of soluble plant fibres as ‘contrabiotics’: working to prevent bacterial adherence to the epithelium**

Work by our group has shown that soluble plant fibres, including those from plantain (bananas) and broccoli, are able to block adherence of various bacteria, including AIEC, to the gut epithelium and also to block translocation across M cells and Peyer’s patches in *ex vivo* experiments [67] (Figure 3). This effect has been shown against a range of pathogens and seems likely to reflect a broad beneficial effect of soluble dietary fibre, an effect that we have termed ‘contrabiotic’. Clinical trials are underway to determine whether dietary supplementation with soluble plantain fibre might reduce the risk of relapse in Crohn’s disease. Conversely, food emulsifiers increase bacterial translocation *in vitro*. Dietary intake of such factors might therefore be one of the environmental factors that could account for the major variations in Crohn’s disease incidence.

**Future directions**

The literature now suggests a significant role for defective host immunity and bacterial dysbiosis in the pathogenesis of IBD. Whereas specific organisms have been associated with disease, the overall structure of the gut microbiome (increases in potentially pathogenic bacteria and a reduction in protective bacteria) and the interplay between organisms may be more significant. To establish the pathogenic importance of these microbial changes, we now need clinical trials, with specific outcome measures, targeting each of these pathways. Proof that *Helicobacter pylori* caused ‘peptic’ ulcers only came with evidence that eradication therapy led to permanent cure and the bacterial hypotheses for IBD will...
Figure 3 | Plantain fibre blocks translocation of *E. coli* across the human intestinal epithelium in Ussing chambers


likewise only become accepted once targeted therapies have been shown to be effective.

References

8 The Wellcome Trust Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 controls Nature 447, 661–678

Received 26 April 2011  
doi:10.1042/BSRT391067