**Host–microbiota interactions in inflammatory bowel disease**

**Bacteria, good and bad**

Inflammatory bowel disease (IBD), a term encompassing the conditions ulcerative colitis (UC) and Crohn’s disease, affects around 1 in 300 people in the UK and causes significant morbidity, although, thankfully, little mortality. Although UC is limited to the colon and causes mucosal ulceration, Crohn’s disease can affect any part of the intestine and causes ulceration, transmural inflammation, stricturing, fistula and abscess formation. Both are relapsing and remitting diseases and, despite advances in medical treatments, including immunosuppressants and drugs which specifically block pro-inflammatory molecules (tissue necrosis factor; TNF), about 25% with UC and 50–80% with Crohn’s disease will require major surgery at least once in their lives.

IBD is usually said to be due to environmental triggers in genetically susceptible patients, but only about 25% have a family member affected, so some cases may result from environmental triggers without any definite genetic predisposition. Our understanding of the genetic factors has been greatly advanced by the application of modern DNA technology through genome-wide association scanning (GWAS) and the genes identified fall into three distinct groups: those affecting the innate immune system, those affecting the immune response, and those affecting the integrity of the mucosal barrier. There are striking geographical variations in the incidence of IBD and, as IBD develops in a ‘Westernizing’ population, UC is initially more prevalent with a subsequent rise in Crohn’s disease. Animal models of IBD almost all require the presence of the normal commensal microbiota, and there is growing support for the hypothesis that IBD results from an inappropriate reaction to the gut microbiota.

**The human gut microbiota**

Study of the human gut microbiota (that is, the community of micro-organisms colonizing the gastrointestinal tract) has advanced greatly with the advent of molecular techniques which allow culture-independent identification of species (only 20–30% can be cultured). Many of these techniques take advantage of the highly conserved nature of the 16S subunit of bacterial rRNA to amplify bacterial DNA extracted from faecal or mucosal samples. Subsequent sequencing of the variable DNA regions then allows species identification. Using this approach, we now know that the total human gut microbiota consists of around 1150 bacterial species. At an individual level, the gut contains two distinct bacterial populations, the faecal and mucosa-associated microbiota, comprising around 160 species in total and typified by a predominance of the Firmicutes and Bacteroidetes phyla. The microbiota is established early in life, particularly following weaning, and then changes relatively little over time. It plays a role in maintaining gut health via the production of epithelial metabolic substrates, including butyrate, as well as including beneficial bacteria with anti-inflammatory properties.

**The microbiota in IBD**

The faecal microbiota in IBD differs from that in health by demonstrating significant inter-individual variation, instability over time, reduction in biodiversity and changes in the dominant phyla, all of which may contribute to the disease state. Even in health, our environment (including diet) can alter the microbiota, but twin and family studies allow us to control for this and have added to the weight of evidence for an abnormal microbiota in IBD. Studies looking at monozygotic twins discordant for disease and other studies comparing patients with Crohn’s disease to unaffected relatives in families with a high incidence of disease have both demonstrated significant differences between health and the disease state, with changes perhaps most marked in those with ileal disease. The phyla which dominate in health are reduced in Crohn’s disease with lower levels of Bacteroidetes and Firmicutes and an increase in Proteobacteria, particularly Enterobacteriaceae. Although changes are also seen in UC, no clear pattern has yet emerged, although reduced *Clostridium* spp. (members belonging to the Firmicutes) and increased *Escherichia coli* (a member of the family Enterobacteriaceae, belonging to the Proteobacteria) has been reported.

The evidence for alterations in the faecal microbiome is now fairly well established, with several independent groups reporting similar
findings, but it has not yet been determined whether these changes have a primary pathogenic role or arise as a consequence of inflammation. These differences have been demonstrated in both active and quiescent Crohn’s disease, suggesting a primary role, but countering this, similar findings, including particularly a reduced diversity, can be seen in both infectious colitis and chemically induced murine models of colitis, raising the possibility that the observed variations are simply a consequence of inflammation.

Similar variations are also observed in the mucosa-associated microbiota and have best been described in Crohn’s disease with more variable findings in UC. The large bowel epithelial surface has a continuous layer of adherent mucus with a thinner intermittent layer found covering the small bowel, and it seems increasingly likely that the bacteria found in association with this mucosal surface are important in the pathogenesis of IBD. In Crohn’s disease, the observed alterations are typified by a reduction in Firmicutes and increase in Proteobacteria, with significant numbers of bacteria, particularly E. coli, found in the normally sterile inner mucus layer. Indeed there is strong evidence for an increase in mucosa-associated E. coli in both the colon and ileum of patients with Crohn’s disease. These E. coli, termed adherent, invasive E. coli (AIEC), lack classical markers of pathogenicity, but are able to invade both epithelial and macrophage cell lines and furthermore can not only avoid killing by, but also replicate within, macrophages.

Evidence for a primary pathogenic role for these E. coli is given by their presence within granulomas, an organized collection of macrophages and the histological hallmark of Crohn’s disease. Moreover, AIEC, when incubated in vitro with macrophages, can induce granulomas, and E. coli with a similar phenotype are found in association with a granulomatous colitis in boxer dogs. Although AIEC can invade epithelial cell lines in vitro, they are not normally observed in the epithelium in vivo which raises the question: if they are primarily pathogenic, what is the route of entry? To answer this, we must look at the site of the earliest lesion seen in Crohn’s disease, the aphthous ulcer, which frequently occurs overlying Peyer’s patches. Peyer’s patches have an overlying ‘dome’ epithelium within which about 5% of cells are specialized microfold (M) cells which act as a major route of entry for gut pathogens. AIEC have been shown experimentally in in vitro and ex vivo studies to translocate across M cells. Thus there is a plausible hypothesis that Crohn’s disease lesions may be initiated by AIEC crossing the mucosa at M cells overlying Peyer’s patches in the ileum, or smaller lymphoid follicles in the colon, with subsequent uptake by macrophages and granuloma formation (Figure 1).

Not all bacteria are harmful, and it is likely that the interplay between bacterial populations is also important. There are particularly intriguing findings in relation to Faecalibacterium prausnitzii, a member of the Firmicutes. Patients with ileal Crohn’s disease have significantly reduced F. prausnitzii in both ileal and colonic mucosal samples, with concomitant increases seen in E. coli numbers. F. prausnitzii releases an anti-inflammatory factor, as yet unidentified, into its surroundings. This appears to have relevance to disease behaviour, as low levels of mucosal F. prausnitzii in surgical resection specimens are associated with a much greater risk of recurrent Crohn’s disease 6 months after surgery, and its supernatant attenuates the severity of chemically induced colitis in mice.

**Mycobacterium**

Mycobacterium avium subsp. paratuberculosis (MAP) has long been incriminated in Crohn’s disease, mainly because it is known to cause granulomatous intestinal disease in cattle (Johne’s disease). MAP DNA has been demonstrated in Crohn’s-related granulomas, but strong evidence for a primary pathogenic role has been lacking. In particular the significant improvement in Crohn’s disease seen with anti-TNF agents very much argues against MAP as a pathogen given that anti-TNF agents can lead to the reactivation of the classical Mycobacterium infection tuberculosis. However, interest has reignited in this area following the discovery of an association between NOD2 polymorphisms and failure to respond to TNF agents very much argues against MAP as a pathogen given that anti-TNF agents can lead to the reactivation of the classical Mycobacterium infection tuberculosis. 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**Genetics of IBD: links to host–bacteria interaction**

Only a relatively small proportion of patients with IBD have an affected relative (6–33%) and concordance between monozygotic twins is low in both UC (15%) and Crohn’s (27%). Genome-wide association studies have identified disease-associated single nucleotide polymorphisms at many gene loci (71 in...
Crohn’s disease, 47 in UC), and it has been suggested that these may only explain around 23% of disease hereditability. Many of the gene associations identified relate to the innate immune response, particularly to the production of defensins, potent antimicrobial molecules, and to autophagy, an important mechanism relevant to killing of bacteria within macrophages. Thus the protein product of the NOD2 gene that is altered in association with ileal Crohn’s disease acts as an intracellular receptor for a component of bacterial cell wall peptidoglycan, muramyl dipeptide. Crohn’s disease-associated mutations in NOD2 have been associated with reduced defensin production, impaired autophagy and increased intra-macrophage bacterial survival.

Autophagy is the process whereby a phagosome fuses with a lysosome forming a mature phagolysosome and is an important mechanism for dealing with intracellular bacteria. Two specific autophagy genes have been associated with Crohn’s disease; autophagy-related 16-like 1 (ATG16L1) and immunity-related GTPase family M protein (RMIRGM). Polymorphisms of these genes have been associated with increased intracellular bacterial survival, perhaps in part due to a failure to form mature phagolysosomes.

Given the possibility, mentioned earlier, that not all IBD may necessarily have an underlying genetic component, it is intriguing that phenotypic defects have also been reported in Crohn’s disease that are not obviously accounted for by genetic abnormalities. Thus patients with Crohn’s disease have reduced recruitment of neutrophils when injected subcutaneously with inactivated E. coli and a reduced ability to clear the bacteria. There is also recent evidence, so far only reported in abstract, that Crohn’s patients’ peripheral blood monocyte-derived macrophages may be defective at killing internalized Gram-negative bacteria. Smoking, a well-established risk factor for Crohn’s disease, has been shown to impair macrophage function and reduce the patient’s ability to deal with intracellular bacteria. Furthermore, vitamin D deficiency also reduces macrophage function and, when given to patients with quiescent disease, has been shown clinically to reduce the risk of relapse.

### Bacteria as targets for treatment

Given the potential role for bacteria in pathogenesis, a clinical response to antibiotics might be expected, but results are mixed. Experimentally, a range of antibiotics...
has been shown to have effect against intra-macrophage AIEC, but there are few good-quality clinical trials on which to base clinical practice\textsuperscript{32}. Antibiotics have been shown to be effective in treating perianal Crohn’s disease and for preventing post-operative recurrence of Crohn’s disease, but the evidence to use them in other settings is lacking despite widespread clinical use\textsuperscript{33}. These inconclusive results may reflect the fact that, until now, the nature of the bacterial target and its site, e.g. intracellular, has been unclear and antibiotic regimens tested are likely to have been suboptimal.

An alternative strategy is to prevent bacteria adhering to the mucosal surface with a view to preventing translocation across M cells. Experimentally, this has been demonstrated using soluble plant fibres, an effect we have termed ‘contrabiotic’, and a clinical trial is currently in progress to assess the ability of supplementation with soluble plantain fibre to prevent recurrence of Crohn’s disease\textsuperscript{34} (Figure 3).

Concluding remarks

Genetic studies have highlighted alterations in pathways within the innate immune response; however, it is possible that similar phenotypic changes can arise directly as a result of environmental factors. The evidence strongly supports an altered microbiome in IBD, and, although some of these alterations may be secondary to inflammation, it is likely that they also play a role in pathogenesis. We now need clinical trials specifically targeting these mechanisms to establish their true importance.

Conflict statement: Jonathan M. Rhodes, together with the University of Liverpool, holds a patent for the use of soluble plantain fibre in treatment of Crohn’s disease.

Professor Jonathan M. Rhodes was one of the speakers at the recent Biochemical Society Focused Meeting ‘The Molecular Biology of Inflammatory Bowel Disease’. Reviews by speakers at this meeting will be published this year in volume 39 (part 4) of Biochemical Society Transactions (www.biochemsoctrans.org).

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