



Conference on ‘Diet, gut microbiology and human health’ Symposium 2: Changes in the microbiome in disease and lifecourse

The microbiome in inflammatory bowel disease and its modulation as a therapeutic manoeuvre

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Inflammatory bowel disease (IBD) is increasing in incidence in both the developed and the developing world. Genetic, immunological and environmental factors are known to be involved. Genome-wide studies have examined the contribution played by host genetics in the development of IBD and have estimated that genetic factors are responsible for about 25 % of the disease risk. Having an IBD-associated genotype does not always lead to development of the disease phenotype, and hence it seems likely that environmental factors are key to triggering development of the disease in genetically susceptible individuals. The gut microbiota contains more cells than its human host, and mounting evidence attests to the importance of the microbiota in the development of several diseases, including IBD, metabolic syndrome and CVD. The present paper reviews the interplay between the microbiota and the mucosal immune system in health and in IBD; and discusses the evidence base for the use of therapeutic modulation of the microbiota to prevent and treat IBD.

Inflammatory bowel disease: Probiotics: Dysbiosis: Faecal microbiota transplant: Prebiotics

A diverse community of micro-organisms inhabits the human intestine. The majority of these are bacteria, but eukaryotes, viruses and archaea are also present. Intestinal bacterial communities comprise up to 1000 different species constituting a diverse ecosystem⁽¹⁾. It is estimated that the human microbiota contains up to 10^{14} bacterial cells, an order of magnitude greater than the number of human cells in our body⁽²⁾. This perception of ourselves has given rise to the view of the microbiota as another organ and that we are ‘supraorganisms’ whose genome is a combination of human and microbial genes⁽³⁾.

Our understanding of the microbiome has increased greatly with the introduction of high-throughput metagenomic processing⁽⁴⁾. The gut ecosystem is dominated by bacteria. More than fifty bacterial phyla have been described, although in the gut two phyla predominate: Bacteroidetes and Firmicutes. Proteobacteria and Actinobacteria are present in lesser proportions and other phyla are represented in minor extents⁽²⁾. Significant differences exist between the magnitude and composition of bacteria both longitudinally from stomach to colon as well as

laterally from the mucosal mucus layer to the intestinal lumen. Many species present in the intestinal lumen do not access the mucus layer and epithelial crypts⁽⁵⁾.

Much of our knowledge of gut microbiota function is derived from studies of germ-free animals. These demonstrate that the commensal microbiota modulates nutrient absorption, mucosal barrier function, angiogenesis and intestinal maturation⁽⁶⁾. The mucosal immune system is required to be simultaneously tolerant of the microbiota while still able to prevent its overgrowth and translocation to systemic sites as well as able to respond appropriately to pathogens. This has led to the development of a finely tuned homeostasis between the huge microbial load of the intestine and the host immune system.

The microbiota–mucosal immunity interface

The intestinal microbiota is protective against invasion by pathogens. Gut microbiota provide the host with a physical barrier against incoming pathogens by competitive

Abbreviation: IBD, inflammatory bowel disease.

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exclusion such as: occupation of adherence sites; competitive consumption of nutrients; and stimulation of host production of antimicrobial substances^(1,6). The commensal microbiota enhance epithelial repair and cross-talk between components of the innate immune responses.

At the same time the intestinal immune system has developed a number of strategies to preserve ignorance as well as tolerance of the commensal microbiota. The inner layer of mucus secreted by goblet cells is resistant to bacterial penetration whereas the outer layer is colonised with bacteria⁽⁴⁾. The epithelial layer is bound together by tight junction proteins which allow nutrient flux into tissues whilst preventing bacterial penetration. Epithelial cells also produce a number of antimicrobial peptides including the cathelicidin, defensin and histatin classes⁽⁷⁾. IgA-producing B cells secrete bacteria-specific IgA which is transcytosed to the apical layer of the epithelium, confining bacteria to the mucus layer⁽³⁾. Symbiotic bacteria that do breach the mucosa are rapidly phagocytosed and killed by macrophages in contrast to pathogens which may actively interfere with macrophage function⁽⁷⁾. Dendritic cells sample penetrating and apical bacteria and then present the sample to B and T cells which, in turn, direct appropriate immune responses, being either tolerance or inflammatory⁽⁷⁾. Innate IL-22 producing lymphoid cells are also essential for the containment of lymphoid resident bacteria to the intestine, preventing their spread to systemic sites⁽⁷⁾. Animal studies have revealed an immune-driven dysbiosis demonstrating the control the immune system exerts on the structure of the intestinal microbiota⁽⁷⁾. Furthermore, human subjects with genetic polymorphism coding for immune components have an altered microbiota when compared with controls with wild-type alleles^(8,9).

Despite advances in our understanding of the microbiota and host–microbiota relationships, much of the detail regarding structure and function of the microbiota are as yet unknown. The human microbiome project was established to enable comprehensive characterisation of the human microbiota and its role in health and disease. In the coming years, our knowledge of the microbiota will significantly expand. With the application of molecular techniques to the study of gut microbiology, mounting evidence is emerging regarding the relationship between dysbiosis of the human gut microbiota and a number of gastrointestinal diseases as well as diseases beyond the gut, including obesity and metabolic syndrome^(3,10).

Dysbiosis in inflammatory bowel diseases

Inflammatory bowel diseases (IBD; Crohn's disease, ulcerative colitis and pouchitis) are considered to be due to an inappropriate immune response to the intestinal microbiota. Genome-wide association studies have identified genes contributing to susceptibility to Crohn's disease and ulcerative colitis. Many of these susceptibility loci play a role in immune responses to microbial signalling and processing⁽¹¹⁾ as well as epithelial barrier integrity^(12,13). Germ-free animal models of colitis indicate that the microbiota drives inflammation in genetically

susceptible hosts⁽¹⁴⁾. Knockout mouse models of colitis have also shown a transferrable colitogenic microbiota to wild-type mice⁽⁸⁾. Clinical evidence also points towards the microbiota-driving inflammation. Faecal diversion ameliorates inflammation in Crohn's disease while reintroduction of the ileal contents to the diverted bowel induces inflammation⁽¹⁵⁾. Pouchitis only occurs following closure of the ileostomy when the pouch is exposed to significantly higher concentrations of bacteria.

A number of theories exist regarding the role of the microbiota in IBD. These range from abnormalities of single organisms to a dysbiosis of the overall composition and diversity of the microbiota, to functional shifts in the microbiota. *Mycobacterium avium* ssp. *paratuberculosis* has long been associated with Crohn's disease; however, a blinded study showed no difference in the rate of culture recovery by two independent laboratories^(14,16) and clinical trials failed to show sustained response in Crohn's disease patients treated with combination antituberculous therapy⁽¹⁷⁾. Other investigators have demonstrated an increased persistence of adherent/invasive *Escherichia coli* in ileal Crohn's disease⁽¹⁸⁾. Others suggest a reduction in protective species of bacteria such as *Faecalibacterium prausnitzii*⁽¹⁹⁾. Molecular analyses suggest that the overall composition and diversity of the microbiota is altered in IBD. More recent studies have assessed the functional metabolic outcomes of disease associated dysbiosis. These demonstrate alterations in bacterial carbohydrate metabolism, bacterial–host interactions, as well as human host-secreted enzymes. However, it remains uncertain as to whether any of these changes are primary or secondary events⁽²⁰⁾.

Modulating the microbiota to prevent and treat disease

The composition of the gut microbiota is determined by many interplaying factors including age, geographical location, host genetics, host immunity, diet and smoking status. Since it is widely accepted that IBD is driven by dysbiosis in genetically susceptible individuals, this list offers a number of potential opportunities to modify the microbiota and prevent or ameliorate the disease.

Moderating neonatal risk

A neonate is born with an immature microbiota which receives early modulation during birth and with feeding. The mode of delivery determines the makeup of the early microbiota, with Caesarean section delivered babies colonised with skin flora, and vaginally delivered babies colonised with bacteria from the birth canal⁽²¹⁾. There is some evidence that Caesarean section is a risk factor for developing IBD⁽²²⁾. Similarly, breast milk and formula feed will lead to establishment of differing microbiota profiles⁽²³⁾. There are conflicting data regarding whether breast-feeding is protective against IBD^(24,25).

Diet and nutrition

Nutrition is vital in the management of adult and paediatric IBD, and modulates the microbiota, but its role in

the development of the disease is still to be clarified⁽²⁵⁾. Patients are prone to malnutrition for a number of reasons: intestinal malabsorption, post-surgical short gut length, strictured bowel lumen and food avoidance behaviour due to meal-related symptoms. Thus patients are routinely monitored from a nutritional viewpoint, and receive supplementary nutrition when required. Nutritional status determines key outcomes including surgical recovery rates. In the paediatric population, diet may be the only treatment required. Elemental and polymeric diets may be successful in not only healing the intestinal mucosa but also in achieving and maintaining remission in IBD^(26,27). In the adult population, a low-fibre diet is often employed to prevent obstruction of a narrowed lumen. The association between specific dietary components and IBD has been researched, but the data are frequently conflicting. Butyrate, a SCFA, is derived from microbial fermentation of dietary starches. It is the major energy source for colonocytes and both butyrate and butyrate producing bacteria have been shown to be deficient in the inflamed colon^(28–30). Butyrate enemas, although not in common clinical use, have been used as a therapy in IBD, highlighting the importance of the microbiota⁽³¹⁾. Other studied nutrients include *n* 3 and *n* 6 fatty acids^(32,33) and vitamin D⁽³⁴⁾.

Antibiotics

The use of antibiotics to target the microbiota is well established in the management of IBD. Antibiotics, including metronidazole, are effective in prevention of post-operative recurrence of Crohn's disease^(35,36) and antibiotics such as metronidazole, ciprofloxacin and rifaximin are the mainstay of treatment for acute and chronic pouchitis⁽³⁷⁾.

Probiotics

Probiotics are generally considered to be safe, and do have some proven clinical therapeutic uses in IBD. They reduce the risk of disease onset⁽³⁸⁾ and maintain disease remission⁽³⁹⁾ in pouchitis. *E. coli* Nissle 1917 has similar efficacy to 5-aminosalicylic acid therapy in the maintenance of remission in ulcerative colitis⁽⁴⁰⁾ and some studies suggest VSL#3 to be effective in the treatment of active mild-to-moderate ulcerative colitis⁽⁴¹⁾. There is little evidence for the use of probiotics in Crohn's disease, however, possibly due to the smaller microbial load in the small bowel as compared with the colon^(42–45). One confounding factor of the probiotic approach is the comparatively low number and diversity of bacterial species available in a typical commercial probiotic in comparison with the gut microbiota. The commercially available products usually contain at least three orders of magnitude fewer bacteria than the microbiome which they are attempting to modify; for example a patient would need to take 1000 VSL#3 capsules to ingest a microbial load equivalent to the gut microbiota⁽²⁾. Furthermore, probiotic bacterial strains may not be able

to compete against the complex interactions of an established and adapted indigenous gut microbial community. The diversity of probiotic products on offer renders most meta-analyses irrelevant, as each trial should only be compared with trials studying the same species or combination of species.

Prebiotics

Prebiotics are non-digestible dietary components which promote the growth of beneficial gut micro-organisms. Fructo-oligosaccharides have been postulated to have a beneficial effect in active Crohn's disease, but initial trial success⁽⁴⁶⁾ has not been reproduced in larger subsequent studies^(47,48).

Faecal microbiota transplantation

The concept of transplantation of the whole gut microbiota has been described in ruminants for some time⁽⁴⁹⁾. Use as therapy in human subjects was first reported by Eisemen *et al.* in 1958 in the treatment of fulminant pseudomembranous enterocolitis⁽⁵⁰⁾. Over the subsequent decades, there have been an increasing number of case reports and case series of the potential therapeutic uses of faecal transplantation not only for *Clostridium difficile* infection⁽⁵¹⁾ but also for constipation, irritable bowel syndrome and IBD⁽⁵²⁾. There also seems to be potential for faecal transplantation in the treatment of diseases beyond the gut such as metabolic syndrome^(53,54). The first randomised controlled trial of faecal transplantation for recurrent *C. difficile* demonstrated 94 % remission at 10 weeks following duodenal infusion of donor faeces⁽²⁹⁾. Studies have shown a significant and durable change in the microbiota following faecal transplantation⁽⁵⁵⁾. In a mouse model of pseudomembranous colitis⁽⁵⁶⁾ suppression of *C. difficile* following faecal transplantation was associated with a change of the recipients' microbiota to a composition similar to that of the healthy input bacterial community and this was closely linked to a rapid increase in species diversity⁽⁵⁶⁾. There are case reports of the use of faecal microbiota transplantation in IBD, but currently there are no published randomised controlled data for faecal microbiota transplantation in IBD.

Faecal transplantation, a therapy used for more than half a century, could hold great promise as a future treatment in the many diseases where dysbiosis of the gut microbiota contributes to disease. While it appears that in the short-term this therapy is safe, there remains concern regarding the long-term health risks that may be posed by faecal transplantation.

Conclusions

Our understanding of the role of dysbiosis in the aetiology of many diseases is increasing, but is currently limited. The prospect of 'mining the microbiota' for novel therapies and to enhance the efficacy of present drug therapies is also a tantalising prospect for the future. The gut microbiota offers a vast undiscovered ecosystem

with a wealth of potential opportunities for the treatment of intestinal and systemic diseases⁽⁵⁷⁾. Enhanced knowledge and understanding of the gut microbiota as an ecosystem of importance comparable with other vital organs will likely lead to greater opportunities for the treatment of IBD, other intestinal disorders and diseases beyond the gut in the near future.

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None.

Authorship

A. L. Hart and P. Hendy co-wrote the paper.

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