

# The microbiome in inflammatory bowel disease and beyond

Jonathan Landy and Alisa Hart

**ABSTRACT** – The diverse and complex community of microorganisms that has co-evolved with the human gut is vital to intestinal functioning, and disturbances in the microbiota and its relationship with the host immune system have been linked to inflammatory bowel diseases, including Crohn's disease and ulcerative colitis. This has suggested several treatment options, including antibiotics, probiotics and faecal transplantation. The human microbiome project has been established to enable comprehensive characterisation of the human microbiota and in the coming years, knowledge in this area is expected to continue to expand.

**KEY WORDS:** Microbiota, inflammatory bowel disease, ulcerative colitis, Crohn's disease

## Introduction

The human gut has coevolved to interface with a diverse and dynamic community of microorganisms. These are mainly bacteria, but eukaryotes, viruses and Archaea are also present. Intestinal bacterial communities comprise up to 1,000 different species, constituting an incredibly diverse ecosystem.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup>

## The structure and function of the microbiota

The gut ecosystem is dominated by bacteria. More than 50 bacterial phyla have been described, although in the gut two phyla predominate: Bacteroidetes and Firmicutes. Proteobacteria and Actinobacteria are present in lesser proportions, as well as other phyla, represented in minor proportions.<sup>2</sup> The distribution of the gut microbiota is not homogenous. Significant differences exist between the magnitude and composition of bacteria both longitudinally from the stomach to the 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.<sup>4</sup>

The coevolution of the mammalian intestinal microbiota has primarily been driven by the metabolic advantages in enhancing

host digestive capabilities. The complex community of bacteria, which is rapidly adaptable and evolving, harbours a diversity of saccharolytic enzymes far beyond the limited repertoire available from the human genome alone. Consequently, this association enables flexible dietary changes and optimises the efficiency of energy harvesting from our diet.<sup>1</sup>

Millions of years of coevolution have led to an integral intertwining of host–microbial physiology. Much of the 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.<sup>5</sup> The mucosal immune system needs to be tolerant of the overlying microbiota and, simultaneously, to control the gut microbiota to prevent its overgrowth, to translocate it to systemic sites and to ensure that it responds 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 intestinal microbiota protects against invasion by pathogens. Gut microbiota provides the host with a physical barrier to incoming pathogens by competitive exclusion, such as occupation of attachment sites, competitive consumption of nutrients and stimulation of host production of antimicrobial substances.<sup>6</sup> The commensal microbiota signals via Toll-like receptor (TLR) MyD88-dependent pathways, enhancing epithelial repair and cross-talk between components of the innate immune responses.<sup>7</sup> Commensal species have been shown to influence the composition of T lymphocyte subsets, with distinct effector functions both locally and systemically.<sup>7</sup>

At the same time, the intestinal immune system has developed several 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 by bacteria.<sup>4</sup> The epithelial layer bound together by tight junction proteins enables nutrient flux into tissues while preventing bacterial penetration. Epithelial cells also produce several antimicrobial peptides.<sup>7</sup> Immunoglobulin (Ig) A-producing B cells secrete bacteria-specific IgA that is transcytosed to the apical layer of the epithelium, confining bacteria to the mucus layer.<sup>3</sup> Symbiotic bacteria that do breach the mucosa are rapidly phagocytosed and killed by macrophages, in contrast to pathogens that actively interfere with macrophage function.<sup>7</sup> Dendritic cells sample penetrating and apical bacteria and interact with B and T cells to direct appropriate immune responses.<sup>7</sup> Innate lymphoid cells that produce interleukin (IL)-22 are also essential for the containment of lymphoid-resident bacteria to the intestine, preventing their spread to systemic sites.<sup>7</sup> Animal studies have shown an immune-driven

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dysbiosis, demonstrating the control that the immune system exerts on the structure of the intestinal microbiota.<sup>8</sup>

## The role of the microbiota in gastrointestinal disease

Despite advances in understanding of the microbiota and host–microbiota relations, many of the details of the 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 their role in health and disease. In the coming years, knowledge of the microbiota will expand significantly. With the application of molecular techniques to the study of gut microbiology, mounting evidence is emerging regarding the relation between a dysbiosis of the human gut microbiota and several gastrointestinal diseases, as well as diseases beyond the gut, including obesity and metabolic syndrome.<sup>2,9–11</sup>

Inflammatory bowel diseases (eg Crohn's disease, ulcerative colitis [UC] and pouchitis) are considered to be the result of an inappropriate inter-relation between the immune responses and intestinal microbiota. Genome-wide association studies have identified genes contributing to susceptibility to Crohn's disease and UC. The elucidation of these susceptibility loci highlights the role of the immune responses to microbial signalling and processing,<sup>12</sup> as well as epithelial barrier integrity.<sup>13,14</sup> Germ-free animal models of colitis suggest that the microbiota drives inflammation in genetically susceptible hosts.<sup>9</sup> Knockout mouse models of colitis have also shown a transferrable colitogenic microbiota to wild-type mice.<sup>8</sup> Clinical evidence also points towards the microbiota driving inflammation. Faecal diversion ameliorates inflammation in Crohn's disease, whereas reintroduction of the ileal contents to the diverted bowel induces inflammation.<sup>15</sup> Pouchitis only occurs following closure of the ileostomy, when the pouch is exposed to significantly higher concentrations of bacteria.

Several theories exist regarding the role of the microbiota in inflammatory bowel diseases. 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* 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<sup>16</sup> and clinical trials failed to show sustained response in patients with Crohn's disease treated with combination antituberculous therapy.<sup>17</sup> Other investigators have demonstrated an increased persistence of adherent and/or invasive *Escherichia coli* in ileal Crohn's disease.<sup>18</sup> Others suggest a reduction in protective species of bacteria, such as *Faecalibacterium prausnitzii*.<sup>19</sup> Molecular analyses suggest changes in the overall composition and diversity of the microbiota to be altered in inflammatory bowel diseases. More recent studies using a combination of 'omics' approaches 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.<sup>20</sup>

## Treatment options targeting the microbiota

Several treatment options targeting the microbiota are well established in the management of inflammatory bowel diseases. Antibiotics are effective in the prevention of post-operative recurrence of Crohn's disease<sup>21</sup> and are the mainstay of treatment for acute and chronic pouchitis.<sup>22</sup> Probiotics reduce the risk of disease onset<sup>23</sup> and maintain disease remission<sup>24</sup> in pouchitis. *Escherichia coli* Nissle 1917 has similar efficacy to 5-aminosalicylic acid (5-ASA) therapy in the maintenance of remission in UC<sup>25</sup> and some studies suggest VSL#3 to be effective in the treatment of active mild to moderate UC.<sup>26</sup> One confounding factor of the probiotic approach is the comparatively low number and diversity of bacterial species available in a typical commercial probiotic compared with the gut microbiota. Furthermore, probiotic bacterial strains might not be able to compete against the complex interactions of an established and adapted indigenous gut microbial community.

An alternative approach is transplantation of the whole gut microbiota. This is a concept that has been described in ruminants for some time.<sup>27</sup> Use as therapy in humans was first reported by Eiseman *et al* in 1958 in the treatment of fulminant pseudomembranous enterocolitis.<sup>28</sup> Over the subsequent decades, there has been an increasing number of case reports and case series of faecal transplantation for *Clostridium difficile*<sup>29</sup> and also constipation, irritable bowel syndrome and inflammatory bowel diseases,<sup>30</sup> as well as diseases beyond the gut, such as metabolic syndrome.<sup>31</sup> The recently published first randomised controlled trial of faecal transplantation for recurrent *C difficile* demonstrated 94% remission at 10 weeks following duodenal infusion of donor faeces.<sup>29</sup> Studies have shown a significant and durable change in the microbiota following faecal transplantation.<sup>32</sup> In a mouse model of *C difficile*,<sup>33</sup> suppression of *C difficile* following faecal transplantation was associated with a change in 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.<sup>33</sup>

Faecal transplantation, a therapy used for more than half a century, could hold great promise as a future treatment where a dysbiosis of the gut microbiota is responsible for disease. Although it appears that, in the short term, this therapy is safe, there remains concern regarding the long-term health risks that might be posed by faecal transplantation. Understanding of the role of a dysbiosis in the aetiology of many diseases is increasing, but is currently limited. We advocate the institution of national registries of donors and recipients of faecal microbiota transplantation with the potential to monitor for any long-term adverse effects. The prospect of 'mining the microbiota' for novel therapies and to enhance the efficacy of current 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.<sup>34</sup> Enhanced knowledge and understanding of the gut microbiota as an ecosystem as essential to our existence as other vital organs is likely to lead to greater opportunities for the treatment of inflammatory bowel diseases, other intestinal disorders and diseases beyond the gut in the near future.

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