The gut microbiome: what every gastroenterologist needs to know

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ABSTRACT

The mucosal surfaces of the body are characterised by complex, specialised microbial communities, often referred to as the microbiome. However, only much more recently—with the development of technologies allowing exploration of the composition and functionality of these communities—has meaningful research in this area become feasible. Over the past few years, there has been rapid growth in interest in the gut microbiome in particular, and its potential contribution to gastrointestinal and liver disease. This interest has already extended beyond clinicians to pharmaceutical companies, medical regulators and other stakeholders, and is high profile within the lay public in general. Such expansion of knowledge holds the intriguing potential for translation into novel diagnostics and therapeutics; however, being such a nascent field, there remain many uncertainties, unanswered questions and areas of debate.

INTRODUCTION

There is a need for gastroenterologists and hepatologists to have an understanding of some of the key principles underlying the gut microbiome, given the implications it has for clinical medicine and the considerable interest that it increasingly holds for patients. The fast-moving nature of the field and sometimes complex terminology can create confusion. This review aims to give an overview of the central areas of knowledge about the gut microbiome, as well as highlighting the major future directions in this area.

OVERVIEW OF THE GUT MICROBIOME

What is the ‘microbiome’ and ‘microbiota’?

First coined by Joshua Lederberg in 2001, these terms are often used interchangeably. The human microbiota consists of up to 100 trillion microbial cells harboured by an individual, whereas the microbiome is the catalogue of these microbes and their genes living within a specific niche such as the human gut (table 1). The key technology used to study the composition of the gut microbiome is next-generation sequencing of bacterial genomes, using DNA extracted from samples (eg, stool, colonic mucosal biopsies) as starting material. This may be done using different techniques, each with their own associated strengths and weaknesses, but all relying on the principle of comparing the DNA sequences obtained from a biofluid with reference databases of microbial genomes (table 2). Bioinformatic tools then help to identify the specific bacterial taxonomic composition within the sample. However, while these sequencing technologies are very helpful for identifying ‘what is there’ in a sample, this does not give the full perspective as to what these bacteria are doing functionally, and how they are interacting with their mammalian host. As such, study of microbiome composition is now often supplemented by other systems biology techniques that give insight into microbiome function, such as metabolomics and proteomics (figure 1); these are also reviewed elsewhere.

Acquisition of the gut microbiome remains controversial, with the most robust evidence suggesting that it is a dynamic process that starts immediately after birth. The first (and most critical) contribution to the establishment of a microbiome is the vertical transmission of maternal microbiome at the time of birth. The specific patterns of colonisation of the gut microbiome within the first few weeks of life are thought to have crucial effects on its future composition. Following this, the infant gut microbiome undergoes rapid development over the first year of life, and appears to be established in an adult form by 3 years of age. Multiple factors play a key role in determining the
gut microbial composition during its development, starting from mode of delivery (vaginal vs caesarean), to early feeding (breast vs formula), use of antibiotics in early life, diet and the environment.7–9

Of all the environmental influences on the gut microbiome, the contribution of diet is among the most important. It is well-established that diet can rapidly and reproducibly alter the gut microbiome, making human studies which do not control for diet difficult to interpret, and also implicating diet as a therapeutic target for altering the gut microbiome.10

What is the significance of a gut microbiome?
The gut microbiome is not a bystander, and has co-evolved with the host over thousands of years to form a complex and mutually beneficial relationship. Through this relationship, the microbiome provides a multitude of benefits to the host, including shaping the intestinal and systemic immune system,11 maintaining the healthy intestinal epithelium, harvesting energy from food12 and protection against pathogens.13 The alteration of the composition of the microbiome that results in disruption of these important physiological functions has sometimes been referred to as ‘dysbiosis’.

What is ‘dysbiosis’?
Dysbiosis is used to describe a change in diversity of microbiome, loss of specific beneficial microbes (symbionts) and expansion of potentially harmful ones (pathobionts). It is, however, a very non-descriptive and perhaps unhelpful term that often gets used to describe any shift in the gut microbial composition away from that seen in a ‘healthy host’ despite there being no agreed definition of what a ‘healthy gut microbiome’ is.14 The gut microbiome is largely composed of two groups at the phyla level, being dominated by the obligate anaerobic Bacteroidetes and Firmicutes, which make up 90% and are generally associated with health (beneficial). Other phyla present at lower levels are Actinobacteria, Fusobacteria, Verrucomicrobia and Proteobacteria, facultative anaerobes. The latter phylum comprises the common Gram-negative pathobionts such as Salmonella spp, Shigella and Escherichia coli. Despite uncertainty regarding the normal microbiome, remarkably congruent signals of ‘dysbiosis’ have been described (at least at a high taxonomic level) for nearly all chronic gastrointestinal (GI) and liver diseases including inflammatory bowel disease (IBD),

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<thead>
<tr>
<th>Table 1</th>
<th>Glossary of common terms related to the gut microbiome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term</td>
<td>Further details</td>
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<tr>
<td>Gut microbiota</td>
<td>The total assemblage of microorganisms present within the gut mucosal environment.1</td>
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<tr>
<td>Gut microbiome</td>
<td>The entire gut ecological habitat, including the microorganisms themselves, as well as their genomes, and the surrounding environmental conditions.</td>
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<tr>
<td>Dysbiosis</td>
<td>A term still used commonly to describe perturbation of the composition of the gut microbiome compared with what might be expected in the healthy host. However, in reality, often a poorly descriptive term without a specific biological definition.</td>
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<tr>
<td>Pathobiont</td>
<td>Microorganisms that usually interact with the host symbiotically, but have the potential to act as pathogens and cause disease under certain conditions.</td>
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<td>Faecal microbiota transplant (FMT)</td>
<td>Typically, a liquidised suspension of microbes (and their associated environmental milieu) derived from manipulated whole stool, administered to the gut of affected patients. Stool is obtained from healthy screened donors, and prepared using homogenisation, mixing with a diluent (eg, normal saline) and filtration. However, lyophilised FMT preparations are now also increasingly being used.</td>
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</table>

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<th>Table 2</th>
<th>Overview of common modalities of microbiome sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of technique</td>
<td>Overview</td>
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<tr>
<td>Metataxonomics (16S rRNA gene sequencing)</td>
<td>The 16S rRNA gene is present in all prokaryotes, and consists of highly conserved regions interspersed by nine ‘hypervariable’ regions. Hypervariable regions are of variable sequence and length between different bacteria.</td>
</tr>
<tr>
<td>Shotgun metagenomics</td>
<td>This technique involves fragmentation and random sequencing of DNA from the collection of genomes and genes within the sample, and the use of advanced bioinformatic techniques to analyse the sequencing data obtained.</td>
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irritable bowel syndrome (IBS), primary sclerosing cholangitis and non-alcoholic fatty liver disease.\textsuperscript{15} It should however be emphasised that in most cases, dysbiosis merely shows an association rather than a cause. Furthermore, there is also increasing evidence to support a significant but less well-characterised contribution from the microbial communities of viruses and fungi (often referred to as the ‘virome’ and ‘mycobiome’, respectively).\textsuperscript{16, 17}

THE CONTRIBUTION OF THE GUT MICROBIOME TO DISEASE: SPECIFIC EXAMPLES

Introduction

There are an ever-growing number of observational studies that have identified distinctive differences in the gut microbiome between patients with particular conditions and matched controls. Such changes are complex and nuanced, and are typically influenced by severity and distribution of disease, underlying treatment at the time of sampling, and region of the world in which the study is being performed, among other factors. However, in this section, a general summary is given of the key microbiome changes that accompany major GI and liver conditions.

Clostridioides difficile infection

Clostridioides difficile is a Gram-positive sporulating obligate anaerobe belonging to the Firmicutes phylum. This can sometimes apparently be a normal component of the healthy gut microbiome, but in the context of treatment with broad-spectrum antibiotics, the colonic microbiome is damaged, with an almost total loss of Bacteroidetes, reduction in Firmicutes and an overgrowth of Proteobacteria; these changes allow C. difficile spores to germinate and for growth (and subsequent toxin production and infection) to occur.\textsuperscript{18} The contribution of faecal microbiota transplant (FMT) to restoration of the gut microbiome and treatment

of C. difficile is discussed in ‘The gut microbiome as therapy’ section.

**Inflammatory bowel disease**

Congruent signals with regard to microbiome disruption are seen in ulcerative colitis (UC) and Crohn’s disease (CD). It is well-recognised that there is a loss of bacteria belonging to the Firmicutes phylum and most studies (although not all) show the same with respect to Bacteroidetes. Faecalibacterium prausnitzii has often been highlighted as a bacterial species which can ferment non-absorbed dietary components into compounds beneficial to the host called short chain fatty acids; F. prausnitzii is reduced in prevalence in both CD and UC. Data have suggested that alteration in the gut microbiome plays a central role in driving UC; datasets highlighting the importance of Roseburia hominis, F. prausnitzii and Akkermansia muciniphila in the inflammation in UC have been published. An over-representation of Gram-negative pathobionts has been described in the gut microbiome of people with IBD, with species of note being E. coli and Fusobacterium spp.

There are a lack of inception data in IBD, which leaves the question of which is the ‘chicken’ and which the ‘egg’ with respect to dysbiosis and inflammation/treatment effects in the context of IBD. This was partially addressed by Gevers et al. who examined a large cohort of treatment-naïve children with new-onset CD. They were able to show a clear difference in the gut microbiome in this cohort compared with healthy controls, and therefore make a link between disease severity and dysbiosis. Recent efforts towards unravelling causal relationships were explored in a study that demonstrated induction of colitis in germ-free mice following transplantation of stool from patients with IBD.

Regarding the ‘IBD microbiome’, much remains to be discovered regarding the contribution of non-bacterial components of the microbiome (such as phages and fungi), the effect of host genetics on the microbiome and mechanisms underlying the observed observations.

**Ileal pouch anastomosis**

Up to 40% of patients who undergo ileal pouch anastomosis (IPAA) following subtotal colectomy for UC will experience pouchitis within 5 years. It is now established that the microbiome in the pouch is the trigger for this, and broad-spectrum antibiotics are very effective for acute pouchitis. However, 10%–15% will go on to have chronic pouchitis, which may or may not respond to current therapy.

The bacterial community present in the ileal mucosa starts to resemble that of the colon immediately after ileostomy closure, and this microbial dysbiosis is associated with the development of pouchitis. Comparing non-inflamed with inflamed pouches, the latter show reduced microbiome diversity, similar but somewhat more pronounced than that seen comparing the bacterial diversity seen in UC compared with normal colon. In particular, in pouchitis, Bacteroidetes are reduced, whereas members of the Proteobacteria family are relatively increased. More recently, it was demonstrated that certain bacterial genera (Blautia, Dorea, Moryella, Suterella and Bacteroides) are associated with a better outcome in relation to treatment in patients with pouchitis. Furthermore, again similar to the situation in UC, it seems that generally, a reduction in families of the Firmicutes phylum and an increase in Proteobacteria are associated with inflammation. It appears that the microbiome dysbiosis seen in pouches is similar when comparing newly formed with more mature pouches. Recently, in a prospective study of 19 patients undergoing colectomy and IPAA, 43% developed pouchitis in a year. Interestingly, in these patients, the microbiome in the colon prior to colectomy was highly predictive of the development of pouchitis.

**Liver disease**

Perturbation of the gut microbiome has been demonstrated in liver diseases including non-alcoholic steatohepatitis/non-alcoholic fatty liver disease, alcoholic hepatitis, primary sclerosing cholangitis, cirrhosis and hepatocellular carcinoma. This is an active area of interventional research using FMT. A recent open-label pilot study of FMT to treat patients with hepatic encephalopathy has shown an interesting signal regarding potential improvement in cognition. An association between specific toxin-producing bacteria and alcoholic hepatitis has recently been described, and bacteriophages were shown as a targeted route to remove these bacteria and possibly reverse alcohol-related liver disease in a rodent model.

**Irritable bowel syndrome**

Dysbiosis has been shown between patients with IBS and healthy controls, with a change in the Firmicutes-to-Bacteroidetes ratio and reduction in bacterial diversity, although certain recent data did not confirm this finding. As such, further research is needed to understand the significance of the contribution of the gut microbiome to IBS.

**Colon cancer**

Association studies in human cohorts have strongly associated several bacterial species with colon cancer. Bacteroides fragilis and E. coli have both been implicated, but the predominant bacterial species linked with colon cancer has been Fusobacterium nucleatum. This has been associated with a worse phenotype and prognosis. F. prausnitzii has been found to be under-represented in patients with human colon cancer. Guo et al examined the use of bacterial species as biomarkers to differentiate patients with colon
cancer from controls with benign GI disease (polyps and IBD) and healthy controls. They looked at ratios of *E. prausnitzii/F. nucleatum* and *E. nucleatum/Bifidobacterium*. Interestingly, *F. nucleatum/Bifidobacterium* was found to be highly discriminatory versus all controls, raising the possibility of a microbiome-based biomarker for colon cancer. Further larger studies are needed, including interventional studies in humans.

**Immune checkpoint inhibitor complications**

It is well-established from animal models that responsiveness to treatment with checkpoint inhibitors is dependent on baseline microbiome profile and the impact of human gut microbiome on the efficacy and side-effect profile of anticancer therapies has also been recognised. Three recent independent studies on humans with solid tumours undergoing checkpoint inhibition showed the remarkable treatment responsiveness of the patients depending on baseline microbiome, although a consistent signal was not seen; this perhaps reflects the differing methodologies employed in these studies, or may reflect a focus on microbiome composition rather than functionality. While it appears that the composition of the gut microbiome is important in laying the foundation for successful checkpoint inhibitor treatment, another aspect of this treatment in which the microbiome seems key is toxicity. Diarrhoea is the main side effect of this highly effective cancer treatment, affecting 40% patients. Of these, a significant proportion go on to develop severe colitis, presumably related to the unfettered action of pro-inflammatory T cells. There have been several intriguing case series where FMT has been shown to be of great benefit in the setting of refractory checkpoint inhibitor colitis and this is the subject of ongoing randomised trials.

**THE GUT MICROBIOME AS THERAPY**

**Dietary manipulation of the gut microbiome**

Detailed discussion of this area is outside the remit of this review, although this is a growing area of interest. In particular, there is an expanding field of research demonstrating that the efficacy of established dietary interventions for GI disease—including the low fermentable oligo, di, mono-saccharides and polyols (FODMAP) diet and enteral nutrition for active CD may be partly mediated by alterations in the gut microbiome.

**Prebiotics**

Prebiotics are dietary components that are fermentable, which have a health benefit to their host. The most common of these are non-absorbable dietary fibres. They have been specifically defined as ‘selectively fermented ingredients that allow specific changes, both in the composition and/or activity of the GI microflora that confers benefits on host well-being and health’. Prebiotics are neither hydrolysed nor absorbed in the upper GI tract, but are selectively fermented by intestinal bacteria, and are able to alter the colonic bacteria. Animal models have shown that prebiotics can enhance mucosal integrity, and furthermore have been shown to reduce pro-inflammatory cytokines and reduce colitis. Prebiotics have an overall excellent safety profile but can be associated with abdominal pain, flatulence and diarrhoea at high doses. Specific prebiotics—such as the short chain fatty acids, including butyrate, acetate and propionate—have demonstrated benefits for reducing inflammation in IBD.

**Probiotics**

Probiotics are live microorganisms that, when administered, are intended to have a beneficial effect on the intestinal microbiome, and therefore confer a health benefit effect. Animal models have suggested that probiotics help promote the intestinal barrier. The studies to date looking at probiotics in IBD have been quite mixed, with some studies highlighting that both maintenance of remission or induction of remission could be achieved with probiotics, with others showing little or no benefit in IBD. A systematic review in 2017 suggested that VSL#3 may be effective in inducing remission in active UC. Probiotics may be as effective as 5-ASAs in preventing relapse of quiescent UC, but currently, the efficacy of probiotics in CD remains uncertain.

**Faecal microbiota transplant**

**Overview**

Initial studies of FMT for recurrent *C. difficile* infection (CDI) were observational, but these have now extended to a number of randomised trials since 2013. These studies have collectively demonstrated that a single FMT for recurrent CDI has an efficacy of >80% in causing remission from the condition almost regardless of many variables related to administration, including whether upper GI or lower GI administration is used. FMT may also have superior efficacy compared with either vancomycin or fidaxomicin in the treatment of CDI. Other developments over this time have been the development of ‘frozen’ FMT protocols (allowing FMT to be pre-prepared and stored in freezers until the time of need), and the emergence of FMT capsules.
While regulation of FMT has been approached differently in various countries, FMT regulation in the UK is within the remit of the Medicines and Healthcare products Regulatory Agency (MHRA), who regulate it as a medicinal product. Within the UK, FMT for recurrent CDI has also been approved by the National Institute for Health and Care Excellence and Public Health England; best practice regarding all aspects of FMT preparation and administration—including donor selection, care of the recipient and governance aspects of running FMT services—have recently been described in joint BSG/Healthcare Infection Society guidelines. European and US guidelines addressing the role of FMT in the treatment of recurrent CDI have also been published. Key best practice aspects of FMT administration are summarised in figure 2.

Donor selection is an area that has been particularly high profile of late, given the described transfer of an extended-spectrum beta-lactamase-producing E. coli from donor to two FMT recipients in the USA, with bacteraemia occurring in both patients, and one of the patients dying. The protozoan intestinal parasite Blastocystis has also been shown to be transmissible via FMT in humans. Given some of the complexities in running FMT services (including logistics related to maintaining a donor pool, costs in screening and preparing material, need for sufficient freezer capacity, etc), there is a growing interest in ‘stool banks’, whereby a ‘hub’ centre provides FMT to ‘spoke’ centres. However, there are still gaps in knowledge related to FMT for the treatment of recurrent CDI. While FMT appears relatively safe over the short term, it is unknown if there are any potential long-term sequelae related to gut microbiome manipulation; the development of FMT registries in certain countries (including the USA and Germany) is an attempt to monitor for this. Furthermore, mechanisms of action of FMT remain poorly understood, although there has been progress made in this area of late.

FMT in the non-CDI setting

There have been four randomised studies using FMT as treatment for UC; these have collectively demonstrated that FMT appears to be a relatively safe and effective modality for induction of remission for this condition (OR=2.89, 95%CI=1.36 to 6.13, p=0.006). However, these studies are markedly heterogenous with regard to key variables (including number of treatments, and mechanics of preparation...
and administration), limiting their interpretability and applicability. Within the UK, the multicentre STOP-Colitis trial is ongoing, which is methodologically investigating the optimal route of FMT administration to patients with UC, and further establishing the efficacy of this treatment for the condition. Further recent data suggest that FMT may have potential as a treatment for IBS, but that appropriate donor selection may be much important than is the case for recurrent CDI. Small single-centre studies of FMT for the treatment of hepatic encephalopathy show promising results, and a UK study investigating the safety of administration of FMT to patients with cirrhosis (Prospective, randomised placebo controlled feasibility trial of faecal microbiota Transplantation in cirrhosis (PROFIT)) has now finished recruitment. The range of other conditions in which FMT has been reported to be of potential benefit are very wide in scope, including primary sclerosing cholangitis, obesity/metabolic syndrome, autism and in the intestinal decolonisation of multidrug-resistant bacteria (table 3).

**CONCLUSION**

New technologies to explore the gut microbiome have resulted in major developments in knowledge over a short period of time. The association between a perturbed gut microbiome and a range of different GI and liver diseases is now well-recognised, but whether the microbiome changes are cause, consequence of incidental remains largely unclear while further mechanistic studies are awaited. The success of FMT for the treatment of CDI has created enthusiasm about a new paradigm of ‘microbiome therapeutics’. However, it is also clear that there remains layers of complexity and uncertainty in the biology of the gut microbiome that were not initially recognised. These will require careful deconvolution over the coming years in order to enable the maximum potential translation of this knowledge base into clinical benefit.

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