The gut microbiome in Myalgic Encephalomyelitis

Over the last dozen years, increasingly powerful DNA sequencing methods have allowed characterization of the microbes residing on and in humans in much greater detail than ever possible before. Abnormalities present in the gut microbiome—those microbial communities residing in our intestines—have now been observed in a number of diseases. One such illness is Myalgic Encephalomyelitis (ME), also known as Chronic Fatigue Syndrome (CFS). CFS was a name coined by the US Centers for Disease Control (CDC) in 1988, and reviled by patients for the resultant trivializing of this serious illness. Recently, the US National Academy of Medicine (NAM) recommended a new name: Systemic Exertion Intolerance Disease, though this name is not yet widely used. In ME, as in other diseases, the diversity of the bacterial species in the gut microbiome is lower than in healthy individuals. Furthermore, the abundances of different bacterial residents of the gut, which influence health both favourably and negatively, differ between ME patients and healthy controls. Bacteria translocate into the blood in greater amount in ME, leading to inflammation. Dysbiosis in the gut likely contributes to symptoms in this life-limiting disease.

Three to four times more women than men have ME. Children and adolescents as well as adults are susceptible to the disease. Prevalence is difficult to determine because of the lack of a simple, objective diagnostic test. While physicians experienced with the disease are readily able to make correct diagnoses, the clinical criteria often vary between studies, making enumeration of patients difficult. An investigation of ME in three regions of England found that about 0.2% fit a widely used 1994 CDC definition. A meta-analysis of 14 studies found the prevalence by clinical assessment to be 0.76%. These numbers translate into 128,000 to 486,000 ME patients in the UK. Thus, even if the lower figure is used, ME does not fit the definition of a rare disease (see www.raredisease.org.uk). An example of a rare but serious disorder that affects intestinal function is Clostridium difficile infection which is at least 10 times less common than ME.

The severity of the disease varies, though most affected individuals are unable to work or attend school full time. For example, a small survey of 25 children with ME in the UK found that only one could attend a full day. Indeed, another study found that ME was responsible for 42% of the medically certified, extended school absences in the UK over a five-year period.

While the ‘fatigue’ element in the name emphasizes a major symptom of ME, most patients report that the fatigue is not the same as that experienced by healthy individuals after vigorous physical exercise or inadequate sleep. Instead, the fatigue is described as a profound lack of energy, more akin to the sensation of exhaustion that occurs during a severe case of influenza or mononucleosis. Two additional symptoms were identified by the National Academies of Medicine committee in a 2015 report (http://www.nationalacademies.org/hmd/Reports/2015/ME-ME.aspx) as hallmarks of the disease: post-exertional malaise and unrefreshing sleep. The new diagnostic criteria also require either cognitive impairment or orthostatic intolerance. The latter refers to a surge of symptoms when upright that improves when the patient reclines, likely due to a disturbance in the autonomic nervous system. With regard to cognitive impairment, patients often report ‘brain fog,’ like the impaired mental capacity, poor memory and concentration that healthy individuals experience when they have been awake all night.

Most people with ME reach a steady-state level of physical and/or mental activity they can sustain without inducing an ensuing increase in symptoms known as post-exertional malaise. Many are home-bound – simple acts such as shopping for groceries can result in worsening of their symptoms. For those who are bedbound, any sort of stimulation, even the mental and physical effort to carry on a conversation, can intensify their symptoms. Many ME patients, whether bedbound or not, are unusually sensitive to light and sound. Bedbound patients often require eyeshades and sound-protecting headphones to cope with those stimuli. Among the most severely ill ME patients (Figure 1), some must be supported at the level of those who are comatose. Some are too impaired to speak and cannot eat nor digest food normally and must be tube fed.
Possible roles of the gut microbiome in ME

Gastrointestinal disturbance is a symptom often reported by ME patients. This fact has encouraged several investigators to compare the gut microbiome in patients versus controls.

Our research group undertook a study of the bacterial gut microbiome by comparing 16S rRNA from faecal samples of 48 ME patients and 39 controls. The 16S rRNA sequence is commonly used to identify bacterial species, as the presence of very variable regions in the 16S rRNA gene provides species-specific signature sequences. We obtained an average of 98,000 sequence reads per sample, more than ample to identify almost all of the bacterial diversity. To determine how many reads are needed, the number of species detected per number of sequences can be graphed to produce a ‘rarefaction curve’ (Figure 2). As more sequences are obtained, the number of species detected increases until a plateau is reached, where few additional species will be found despite a large number of additional sequence reads. For our samples, it is evident that 30,000 reads would be more than sufficient. For the example shown of a theoretical sample with low diversity, 5000 reads would have been adequate, while the high-diversity example indicates that even 30,000 reads would not suffice.

A conclusion that can be drawn from Figure 2 is that ME cases have reduced bacterial diversity in comparison to healthy controls. Such reduced diversity has been observed in other diseases such as Clostridium difficile infection, inflammatory bowel disease and necrotizing enterocolitis.

The sequence data can also be analyzed for differences in the abundance of various species between cases and controls. Some species that we found to be differentially abundant represented a very small fraction of the bacteria present and thus may not have a large effect on gut ecology and function. In Figure 3, we show those genera that a) represent more than 1% of the gut microbiome and 2) varied significantly among faecal samples between ME and healthy controls. The reduced abundances of Bifidobacterium and Faecalibacterium species in patients have also been reported in inflammatory bowel disease and other conditions. Faecalibacterium species produce butyrate, a short-chain fatty acid that has anti-inflammatory properties, and thus its reduction would predict lower levels of butyrate. While we did not measure butyrate in our samples, when faecal samples of 34 female cases and 25 controls were examined by Armstrong et al. in another study, surprisingly, butyrate was higher in the ME patient samples. Determining which metabolites are actually present in the gut can be difficult to predict merely from a list of species that reside there, given the complex interactions among different microbial communities and with the cells in the intestine.

Figure 1. ME devastates lives. A healthy college student one month before becoming ill with ME (left), soon becomes too debilitated to sit up, unable to tolerate light nor sound. Bedbound for two years, he becomes unable to chew and must enter hospital to receive a feeding tube because he is too weak to eat.

Figure 2. Rarefaction curve, graph of species detected per sample sequenced. When the slope is steep, many species remain to be discovered. At the plateau, most species have been detected and additional sequences will not reveal many more.

Figure 3. Differences between abundance of members of bacterial genera in ME patients versus healthy controls. A log10 scale is used in order to fit genera with large ranges of abundance onto the same graph. All differences shown are statistically significant (p<0.05).

Several studies in which bacteria were cultured also demonstrated differences between ME patients and controls. However, many gut microbial residents cannot be cultured and are known only by their DNA sequences, so that high-throughput sequencing of 16S ribosomal DNA for identifying bacterial taxonomic groups is beginning to supplant culture methods. Nevertheless, there are also limitations to knowledge from DNA
sequences of intestinal contents. For example, while ribosomal DNA sequencing can detect that *Escherichia coli* is present, it doesn’t reveal whether one of the highly virulent *E. coli* strains is present in addition to benign or beneficial *E. coli* strains that reside in most individuals. To find pathogenic *E. coli*, bacteria are grown on specific culture media and then tested with an antibody that reacts with proteins present in disease-causing *E. coli* strains. Thus, a harmful bacterium could be present in ME patients and go undetected by ribosomal DNA sequencing.

**Leaky gut problems**

When the intestinal lining is inflamed, bacteria can translocate into the bloodstream through loosened intestinal tight junctions leading to a ‘leaky gut’ (Figure 4). The immune system then detects the presence of bacteria or bacterial components in the blood and mounts an immune response to counter this apparent invasion. There can be collateral damage from the immune system’s attack on perceived threats. ME patients often have symptoms of chronic inflammation such as muscle and joint pain and swollen lymph nodes.

In order to find out whether ME patients might have more bacterial products in their blood than healthy people and could be responding to them, we tested whether the levels of certain molecules were different in the blood of the same ME patients and healthy controls whose faecal samples were sequenced. We found that patients had higher levels of lipopolysaccharides (LPS), a large molecule comprised of both lipid and sugar components. LPS are present on the outer membrane of some bacteria and cause a strong immune response. We found that levels of LPS, LPS-binding proteins and a receptor for LPS-binding protein (soluble CD14), which signals the presence of LPS to the immune system, are increased in ME patients. Thus, the abnormal gut microbiome in ME patients likely contributes to their chronic inflammation and ensuing symptoms. While most often comes to mind when considering intestinal bacterial species, there is increasing evidence that the gut microbiome affects the risk of colorectal cancer, obesity and abnormal mental function. Metabolites and proteins from the gut enter the bloodstream in healthy as well as diseased individuals, and some can affect the central nervous system and brain.

**Prospects for treatment**

Oral prebiotics and probiotics are being investigated for restoration of bacterial diversity and resolution of gastrointestinal diseases. Prebiotics are substances thought to improve growth of beneficial species, while probiotic supplements contain microbes known to be present in healthy guts. In order to be incorporated into a probiotic pill, bacteria must be grown in culture, but culture conditions for growing many of the bacterial species present in the human gut are not known. Thus, only a selection of certain species can be incorporated into commercially available probiotics. How these different species affect people with different types of gut microbiomes, and whether gastrointestinal illnesses can be improved with their aid is an important topic that is currently being explored in the research community.

Because pure cultures of many gut microbes cannot be obtained, researchers have turned to faecal transplants, i.e. introduction of faecal material from healthy human donors into recipients. This treatment has cured some individuals with severe gastrointestinal dysfunction from *Clostridium difficile* infection. Whether this process can also help patients with other types of intestinal diseases and ME is less clear. Promising reports have appeared about improvements in ulcerative colitis, Crohn’s disease and autism. With regard to ME, anecdotal reports from
patients who have tried faecal transplantation indicate some reduction in symptoms, but not complete recovery nor persistent improvement in their conditions. One study of faecal transplantation indicated that 42/60 ME patients had a favourable response. The results are sufficiently promising to suggest that a clinical trial of faecal transplants in ME would be worthwhile.

**Future directions**

Multiple studies now show that the gut bacterial composition is abnormal in patients with ME, a life-limiting disease. These findings are now among many discoveries of biological differences between ME patients and healthy individuals, all of which should dispel any remaining notions that the illness is psychological in nature. Future studies on the eukaryotic microbiome and virome may reveal additional disturbances in the microbial communities of people with the disease. While these gut abnormalities may be a response to some other inciting factor, rather than the basal cause of disease, learning how to ameliorate them could have clinical benefits for patients and help promote recovery, perhaps in conjunction with other treatments.

**Further reading**