How do we benefit from the microorganisms that live within us?

In 1683 a Dutchman, Antonie van Leeuwenhoek, having developed microscopes many times more powerful than any before, examined the white matter from his teeth and observed, 'the number of animals in the scruff of a man's teeth are so many that I believe they exceed the number of men in a kingdom.' After his death in 1723, no other scientists were able to see these 'animalcules' without the impressive magnification unique to Leeuwenhoek's microscopes (bequeathed to, and promptly lost by, the Royal Society) leaving his discovery to be dismissed for almost 150 years[2,3]. Microbes only reclaimed the scientific spotlight 1860s when Louis Pasteur proved they could cause disease. However, this was the genesis of 'germ theory,' which indiscriminately cast all our resident bacteria as malicious invaders, and ensured that the microorganisms living within our bodies became known only as enemies to our health. Since then, modern medicine has focused on the war against microbes - through sanitation, antiseptics, and the miraculous development of antibiotics. The idea that these supposed "enemies" could actually be good for us only emerged this century, gaining prominence when the Human Microbiome Project in 2007 - a 5 year program to sequence the genomes of microbial populations of 300 healthy Americans - helped to unveil the myriad ways our resident bacteria benefit us.

Our body's flora has frequently been described as an entire organ. It harbours a diverse community of intricately connected microbes in a precarious state of symbiosis. In exchange for a favourable habitat, these microbes fulfil many functions we can't perform ourselves: helping to metabolise otherwise indigestible compounds, regulating our immune response, and even influencing brain activity. A 2013 study found that one third of small molecules, including vitamins, amino acids, and neurotransmitters, in our body originated from gut bacteria[3]. Many studies comparing germ-free mice to those with a healthy microbiome have highlighted the role of microorganisms in preventing disease, maintaining a healthy weight, and improving mood[4]. Whilst microbes of all kind are found throughout our body, the most researched and arguably the most influential are the bacteria living in our gut.

Perhaps the most obvious role of our gut microbiome is to help digest compounds. One of our top metabolisers is called *Bacteroides thetaiotaomicron (B-theta).* Found only in the gut, this bacterium has genes for 260 different enzyme which help degrade carbohydrates, specifically glycoside hydrolases and starch binding proteins[1], releasing nourishing molecules called short-chain fatty acids (SCFAs). In comparison, the human genome only has 95 carbohydrate-digesting genes, despite containing 1000 times more DNA[3]. When infused into germ-free mice, *B-theta* was found to activate a range of mouse genes known to be necessary for a healthy gut by improving absorption of nutrients, building an impermeable barrier, breaking down toxins, and the formation of blood vessels[2].

Another exciting area of research explores the role of our gut bacteria in controlling our mood, and its implications for potential treatments of depression, anxiety, and other mental health disorders. This field was sparked by a Japanese study which showed that in response to stress, levels of the stress hormone Corticosterone were doubled in "germ-free"(GF) mice, compared to mice with

normal microbial (SPF) populations[12]. These results support similar studies showing the significance of gut bacteria in neural pathways and the "gut-brain axis."

The most fundamental way our bacteria protect us from disease is through Darwinistic competition. In a healthy microbiome, beneficial bacteria consume all available nutrients, preventing pathogenic bacteria from invading our gastro-intestinal (GI) tract. When the community is out of balance, for instance following a course of antibiotics, the equilibrium is perturbed and the microbiome is thrown into a potentially pathological state of dysbiosis. Beneficial bacteria are lost, leaving more nutrients available to support the expansion and pathogenicity of opportunistic pathogens.

One such opportunistic pathogen is *Clostridium difficile (C.diff). C.diff* infections can cause debilitating diarrhoea, severe cramps, bowel inflammation and can be fatal, yet this bacteria is carried, without symptoms, by about 1 in every 30 healthy adults[10]. The bacterium uses available nutrients, including fermentable amino acids such as Glycine and Proline, as energy sources, to produce two exotoxins, Tcd A and Tcd B, which damage epithelial cells lining the colon, increasing permeability. At low levels, this is essentially harmless, and kept under control by the vast array of other bacteria present. However, when our gut is in a state of dysbiosis, *C.diff* exploit the increased availability of nutrients and double every 12 minutes, requiring only a few hours to dominate the intestine. When this happens, the volume of toxins released causes severe damage to the GI tract.[1,9] Patients suffering from *C.diff* infections often take antibiotics for years, as *C.diff's* resistant spores cause the infection to return as many as 1 in 5 cases[10]. For some, the only long term solution is a Faecal Transplant (FMT), inserting a stool sample into the patient's gut helping to restore a healthy microbiome and suppress the *C.diff* population. A systematic review in 2014 showed that of 536 patients with recurrent *C. diff* infections, 467 (87%) experienced resolution of diarrhoea following FMT, and therefore concluded that "FMT seems efficacious and safe for the treatment of recurrent" *C. diff* infections, and should be encouraged by hospitals as treatment.[8]

Another way that the microbiome helps prevent disease is through mediating anti-inflammatory pathways of the immune system. The human body is unique in having both a complex microbiome and an adaptive immune response, whereas most other organisms, having a simple microbiome, rely solely on the innate response. Therefore it seems likely that the two have co-evolved over millennia - the need for a complex, adaptive immune response developed in sync with the growing microbiota, and specifically in the gut where the microbial population is highest. Our colon, hitherto thought of as a somewhat superfluous extension of our small intestine, contains 1 trillion microbes per gram. To compare, our stomach has 10 microbes per gram, our duodenum has 1,000 per gram, and our ileum has a million per gram, but the colon's population dwarves these values[3]. Scientists believe that it cannot be a coincidence then, that the gut also hosts 70% of our body's immune cells[3].

The importance of this relationship is shown by investigations into the immune system of germ-free mice, which are severely underdeveloped. For example, these mice show many deficits in their gut-associated lymphoid tissues, including fewer, smaller Peyer's Patches and mesenteric lymph nodes, as well as increased numbers of T helper 17 cells in the colon, which release interleukin 17 (IL-17), a pro-inflammatory cytokine associated with the pathogenesis of Inflammatory Bowel Diseases (IBDs), as shown in Table 1[5]. Immune tolerance to harmless bacteria in the gut is developed

by dendritic cells, which are able to extend across epithelial and mucous layer to sample antigens or cells from the gut and present them to the T cells in the Peyer's Patches for inspection, which can result in tolerogenic activation as specific T cells are converted into an immunomodulatory cell, or a regulatory T cell. The T cells then migrate to the villi, where they secrete IL-10 onto the epithelium, dampening inflammation.[11] Experiments on mice in the 1990s showed that interfering with production of regulatory T cells produced intestinal inflammation that looked like human bowel disease.

Table 1
Intestinal immunologic defects in germ-free mice

Intestinal organ development	Site	Phenotype in Germfree mice
Small Intestine	Peyers Patches	fewer, less cellular
	Lamina propria	thinner, less cellular
	Germinal centers	fewer plasma cells
	Isolated lymphoid follicles	smaller, less cellular
Mesenteric Lymph nodes	Germinal centers	smaller, less cellular

Cellular Defects	Cell Type	Phenotype in Germfree mice
Intestinal epithelial lymphocytes	CD8+ T cells	fewer, reduced cytoxicity
Lamina propria lymphocytes	CD4+ T cells	proportional decrease in number
		decreased Th17 cells (Small intestine)
		increased Th17 cells (Colon)
Mesenteric Lymph nodes	CD4+CD25+ T cells	reduced expression of FO
		reduced suppressive capacity

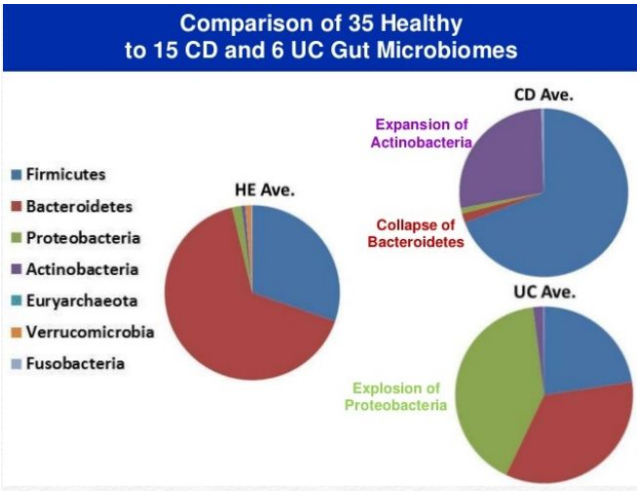

Figure 1

Inflammatory Bowel Diseases, notably Crohn's Disease and Ulcerative Colitis, are commonly known to be an overreaction of the immune system. Whilst a range of factors affect susceptibility to IBDs, current research is converging on the significant role of the microbiome. Patients with IBDs often have a far less diverse microbiota, including conspicuously low numbers of bacteria such as *Bacteroidetes Fragilis* (see Fig. 1[14]), which help mediate anti-inflammatory pathways[2]. This data correlates with a large Danish cohort study that found children who developed early IBD were 84% more likely to have had a course of antibiotics, disrupting the child's microbiome, compared to healthy children, and that each course of antibiotics received increased the risk of developing Crohn's disease by 18%[1,11]. Whilst some of these antibiotics may have been treatment for undiagnosed IBDs, the data is consistent with other investigations. Other research ascribes the sudden increase in rise of Crohn's disease diagnosis since World War II to the elimination of parasitic worms, which in the past may have helped 'train' the immune system to prevent it attacking the

epithelium of our GI tract[3]. The rise of many 'Modern Plagues,' a term coined by Martin Blaser to describe IBDs, allergies, diabetes, and others, may well be a result of increased exposure to antibiotics, the very drugs which helped cure us of historical epidemics[1].

Babies are not born with a complete microbiome. The development of our microbiome powerfully illustrates the intricacies and advantages of the relationship formed between our commensal microbes and us. In order to stimulate the development of a healthy microbiome, a baby's immune system is suppressed for the first 6 months of its life[2], leaving it open to all the microbes it may encounter. To protect against dangerous microbes, breastmilk contains the mother's antibodies, ensuring that harmful microbes are destroyed and unable to colonise the baby. In a study where mice were genetically engineered to lack the ability to produce one of these antibodies in their milk, their offspring contained unhealthy combinations of microbes, similar to those found in people with IBDs[2]. However, breastmilk does more than merely ward off the harmful bacteria - it also enthusiastically welcomes in the "good" bacteria. The third largest component of breastmilk is a group of complex sugar prebiotics called oligosaccharides, 200 of which are unique to humans and known as Human Milk Oligosaccharides (HMOs.) Babies cannot digest these energy-rich compounds, or at least not without help. In the large intestine, HMOs nourish a particular bacterium called *Bifidobacterium longum infantis (B. infantis),* which outcompete any other bacterium on the same diet. *B. infantis* has a genetic cluster of 30 genes that code for certain HMO-degrading enzymes. HMOs in breastmilk are not feeding the baby, but rather the microbes growing inside it, and so unsurprisingly, *B. infantis* very quickly becomes the predominant microbe in the guts of breast-fed infants.

This is just as well, because *B. infantis* is a hugely valuable multi-tasking microbe. As it ferments the HMOs, it releases SCFAs, used as a source of ATP for cells, particularly in the gut epithelium, salvaging energy from otherwise indigestible compounds, providing between 5-15% of our calorie intake. If not immediately absorbed for their energy, SCFAs are used in our muscles or liver, and are essential in dampening the immune response. For instance, If a SCFA binds to a G protein-coupled receptor such as Gpr41, Gpr43, Gpr109a or Olfr78, several cellular processes can be modulated including gene expression, differentiation, or apoptosis, which can, among other things, help reduce inflammation.[6] Further analysis of compounds in human milk have shown that it's not just HMOs that stimulate the development of a healthy microbiome. *B. Infantis* are also one of the best consumers of human milk glycolipids and gangliosides, or acidic glycolipids, in particular GM3 and GD3. A product of this degradation is sialic acid, an essential nutrient for brain growth, which may help explain the unparalleled rate of brain growth in infants. Urea is also found in breast milk, providing a nitrogen-rich source for bacteria to make proteins, without competing with the baby[1].

Ironically, the huge benefits of the microorganisms within us are now only being understood as a result of the consequences arising from their destruction. Nobel laureate Barry Marshal once said "I never killed anyone by giving them antibiotics but I know of plenty who died when they didn't get 'em." However, it is time to challenge the use of antibiotics as a catch-all cure, in order to protect our endangered microbiota. We must embrace our natural defenders in order to protect ourselves from the increasingly prevalent 'Modern Plagues,' and, as we approach a post-antibiotic era, more research is needed to unleash the full potential from the microorganisms living within us.

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