Microbial Dysbiosis in Crohn's Disease:

Causes and Solutions

Scott Harris

1st year Biochemistry BSc

Introduction

Crohn's Disease (CD), along with Ulcerative Colitis (UC), are the two inflammatory bowel diseases (IBDs); illnesses that involve chronic inflammation and pain within the gastrointestinal (GI) tract (Coates *et al.,* 2020). Other symptoms of IBD include: diarrhoea, blood in stool, fatigue, lack of appetite and weight loss (NHS, 2020). Unlike colitis, CD can be found in patches anywhere along the tract, however the ileocolonic region (where the small and large intestine are joined) tends to be the most commonly affected (Day *et al.,* 2012). It was estimated in 2014 by the National Institute for Health and Care Excellence that 157 in 100,000 people have CD (NICE, 2014), but many of those with the condition report stigma and negative psychological effects as a direct result (Gamwell *et al.,* 2020). It is therefore important for awareness for CD to be raised, and its status as a relevant issue recognised. It is known that CD is caused by and/or influences the gut microbiome - the communities of bacteria and small amounts of other microorganisms of the intestines (Daniel *et al.,* 2007). In a healthy individual, the majority of these bacteria are commensal (non-harmful), and many provide benefits, like the production of vitamins and the prevention of too many pathogenic bacteria from growing. The gut microbiome of CD patients is characterised by a lack of diversity and an imbalance, or dysbiosis, of bacteria that perform different functions (Daniel *et al.,* 2007). This is at least partially responsible for inflammation; pro-inflammatory bacteria outweigh anti-inflammatory bacteria (Leylabadlo *et al.,* 2020). Both current treatments for CD – anti-inflammatory treatments and immunosuppressants - only work in a small portion of patients (Hart *et al.,* 2020; Schoepfer *et al.,* 2014; Feagan *et al.,* 2013; Feagan *et al.,* 2016; Rutgeerts *et al.,* 2005; Syal, Kashani & Shih, 2018), with little understanding of why. Research into the reasons why people get CD and possible alternative therapies are therefore essential.

Although CD is rarely fatal, it can cause a severe decrease in quality of life to many who suffer with it. Recurring abdominal pain is one of the most common symptoms of the disease, being found in around 70% of patients (Bielefeldt, Davis & Binion, 2009; Coates *et al.,* 2020). Anti-inflammatory treatments, specifically the usage of the 5-Aminosalicylate (5-ASA) drug group, are one of the most commonly used to treat CD, however there is often disagreement on their efficacy, with a large discrepancy between cases (Hart *et al.,* 2020; Schoepfer *et al.,* 2014). Recently, immunosuppressants have been approved by the US Food and Drug Administration (FDA) for CD treatment, but these are also seen to be ineffective in many patients – between 1/3 and 1/2, depending on the specific drug (Feagan *et al.,* 2013; Feagan *et al.,* 2016; Rutgeerts *et al.,* 2005; Syal, Kashani & Shih, 2018). There is potential for other treatments with more decisive evidence to be developed; this includes the discovery of nutraceuticals that have similar effects (Ortiz *et al.,* 2020), and faecal microbiota transplants. These have already been used to great success within other gastrointestinal diseases, specifically *Clostridium difficile* infections (Costello *et al.,* 2015), but their usage within CD is still in its experimental phase, with the lack of understanding of its pathogenesis causing paradoxical issues, such as the increase of gut microbiota diversity with no apparent effect on the patient's pain levels.

Dysbiosis of the Gut Microbiome

There is a great amount of taxonomic variance within the gut microbiome. *Escherichia coli* can be found in most individuals through culture growth, and the phyla Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia are also present in the majority of cases, though the latter three in smaller numbers. However, the possibility of finding a more specific core set of bacteria than this, such as universal genera or species, is very unlikely, and even the few species that have been grown in great enough numbers to justify such a claim can be present in amounts as low as 0.5% of the total flora (Lozupone *et al.,* 2012). The diversity in patients' microbiomes is the basic premise of bacterial dysbiosis – the abnormal make-up of bacterial colonies, leading to possible over and/or under-presence of certain organisms that leads to disease. Given the huge amount of variation within microbiomes, even being able to characterise a 'normal' and 'abnormal' state seems impossibly complex; therefore, metagenomic approaches have become increasingly popular within this field of research. It is important to know what exact type of bacteria one has in their GI tract much less than simply what they are actually doing, both within the bacterial communities and towards the host system. Functional searching to assess gene profiles can give light to ideas on the main effect of different 'types' of bacteria usually, but not necessarily, related to their taxonomy (aerobic/anaerobic living style, metabolic intake and output, etc.) (Lozupone *et al.,* 2012).

Of course, it is important to note that not all variance is necessarily bad; many factors affect an individuals' microbiome composition. This can include: diet and geographical location (De Filippo *et al.,* 2010); genetics, especially mutations/allelic variation of proteins which

regulate innate immunity to commensal bacteria (Zaruhi *et al.,* 2008); and age. Infants generally possess the most variability between individuals, while taxonomic diversity is seen to increase during pregnancy. There is also the tendency for Bacteroidetes species to dominate the microbiota with old age (Kostic, Howitt & Garrett, 2013; Lozupone *et al.,* 2012) (Figure 1). One of the most notable presentations of CD is the greatly decreased amount of both diversity and overall count of bacteria within the gut (Dicksved *et al.,* 2008; Daniel *et al.,* 2007; Peterson *et al*., 2008). It is well known that the gut microbiome is a multifaceted, key part of human health. Species create short-chain fatty acids (SCFAs), such as butyrate and acetate, which are used as fuel by the colonic epithelial cells (Huda-Faujan *et al.,* 2010). A well-balanced gut microbiome is also shown to stimulate the immune system, leading to a lower rate of autoimmune disease (Nishida *et al.,* 2017). In particular, patients with CD are likely to have a lowered amount of bacteria from the phylum Firmicutes, including the key bacterium *Faecalibacterium prausnitzii*.

F. prausnitzii is known to be a key bacterium species for a healthy gut microbiome for two principal reasons. The first is that it is one of the above mentioned SCFA-producing bacteria, specifically a butyrate-producing bacteria (BPB). This molecules' abundance has been shown to affect multiple areas of the human body, such as the intestinal cell life cycle and the halting of the development of cancer, though the exact mechanisms are not yet determined in most cases (Leylabadlo *et al.,* 2020). Low levels of available butyrate have also been associated with increased levels of pathogenic bacteria, such as *E. coli* (Nishida *et al.,* 2017). The second key aspect of *F. prausnitzii* is its anti-inflammatory and immune system-related effect (Miquel *et al.,* 2013; Stein & Shaker, 2015). In particular, *F. prausnitzii* is known to stimulate the production of a great amount of the primary anti-inflammatory cytokine IL-10 compared to other bacterial strains, while it produces among the lowest amount of the pro-inflammatory cytokine IL-12. The ratio of IL-10:IL-12 stimulation is the metric generally used for determining the anti-inflammatory/inflammatory effect of a species; it seems from this data that *F. prausnitzii* is one of the most potent (Sokol *et al.,* 2008). An additional change in the composition of a CD patient microbiome is the increase in bacteria associated with mucolysis, the breaking down of the gut mucus. Ruminococcus species are known to usually be the most abundant varieties. They are shown to specifically break down the MUC2 protein within mucus, while other species are able to enzymatically catalyse the degradation of the oligosaccharides which surround it (Hoskins & Boulding, 1981; Png *et al.,* 2010).

The Possibility of Faecal Microbiota Transplantation as a Therapy for Crohn's Disease

Faecal Microbiota Transplantation (FMT) is the introduction of faecal matter from a healthy individual into a patient, with the goal of rebalancing the levels of bacteria within the gut. The treatment is currently given as either enemas or colonoscopies, and there have been no disadvantages for the growing use of frozen faecal matter, with the advantage that it can be stored for much longer periods of time (Costello *et al.,* 2015; Ramai *et al.,* 2019). There is also growing support for the use of oral capsules, as their administration is much less invasive and prevents any risk of gut perforation (Ramai *et al.,* 2019). The capsules contain lyophilised faecal powder mixed with a cryoprotectant, such as glycerol, thickly encapsulated to prevent dissolution by stomach acid (Tian *et al.,* 2015). Historically, it was preferred that the donated sample was taken from healthy close relatives, however this has not been shown to be an important factor in its efficacy (Ramai *et al.,* 2019), potentially allowing the treatment to be conducted on a larger amount of patients. FMT is believed to be a potential treatment candidate for CD (and IBDs in general), as it has been repeatedly shown that the treatment restores balance within the gut microbiome. FMT grants the individual a higher amount of colony resistance—the presence of commensal bacteria that are able to prevent the spread of pathogenic bacteria (Ademe 2020; Ooijevaar *et al.,* 2019). It also increases the number of SCFA bacteria which are known to be specifically lacking in IBD patients, as well as the restoration of a healthy bile composition. Primary bile, mainly made up of cholic and taurocholic acid, is the substance secreted by the patient's liver, while bacteria within the gut deconjugate and dehydroxylate these molecules to create

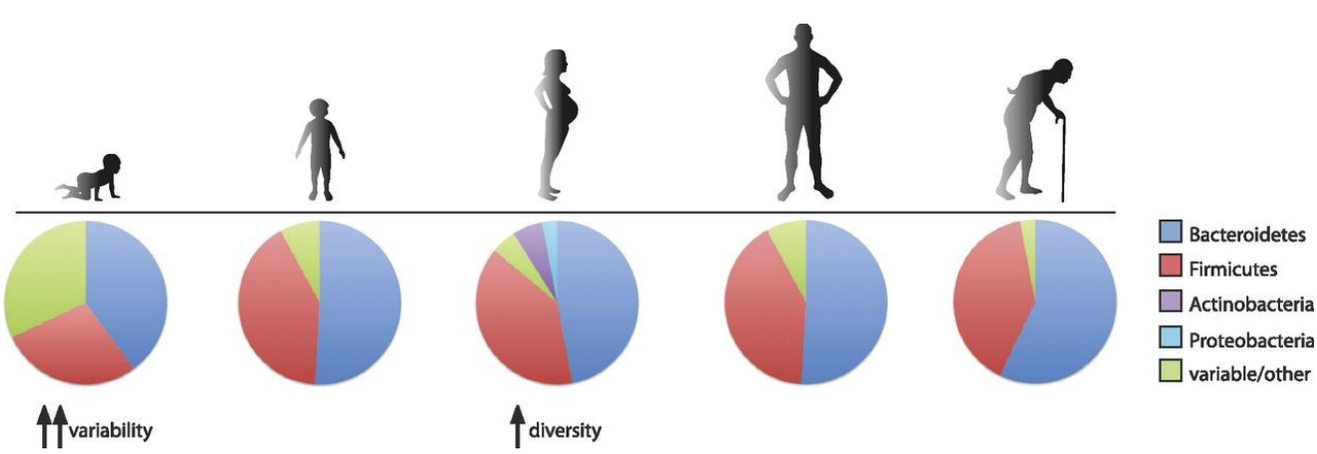

Figure 1. The relative amounts of certain bacterial phyla in healthy individuals at different life stages. There is increased variability between infant individuals, and diversity in pregnant individuals increases, including the growth in proportion of otherwise less common phyla. Adapted from Kostic, Howitt & Garrett, 2013.

secondary bile (Weingarden *et al.,* 2014). Secondary bile is the main constituent of bile within healthy individuals and patients post-FMT, and can prevent the spread of pathogenic bacteria which are known to use primary bile as a germination source, most notable *Clostridium difficile* (Khoruts & Sadowsky, 2016). FMT is most currently used for *C. difficile* infections, where it has been demonstrated to be quite a success (Weingarden *et al.,* 2014), and increasing the therapy's scope to other gastrointestinal diseases has been the focus of great amounts of research. Targeted disorders include CD and UC, but also Irritable Bowel Syndrome (IBS) and issues following low amounts of commensal bacteria after long antibiotic treatments. However, use of FMT for IBD is controversial, with a great variety in its efficacy between patients. It is unknown why the treatment works well in some patients but poorly in others (Angelberger *et al.,* 2013; Rossen *et al.,* 2015). It is usually shown that there is a positive change in the gut microbiome of patients, and often some improvement is seen, but it is not very often a long term change; the best time for patients in terms of their illness is usually 1 month post-FMT (Collins & DeWitt, 2020).

There is a strong possibility that the conflicting conclusions behind studies taken on this subject are at least partly due to the fact that all so far have been of a small-scale with limited numbers of patients; a set of 'well-designed randomised controlled trials' are needed to grant more decisive results (Anderson, Edney & Whelan, 2012). However, it does reinforce the fact that CD, and IBDs in general, are still poorly understood, complex diseases, of which gut dysbiosis is only one part (Caldeira *et al.,* 2020; Khan *et al.,* 2019). It may also be related that CD patients have been found to have a different dysbiosis to other GI diseases, including UC, IBS, etc; there are unique features of the imbalances in each, but CD has been proven to have the most distinct pattern (Bernstein & Forbes, 2017). This could lead to the conclusion that current FMT treatments may not work for CD as often as *C. difficile* infections because the samples do not contain enough of the correct type of bacteria for the disease to be able to go into a state of remission. There has been a list published that details 'Microbes of Interest' specifically within CD and UC (Bernstein & Forbes, 2017), and synthetically altered FMT samples could be generated to create a more personalised approach.

References

Ademe, M. 2020. Benefits of fecal microbiota transplantation: A comprehensive review. *Journal of Infection in Developing Countries*, 14 (10), 1074-1080.

Anderson, J. L., Edney, R. J. & Whelan, K. 2012. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*, 36(6), 503-516.

Angelberger, S., Reinisch, W., Makristathis, A. *et al.* 2013. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *The American journal of gastroenterology*, 108(10), 1620-1630.

Bernstein, C. N. & Forbes, J. D. 2017. Gut Microbiome in Inflammatory Bowel Disease and Other Chronic Immune-Mediated Inflammatory Diseases. *Inflamm. Intest. Dis.*, 2(2), 116-123.

Bielefeldt, K., Davis, B. & Binion, D. G. 2009. Pain and Inflammatory Bowel Disease, *Inflamm. Intest. Dis.*, 15(5), 778-788.

Caldeira, L. d. F., Borba, H. H., Tonin, F. S. *et al.* 2020. Fecal microbiota transplantation in inflammatory bowel disease patients: A systematic review and meta-analysis. *PLoS ONE*, 15(9), 1-17.

Coates, M. D., Johri, A., Gorrepati, V. S., *et al.* 2020. Abdominal pain in quiescent inflammatory bowel disease. *International Journal of Colorectal Disease,* 36(1), 93-102.

Collins, M. & DeWitt, M. 2020. Fecal microbiota transplantation in the treatment of Crohn disease. *JAAPA: Journal of the American Academy of Physician Assistants*, 33(9), 34-37.

Costello, S. P., Conlon, M. A., Vuaran, M. S., Roberts-Thomson, I. C. & Andrews, J. M. 2015. Faecal microbiota transplant for recurrent *Clostridium difficile* infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Alimentary pharmacology & therapeutics*, 42(8), 1011-1018.

Day, A. S., Ledder, O., Lemberg, D. A. & Leach, S. T. 2012. Crohn's and colitis in children and adolescents. *World Journal of Gastroenterology*, 18(41), 5862-5869.

De Filippo, C., Cavalieri, D., Di Paola, M., *et al.* 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 107(33), 14691-14696.

Dicksved, J., Halfvarson, J., Rosenquist, M., *et al.* 2008. Molecular analysis of the gut microbiota of identical twins with Crohn's disease. *The ISME journal*, 2(7), 716-727.

Feagan, B. G., Rutgeerts, P., Sands, B. E., *et al.* 2013. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *New Engl. J. Med.,* 369(8), 699-710.

Feagan, B. G., Sandborn, W. J., Gasink, C., *et al.* 2016. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *New England Journal of Medicine*, 375(20), 1946-1960.

Frank, D. N., St. Amand Allison, L., Feldman Robert, A., *et al.* 2007. Molecular-Phylogenetic Characterization of Microbial Community Imbalances in Human Inflammatory Bowel Diseases. *Proceedings of the National Academy of Sciences of the United States of America*, 104 (34), 13780-13785.

Gamwell, K. L., Roberts, C. M., Espeleta, H. C., *et al.* 2020. Perceived stigma, illness uncertainty, and depressive symptoms in youth with inflammatory bowel disease: The moderating effect of mindfulness. *Psychology, Health & Medicine*, 25(9), 1037-1048.

Hart, A., Ng, S. C., Watkins, J., *et al.* 2020. The use of 5-aminosalicylates in Crohn's disease: a retrospective study using the UK Clinical Practice Research Datalink. *Annals of Gastroenterology*, 33(5), 500-507.

Hoskins, L. C. & Boulding, E. T. 1981. Mucin degradation in human colon ecosystems. Evidence for the existence and role of bacterial subpopulations producing glycosidases as extracellular enzymes. *Journal of Clinical Investigation*, 67(1), 163-172.

Huda-Faujan, N., Abdulamir, A. S., Fatimah, A. B., *et al.* 2010. The Impact of the Level of the Intestinal Short Chain Fatty Acids in Inflammatory Bowel Disease Patients Versus Healthy Subjects, The *Open Biochemistry Journal,* 4, 53-8.

Khan, I., Ullah, N., Zha, L., *et al.* 2019. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? *IBD Treatment Targeting the Gut Microbiome*, 8(3), 126.

Khoruts, A. & Sadowsky, M. J. 2016. Understanding the mechanisms of faecal microbiota transplantation. *Nature reviews. Gastroenterology & hepatology*, 13(9), 508-516.

Kostic, A. D., Howitt, M. R. & Garrett, W. S. 2013. Exploring host-microbiota interactions in animal models and humans. *Genes Dev.,* 27(7), 701-18.

Leylabadlo, H. E., Ghotaslou, R., Feizabadi, M. M., *et al.* 2020. The critical role of *Faecalibacterium prausnitzii* in human health: An overview. *Microbial Pathogenesis*, 149, 104344.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature*, 489, 220-230.

Miquel, S., Martín, R., Rossi, O., *et al.* 2013. *Faecalibacterium prausnitzii* and human intestinal health. *Current Opinion in Microbiology*, 16(3), 255-261.

Nishida, A., Inoue, R., Inatomi, O., *et al.* 2017. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clinical Journal of Gastroenterology*, 11(1), 1-10.

Ooijevaar, R. E., Terveer, E. M., Kuijper, E. J., Verspaget, H. W. & Keller, J. J. 2019. Clinical application and potential of fecal microbiota transplantation. *Annu. Rev. Med.,* 70, 335-351.

Ortiz, T., Arguelles-Arias, F., Illanes, M., *et al.* 2020. Polyphenolic Maqui Extract as a Potential Nutraceutical to Treat TNBS-Induced Crohn's Disease by the Regulation of Antioxidant and Anti-Inflammatory Pathways. *Nutrients,* 12(6), 1752.

Peterson, D. A., Frank, D. N., Pace, N. R. & Gordon, J. I. 2008. Metagenomic Approaches for Defining the Pathogenesis of Inflammatory Bowel Diseases. *Cell Host & Microbe*, 3(6), 417-427.

Png, C. W., Lindén, S. K., McGuckin, M. A., *et al.* 2010. Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of

mucin by other bacteria. *American Journal of Gastroenterology*, 105,(11), 2420-2428.
Ramai, D., Zakhia, K., Ofosu, A., Ofori, E. & Reddy, M. 2019. Fecal microbiota transplantation: donor relation, fresh or frozen, delivery methods, cost-effectiveness. *Annals of Gastroenterology*, 32(1), 30-38.
Rossen, N. G., Fuentes, S., van der Spek, M. J., *et al.* 2015. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. *Gastroenterology*, 149(1), 110-118.
Rutgeerts, P., Sandborn, W. J., Feagan, B. G., *et al.* 2005. Infliximab for induction and maintenance therapy for ulcerative colitis. *New England Journal of Medicine*, 353(23), 2462-2476.
Schoepfer, A. M., Bortolotti, M., Pittet, V., *et al.* 2014. The gap between scientific evidence and clinical practice: 5-aminosalicylates are frequently used for the treatment of Crohn's disease. *Alimentary pharmacology & therapeutics*, 40(8), 930-937.
Sokol, H., Pigneur, B., Watterlot, L., *et al.* 2008. *Faecalibacterium prausnitzii* Is an Anti-Inflammatory Commensal Bacterium Identified by Gut Microbiota Analysis of Crohn Disease Patient. *Proceedings of the National Academy of Sciences of the United States of America*, 105(43), 16731-16736.
Stein, D. J. & Shaker, R. 2015. Inflammatory Bowel Disease. A Point of Care Clinical Guide, Springer International Publishing.
Syal, G., Kashani, A. & Shih, D. Q. 2018. Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Primer for Internists. *The American Journal of Medicine*, 131(9), 1017-1024.
Tian, H., Ding, C., Gong, J., *et al.* 2015. Freeze-dried, capsulized fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *Journal of Clinical Gastroenterology*, 49(6), 537-538.
Weingarden, A. R., Sadowsky, M. J., Chen, C., *et al.* 2014. Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 306(4), G310-G319.
Zaruhi, A. K., Zhanna, A. K., Gayane, P. M., *et al.* 2008. Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS ONE*, 3(8), e3064-e3064.

Insider Imprint

Follow us on social media

Like and follow our pages to see our latest news and receive updates about writing and art opportunities!

twitter.com/insiderimprint

facebook.com/insiderimprint

instagram.com/insiderimprint