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Efficacy of fecal microbiota transplantation in patients with Crohn's disease

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS
WITH CROHN'S DISEASE**

by

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B.S., Marist College, 2008

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requirements for the degree of
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ABSTRACT

Crohn's disease (CD) affects 1.5 million Americans a year. Many patients refractory to standard treatments suffer from life-long detrimental symptoms that reduce quality of life. Chronic inflammation of the gastrointestinal tract, a hallmark of CD, has led to the development of drugs and interventions targeting the immune response. Recent ideology regarding the pathophysiology of CD has implicated a role for the microbiome within the human gut. The advent of metagenomics has allowed for the characterization of the microbiome of both healthy and diseased individuals. The phylogenetic bacterial makeup of the microbiome of patients with CD shows an alteration from that of healthy individuals. Therapeutic methods incorporating the re-establishment of a healthy microbiome are being investigated. Fecal microbiota transplantation (FMT) has become the "gold standard" of care for patients afflicted with *Clostridium difficile* (*C. diff.*) infection who do not respond to a standard antibiotic regimen. *C. diff.*-infection presents with similar symptoms and alterations of the microbiome to that observed in patients diagnosed with CD. To date, only studies of three case and two cohorts utilizing FMT in patients with CD have been reported. Results from these studies show potential usefulness for fecal microbiota transplantation as a therapy for CD. This review offers insight for improved clinical trials in which FMT and immune response therapies are adjunctively utilized to improve CD treatment.

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LIST OF ABBREVIATIONS

AE.....	adverse events
CD.....	Crohn's Disease
CDAI.....	Crohn's disease activity index
CDI.....	Clostridium difficile Infection
CRP.....	C-reactive protein
EEN.....	Exclusive Enteral Nutrition
FDA.....	Food and Drug Association
FMT.....	fecal microbiota transplantation
FOS.....	fructo-oligosaccharide
GI.....	Gastrointestinal
HBI.....	Harvey-Bradshaw Index
IBD.....	Inflammatory Bowel Disorder
IL.....	interleukin
IND.....	Independent New Drug
MDP.....	muramyl dipeptide
NF- κ B.....	nuclear factor kappa-light-chain-enhancer of activated B cells
NOD2.....	Nucleotide-binding oligomerization domain-containing protein 2
PRR.....	pattern recognition receptor
RCT.....	randomized-controlled trial
SCFA.....	short-chain fatty acid

TGF- β 1.....transforming growth factor beta 1
TH.....helper T-cell
TLR.....Toll-like receptor
TNF- αtumor necrosis factor-alpha
UC.....Ulcerative Colitis
VCAM-1.....vascular cell adhesion molecule 1

INTRODUCTION

Crohn's disease (CD) is an idiopathic, chronic inflammatory bowel disease (IBD) of the gastrointestinal (GI) tract of genetically susceptible individuals. It can range anywhere from the mouth to the anus and most commonly presents with diarrhea, abdominal pain, rectal bleeding and in more severe cases, ulcers (Klapproth, 2012). Approximately 1.5 million Americans are afflicted with CD (Ananthakrishnan, 2015). It exerts a major burden on an individual's quality of life due to life-long treatments, frequent hospitalizations, and adverse social functioning. There is no known etiology of the disorder, although understanding of factors involved in its onset has vastly improved in the past thirty years.

Recent research has implicated a combination of genetic and environmental factors in the development of CD. Of the genetic factors, several risk loci have been elucidated as prominent sites of mutation within the CD community including the nucleotide-binding oligomerization domain containing 2 (NOD2) and ATG16L1 genes (Ek, 2014). The gene products function to regulate gut immunity but through wholly different mechanisms. In addition, recent evidence has implicated the alteration of the gut microbiota as crucial to the onset of CD. Certain phenotypic changes to the makeup of this microbial population have led to disease states and morbidity (Jacobs & Braun, 2014).

Chronic inflammation of the GI tract is the underlying cause of CD symptoms. Traditional treatments for CD include the use of immunosuppressants, steroids, and antibiotics with the goal of remission of inflammatory flare-ups in the short and long term

(Bandzar, 2013). Many aims of research have worked to produce drugs that reduce or abrogate these inflammatory pathways. Cytokines and adhesion molecules are major mediators of the inflammatory response and have recently been targeted for inhibition by experimental drugs (Pedersen, 2014). Drugs, such as infliximab and adalimumab, function to inhibit the pro-inflammatory cytokine tumor necrosis factor alpha (TNF- α) while natalizumab targets adhesion molecules of vessel walls. Additional therapies targeting the microbiome include prebiotics and probiotics. These biologic therapies aim to improve the overall health of an individual's gut. While many of these therapeutic options can be successful treatments in alleviating symptoms, the need for therapies that achieve prolonged and even complete remission of Crohn's disease is still the focus of many researchers.

Fecal microbiota transplantation (FMT) has been shown to be a successful mode of therapy in cases of recurrent *Clostridium difficile* infections (CDI) in which dysbiosis of the microbiome occurred (Cammarota, 2014). Recent studies have reported an 86% curative rate of CDI for patients receiving multiple FMTs (Lee et al., 2014). Due to the increasing evidence of positive outcomes for CDI patients, FMT has been sought as a therapeutic means for the two major IBD's, Crohn's disease and ulcerative colitis (UC).

There are limited publications on fecal microbiota transplantation in IBD cases, Crohn's disease in particular. Much research on gastrointestinal disorders is now focusing on the microbiome and its interactions with the host immune system. This review aims to discuss the risk factors for CD, current and experimental treatment options, historically successful results of fecal transplantation and its potential efficacy in

the treatment of Crohn's disease.

PUBLISHED LITERATURE

Genetic Risk Factors

It has been shown that relatives of patients with Crohn's disease experience increased risk of developing the disease than control populations (Calkins, 1986). This supports the notion that there is a genetic aspect of CD. In the past 15 years, 163 risk loci have been identified as potential indicators for CD. Many of these risk loci are shared amongst other disease states including, but not limited to, ulcerative colitis, ankylosing spondylitis, psoriasis, and even diabetes (Jostins et al., 2012). Table 1 details the 30 loci that have been identified as specific to CD including NOD2 and ATG16L1.

The NOD2 gene product has been implicated in association with Crohn's Disease patients (Hugot et al., 2001). Single nuclear polymorphisms (SNP's) and frameshift mutations of the coding region of NOD2 affect the leucine-rich region of the NOD2 receptor, a Nod-Like Receptor (NLR), which is present on antigen-presenting cells (APCs) and Paneth cells of the gut (Lala et al., 2003). Recent studies have shown that muramyl dipeptide (MDP), a component of the peptidoglycan layer of the cell wall of many bacterial cells, serves as the major ligand for the NOD2 receptor (Inohara et al., 2003). Normal function of these receptors stimulate NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), a transcription factor that rapidly initiates pro-inflammatory responses (Baldwin, 1996). Compromised function of these immune response genes could lead to uncontrolled, and potentially damaging inflammation, which

TABLE 1: Crohn's disease Specific Risk Loci

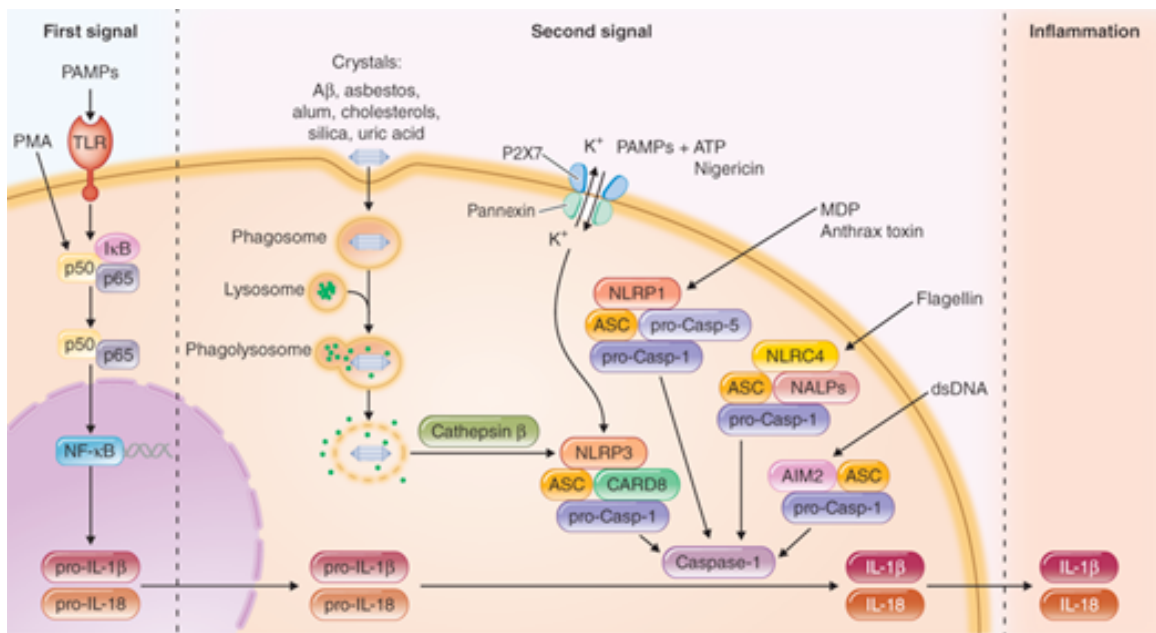
Crohn's disease			
Chr.	Position (Mb)	SNP	Key genes (+ no. of additional genes in locus)
1	78.62	rs17391694	(5)
1	114.3	rs6679677	PTPN22 * (8)
1	120.45	rs3897478	ADAM30 (5)
1	172.85	rs9286879	FASLG, TNFSF18 (0)
2	27.63	rs1728918	UCN (23)
2	62.55	rs10865331	(3)
2	231.09	rs6716753	SP140 (5)
2	234.15	rs12994997	ATG16L1 * (8)
4	48.36	rs6837335	(6)
4	102.86	rs13126505	(1)
5	55.43	rs10065637	IL6ST, IL31RA (1)
5	72.54	rs7702331	(4)
5	173.34	rs17695092	CPEB4 (2)
6	21.42	rs12663356	(3)
6	31.27	rs9264942	(22)
6	127.45	rs9491697	(3)
6	128.24	rs13204742	(2)
6	159.49	rs212388	TAGAP (5)
7	26.88‡	rs10486483	(2)
7	28.17	rs864745	CREB5, JAZF1 (1)
8	90.87	rs7015630	RIPK2 (4)
8	129.56	rs6651252	0
13	44.45	rs3764147	LACC1 (3)
15	38.89	rs16967103	RASGRP1, SPRED1 (2)
16	50.66†	rs2066847	NOD2 * (6)
17	25.84	rs2945412	LGALS9, NOS2 (3)
19	1.12	rs2024092	GPX4, HMHA1 (20)
19	46.85‡	rs4802307	(9)
19	49.2	rs516246	FUT2, (25)
21	34.77	rs2284553	IFNGR2, IFNAR1 (10)

*Gray highlighting indicates newly discovered loci. Bolded rs numbers represent SNP's with p-values less than 1×10^{-13} . Bold genes represent verification with 2 or more sequencing techniques (Taken from Jostins et al., 2012).

may explain the susceptibility of patients with mutant NOD2 loci for chronic CD. Co-stimulation with Toll-like receptor 4 (TLR-4), another molecule known to activate NF- κ B, has also been shown to enhance both pro- and anti-inflammatory cytokine production (Fritz et al., 2005). It is believed that regulating inflammation is an extremely important function that helps to maintain gut immunity, and dysfunction of these receptors has the capacity to allow for translocation of commensal bacteria across the mucosal-epithelial barrier. Displacement of intestinal microbes from the confines of the gut, where they are recognized as non-threatening, to the tissue is likely to induce robust inflammatory responses characteristic of those observed in patients with compromised NOD2 function. In addition, many researchers believe the NOD2 receptor plays a role in immune-regulatory mechanisms that act to suppress TLR activation of pro-inflammatory cytokines (Watanabe, 2004). Having compromised abilities to regulate inflammatory responses in the gut could lead to enhanced recruitment of inflammatory cells and subsequent tissue damage. Figure 1 illustrates TLR and NOD2 co-stimulation leading to an inflammatory response. The NOD2 receptor is contained within the NLRP family in the figure, associating with caspases to induce the release of inflammatory signaling molecules. Whether polymorphisms of the NOD2 gene are causal or resultant in patients with CD is still unclear. Further investigation into the implications of mutated NOD2 and their association with the development and life-long persistence of CD will elucidate possible treatment strategies to correct for compromised regulation of intestinal inflammation.

Another mechanism by which many researchers believe a chronic inflammatory state is sustained in the gut is the disruption of normal autophagic pathways in innate immune cells. Autophagy has been established as a means for phagocytic cells, such as macrophages and dendritic cells, to sample cytosolic material from pattern recognition receptors (PRR's) and effectively clear them via lysosomal degradation (Tal & Iwasaki, 2009). ATG16L1 is a gene linked to autophagy and has been identified as an important risk locus for CD. ATG16L1 T300A mutation in a murine model has been shown to reduce antimicrobial autophagy as well as increase Interleukin-1 beta (IL-1 β) secretion of innate immune cells of the *lamina propria* (Lassen et al., 2014). IL-1 β induces inflammation as shown in Figure 1, and has been implicated as a mediator of intestinal epithelial barrier function, increasing permeability with increased expression levels (Al-Sadi & Ma, 2007). Therefore, targeting IL-1 β in CD patients may help dampen intestinal inflammation and alleviate the painful flare-ups caused by uncontrolled innate immune responses.

Genetic predisposition to Crohn's Disease does not necessarily lead to onset of disease. However, elucidation of risk loci and subsequent gene products that can become dysfunctional allows for further research into the mechanisms that can result in CD. Dysfunction of receptors essential to the inflammatory response (NOD2) or autophagic (ATG16L1) pathways of immune cells of the gut allows for potential dysregulation and onset of CD.



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Figure 1: Co-stimulation between TLR and NOD2 receptors resulting in production and secretion of IL-1 β and a pro-inflammatory response (Taken from Kasper, 2013).

Environmental Factors

The pathophysiology of IBD is not well understood. While many persons with risk variants present with the clinical symptoms of CD, most do not and live healthy lives. Microbiota-host interactions are a symbiotic relationship. Unhealthy diet can lead to colonization of microbes within the gut that are not beneficial to the host, potentially inducing inflammatory responses. As a westernized diet, one of high fat and sugar content, becomes more consumable throughout the world, the incidence of CD and other inflammatory bowel diseases has increased (Ananthakrishnan, 2015). Consequently, rapid growth and expansion of what was typically a European and North American disease to a more world-wide patient base lends to the notion that environmental influences, not solely genetic risk factors, are paramount to the onset of CD. Factors such as a westernized diet and lack of exercise have been targeted as potential warning signs for disease or flare-ups, however, it is the alteration of an individual's microbiome population that has spurred most interest over the past decade (O'Toole, 2014).

Microbiome and Inflammatory Bowel Disorders

The human gut microbiome encodes for thousands of bacterial species that inhabit the gastrointestinal tract of all human beings. They thrive in a symbiotic relationship with the host as either commensal or mutualistic bacteria (Hold et al., 2014). The Human Microbiome Project, commissioned by the National Institute of Health, has used advances in sequencing technologies and metagenomics in order to map the genome of the microbiota of healthy individuals. The purpose of the project is to establish a

documented “healthy cohort” of genetic information which would be used to compare the microbiota populations of persons affected by various diseases with those of healthy individuals (Hold et al., 2014). Knowing which microbial populations are most beneficial and in what combination, will facilitate treatment regimens to help restore the communities that are most beneficial to the well being of the host.

The term, “dysbiosis” refers to the alteration of an individual’s microbiome, and is usually accompanied by a disease-state. Immune cells within the host intestine tolerate homeostatic intestinal microbes. Drastic changes in microbial populations to unfamiliar species may provoke inflammatory responses, leading to the characteristic flare-ups experienced by patients diagnosed with CD. In the last decade it has been shown that the gut of healthy individuals contains a large volume of bacteria of the phyla *Firmicutes* and *Bacteroidetes* (Frank et al., 2007). These two phyla have been shown to decrease in number in individuals who have been diagnosed with CD creating a dysbiotic environment between the host and microbial population. In addition, bacteria of the phyla *Proteobacteria* are more abundant in these patients (Mukhopadhyaya, 2012). Figure 2 is a representation of phyla-level classification of patients who underwent ileo-colic resection surgery and post-operative colonoscopy versus healthy controls. From this particular study, it can be seen that both *Firmicutes* and *Bacteroidetes* dominate both the ileum and colon of healthy individuals while *Proteobacteria* are more prevalent amongst those with CD. Furthermore, lack of diversity of bacterial species amongst the different phyla has been identified as a condition of CD patients. Manichanh et al. (2006) reported

a reduction from 43 ribotypes of *Firmicutes* in healthy patients to just 13 in those afflicted with CD.

While it has been established that a dysbiotic gut is a hallmark of CD, it is still unknown whether this is a cause of the disorder or simply a result of already persisting inflammation, possibly due to mutations in loci known to control inflammatory pathways. How the innate immune system of the gut reacts to changes in the microbiota is a popular field of interest to researchers, as it is the key to subduing damaging inflammatory reactions that plague many patients diagnosed with CD and other inflammatory bowel disorders.

Diet and Crohn's disease

When discussing the microbiome as an indicator for Crohn's disease one must also discuss any means that contains the potential to alter the makeup of the microbiome. Most studies examining the correlation of diet with CD have been retrospective in nature, and no definitive link has been established. However, diet possesses the ability to contribute to the pathogenesis of IBD and must be extensively researched in a controlled setting. Studies of how healthy individuals with CD respond to various changes in controlled diet could elucidate the mechanisms of intestinal immune tolerance.

Fiber has been implicated as an important nutrient in maintaining proper gut health. It has been shown to decrease permeability of *Escherichia coli* across M-cells of Peyer's patches of the gut (Roberts et al., 2010). Fiber is also broken down to short-chain fatty acids (SCFA), which have been known to regulate inflammation of the gut by

Phylogenetic Microbiome Classification Post-Operation

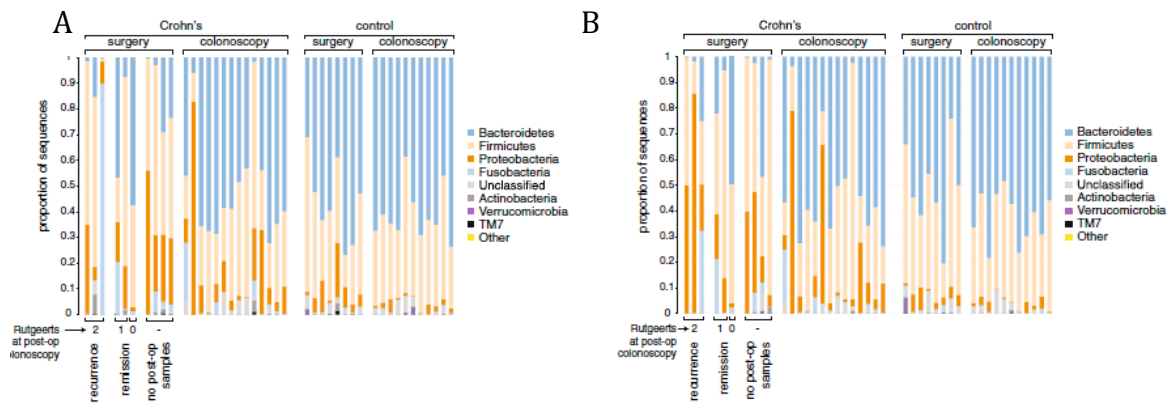


Figure 2: Phyla-level classification of the microbiome of both the (A) ileum and (B) colon of patients with CD undergoing resection surgery and post-operative colonoscopy versus healthy individuals undergoing surgery and colonoscopy. Prevalence of phylum decreasing from top to bottom (Taken from Dey, 2013)

inhibiting certain nuclear factors that actively stimulate pro-inflammatory cytokines (Maslowski, 2011). Studies investigating SCFA's have determined a role for increasing immune tolerance by increasing the production of regulatory T-cells in the gut, which are capable of countering pro-inflammatory cytokine secretion (Thorburn, 2014). A retrospective study of 59 CD patients compared to 477 controls showed an increased dietary intake of sugars amongst the CD population (Jakobsen, 2013). Another independent study of 168 children, of which 53 had CD, confirmed that the children with CD had an increased dietary intake of sugars and meats (Tsiountsioura et al., 2014). These results highlight the risks of high sugar-containing diets and suggest they result in colonization of threatening microbes within the gut, which stimulate inflammatory reactions from intestinal immune cells. Substituting the high sugar diet with one high in fiber may sweep away damaging microbes and activate regulatory T cells that hinder inflammation, thereby restoring intestinal homeostasis and relieving patients of flare-ups.

Dietary vitamins have also been associated with a lower incidence of IBD due to their anti-inflammatory properties. Both vitamin D and calcium are thought to enhance the resistance to epithelial cell dysfunction and injury. The hormonally activated form of vitamin D, 1,25(OH)₂ D₃, has been proven to ameliorate the effects of certain helper T-cells (TH 1 and TH 17) known to induce and maintain inflammatory reactions (Cantorna, 2014). Patients with Crohn's disease display a chronic proliferation of TH 1 and TH 17, which produce pro-inflammatory cytokines. The vitamin D receptor on these cells is only accessible in the activated T-cells that would be indicative of disease (Cantorna, 2014). These are encouraging data that support a role for vitamin D as a beneficial

participant in an anti-inflammatory response of the gut, and suggest it could be utilized to abrogate damaging intestinal inflammation associated with CD.

Certain amino acids are thought to be immunoregulatory in function as well. Glutamine and arginine have been found to mediate inflammation during times of stress and flare-ups. Glutamine is the preferred substrate of enterocytes of the intestine, and recent enteral nutritional intervention studies have observed a therapeutic result of such supplementation (Coëffier, 2010). Similarly, L-arginine supplementation in a murine model displayed reduced serum levels of TNF- α and IL-17, two constitutively elevated cytokines during bouts of inflammation (Ren et al., 2014). Supplemental diets and therapies of arginine and glutamine can be advantageous towards achieving clinical remission of CD flare-ups.

The advent of metagenomic sequencing has allowed for the classification of the gut microbiome in healthy and diseased individuals. Insights into how the manipulation and alteration of the microbiome can affect healthy individuals have potential to inspire development of novel therapeutics for patients with CD.

Current Management

Due to the unknown etiology of Crohn's disease and the variation by which it presents from patient to patient, managing the disease focuses on alleviating symptoms and inhibiting a wide range of inflammatory molecules. Symptoms include chronic flare-ups, diarrhea, weight loss, and abdominal pain to name a few. Anti-inflammatory antibody therapies target TNF- α and alpha-4 integrin molecules to suppress

inflammation. More recently, cytokine specific antibodies are being researched as another means to achieve remission of inflammation. Immunosuppressants, such as Azathioprine and Mercaptopurine function to suppress the immune response and have been utilized in organ transplantation and inflammatory disorders for decades. Approaches targeting the microbiota have been gaining ground in the scientific community as a means to abrogate possible pathogenic stimuli in the gut and resolve a dysbiotic environment. These include antibiotics, probiotics, prebiotics, and fecal microbiota transplantation. Other therapies focus on supplementing much needed nutrition into the diet. Exclusive enteral nutrition (EEN) is a therapy that temporarily suspends 80-90% of a patient's normal diet for a liquid supplement enriched with beneficial nutrients and a majority of the daily caloric intake (Day & Burgess, 2013). It has been extensively studied and shown to induce remission in CD patients (Takagi et al., 2006). All of these therapies offer certain advantages and disadvantages to the patient. Providing a personalized regimen that is effective at achieving remission while minimizing detrimental effects to the patient is the goal of CD management.

Anti-inflammatory Drugs

TNF- α is an inflammatory cytokine that is secreted from helper T-cells, TH 1 cells chiefly among them (Romagnani, 1994). Its presence in CD patients has been well documented. While this cytokine contains pleiotropic properties and affects many different organ systems, the entirety of its importance is still not completely understood. Therapeutic agents targeting TNF- α have been successful in reducing inflammation

within the bowel. Such agents include infliximab, adalimumab, and certolizumab pegol. Infliximab is a IgG1 murine-human monoclonal antibody that binds to the precursor of TNF- α , essentially inhibiting its differentiation and activity (Ebert, 2009). It also functions in the cell- signaling cascade of TNF- α . TNF- α receptor type 2 (TNFR2) binding to its ligand cleaves off the extracellular domain and enters the circulation as a soluble product. Infliximab increases cleavage of TNFR2 from innate immune cells reducing its presence on the surface and tying up the TNF- α (Reinhard, 1997).

Adalimumab is another TNF- α blocker that binds directly to TNF- α and neutralizes its biological activity. It is also pro-apoptotic to mononuclear cells producing TNF- α (Cvetković, 2006). In a retroactive analysis of 124 patients administering adalimumab self-injections, 75% were satisfied with the results of the treatment while 85% continued to adhere to the treatment (Hirai et al., 2014). In 2008, certolizumab pegol was approved for Crohn's disease patients who did not respond well to either infliximab or adalimumab. Certolizumab pegol is a humanized antibody that has a polyethylene glycol (PEG)ylated fragment that binds and inhibits TNF- α (Nesbitt et al., 2007). It has been suggested that certolizumab pegol would be a good alternative to other anti-TNF- α drugs for pregnant women. The polyethylene glycol does not cross the placental barrier protecting the child from unnecessary complications (Pasut, 2014).

A meta-analysis screened 506 studies using anti-TNF-alpha therapies and reported on 10 randomized-controlled studies using infliximab, adalimumab, and/or certolizumab pegol in the treatment of CD (Stidham et al., 2014). Endpoints included induction of remission and response as well as maintenance of remission and response.

All 3 anti-TNF- α agents were successful in significantly inducing and maintaining remission and response compared to placebo controls. Anti-TNF- α agents were 1.66 times more likely to induce clinical remission and 1.68 times more likely to maintain clinical remission in CD patients.

The advantages of TNF- α inhibitors are clear. Clinical remission of flare-ups has been well documented. Certain complications including targeting of antibody to particularly inflamed regions of the bowel have been encountered. In patients who do not respond well to the therapy, endoscopic and serological results have indicated an inability of the anti-TNF- α antibody to migrate to diseased tissues (Yarur et al., 2015). In addition, infliximab resistance has been found in some patients with CD in which doubling the dose or reducing the dose had no particular effect on clinical recurrence or remission rates (Nagata et al., 2015). It is also implicated in the increased risk of post-operative complications (El-Hussuna, 2014), however, there are conflicting views as to the validity of these claims.

Alternative to anti-TNF- α inhibitors, natalizumab is a humanized monoclonal antibody that targets alpha-4 integrin molecules of both innate and adaptive immune cells. Alpha-4 integrin is expressed on basophils, eosinophils, monocytes and lymphocytes. One ligand for this molecule is the vascular cell adhesion molecule-1 (VCAM-1). VCAM-1 is expressed on the endothelial cells of blood vessels. Upon stimulation with various pro-inflammatory cytokines, VCAM-1 is expressed in order to assist innate and adaptive immune cells in migrating across endothelial cells to the sites of inflammation (Schechner, 1999). Natalizumab binds to alpha-4 integrin and inhibits it

from binding VCAM-1 (Wipfler et al., 2011). In doing so, natalizumab inhibits the migration of immune cells to sites of inflammation, dampening the inflammatory response that occurs during flare-ups in CD patients. One benefit of natalizumab is that alpha-4 integrin is not expressed on neutrophils. These innate immune cells can continue to phagocytically clear microbial and bacterial fragments, thus preserving an immune response (Hickey, 2009). In a recent study, 49 CD patients who had previously seen no beneficial returns on anti-TNF- α treatment were administered natalizumab for a minimum 7-month duration. Eighty-five percent of patients found success with the treatment lasting at least 12 months (Sakuraba et al., 2013).

The use of biological agents, including TNF- α and alpha-4 integrin targeting drugs, has vastly improved the quality of life of many patients suffering from CD. Table 2 illustrates the various drugs that target molecules of the inflammatory response as well as certain immune cell populations that are elevated in CD. Brodalumab and tocilizumab are experimental drugs that target pro-inflammatory cytokines IL-17R and IL-6R, respectively. Abatacept does not target inflammatory molecules but instead attempts to inhibit the production of T-cells that would produce such molecules in response to antigen. With all the advances that have been made in the field, there is still 20-40% of patients who do not respond to these therapies (Nielsen, 2012). Prominence of the role of the microbiota in CD has been gaining traction over the years. For this reason, it is paramount that adjunctive therapies targeting the microbiome be investigated concurrently with anti-inflammatory treatments.

Table 2: Common drugs and their target effects in treating inflammation in CD patients (Taken from Bandzar, 2013)

Cellular pathways and contraindications.	
Drug	Cellular pathway
Infliximab	Block TNF-alpha
Adalimumab	Block TNF-alpha
Certolizumab pegol	Block TNF-alpha
Natalizumab	Block alpha-4 integrin
Human growth hormone	Block IL-6
Conjugated linoleic acid	Up-regulate PPAR-gamma
Rifaximin	Inhibit bacterial transcription
Ustekinumab	Block p40 subunit of IL-23, IL-12
AMG 139	Block IL-23
Brodalumab	Block IL-17R
Tocilizumab	Block IL-6R
IL-10	Block IL-6, TNF-alpha, IFN-gamma
IL-11	Preserve intestinal morphology
Visilizumab	Induce apoptosis in T-cells
Abatacept	Block T-cell activation
Mesenchymal stem cells (MSCs)	Block CD80/86, IL-12, TNF-alpha, and increase IL-10

Microbiota Treatments

The dysbiosis of the gut microbiome has been implicated as a possible pathogenesis of CD and has led to the advent of many therapies aimed at re-establishing the symbiotic relationship between microbiota and host consistent with that of a healthy individual. The use of antibiotics has been found to yield conflicting results, with a great many trials resulting in a lack of efficacy. Prolonged antibiotic use has also been implicated with various side effects. Unlike antibiotics, probiotics are living bacterial organisms that are ingested in large amounts in order to re-establish gut homeostasis and provide health benefits to the host. They must be either pathogenic towards inflammation inducing microbes or outcompete these same harmful bacteria for valuable nutrients and space (Gionchetti et al., 2006).

Antibiotics

Metronidazole is an antimicrobial that inhibits nucleic acid synthesis of anaerobic bacterial cells. It has historically been utilized as a treatment against CD as far back as 1978 (Blichfeldt, 1978). In this double blind cross-over study, 22 CD patients receiving steroids were treated with either metronidazole or placebo for 2 months. No significant difference was found in overall clinical condition between the 2 groups, however, patients with colonic disease were found to have improved symptoms. Sutherland *et al.* (1991) also found mixed results in a study of 105 patients who were treated with different doses of metronidazole. The Crohn's disease activity index (CDAI) showed

improvement from baseline in patients on the metronidazole regimen. A CDAI score of less than 150 is considered remission. Clinical remission was not achieved in the groups being treated with metronidazole.

Ciprofloxacin has been studied in conjunction with and independent of metronidazole. In a study of 47 adults with CD, ciprofloxacin was administered 500mg twice daily against a placebo group receiving no treatment. While mean CDAI scores were not significant, patients in the ciprofloxacin group achieved remission with a mean score of 112 (Arnold, 2002). In another study, metronidazole and ciprofloxacin were administered together while the placebo group received a standard therapy of methylprednisolone, a steroid. Remission rates of the treatment group and placebo group were 63% and 45.5%, respectively (C. Prantera et al., 1996). This indicated a possible alternative to steroidal treatment.

More recently, rifaximin has been studied for its efficacious potential in CD patients. Rifaximin has no known drug-drug interactions and is non-systemic leading many to suggest a favorable role in the treatment of CD (Charmot, 2012). In a study of 402 patients, rifaximin was administered in doses of 400mg, 800mg, or 1200mg twice daily for 12 weeks (Prantera et al., 2012). Significant results were seen in the cohort that received 800mg treatment while the 400mg and 1200mg groups were not found to be significantly different from the placebo control. In addition, adverse events (AE) were noted predominantly in the 1200mg group ranging from nausea and vomiting to fever and increased C-reactive protein sera levels. Ten percent of patients in this group ceased the treatment before the endpoint due to these AE's.

The use of antibiotics to treat CD is a double-edged sword. Prolonged use of antibiotics in clinical settings has been implicated in the induction of *Clostridium difficile* infection in patients with otherwise healthy microflora (Theriot, 2014). The idea that antibiotics can induce disruptions in the normal microbiota as well as remedy them limits its potential as an ideal treatment for CD.

Pre- and Probiotics

The ever-growing notion that the microbiota plays a role in the pathogenesis of IBD has spurred research into the use of probiotics and prebiotics as a means to treat CD. The idea of probiotics was first proposed by Nobel prize winner Elie Metchnikoff who believed that *Lactobacilli* in the gut could confer health and longevity to humans (Keszthelyi et al., 1996). *Lactobacilli* and *Bifidobacteria* are among the first bacterial phyla to colonize the GI tract of humans prenatally (Reuter, 2001). They confer homeostatic and immune related health benefits to the gut, chiefly, their antimicrobial property. Many *lactobacilli* and *bifidobacteria* strains are effective at resisting colonization of harmful, pathogenic bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* (Presti et al., 2015). *In vitro* analysis of immune-regulatory function of certain probiotic strains showed an ability to modulate cytokine secretion of a human fibroblast cell-line of the gut (Presti et al., 2015). Figure 3 shows the ability of some lactobacillus strains to inhibit pro-inflammatory cytokine secretion. Another bacterial strain that has recently been found to be beneficial to the gut is *Faecalibacterium prausnitzii*. Its advantage as a probiotic lies in its ability to up-regulate

regulatory T-cells in a murine model (Qiu, 2013). These T-cells suppress inflammation by secreting anti-inflammatory cytokines such as IL-10 and TGF- β 1.

Ten randomized-controlled trials (RCT) were found in which CD was treated using probiotics only (Ghouri et al., 2014). *Lactobacillus*, *Lactobacillus rhamnosis* and *Lactobacillus johnsonii* were the bacterial strains used in these studies. Endpoints of the trials included clinical remission with CDAI score below 150, endoscopic recurrence rate, and clinical relapse rate. All three *Lactobacillus* strains did not significantly lower CDAI scores or relapse rates (Shen, 2009). *Saccharomyces boulardii* is another probiotic that has been studied for its potential benefit. In a murine model study, *Saccharomyces boulardii* was shown to improve enteric barrier defense by decreasing permeability of epithelial cells (Garcia Vilela et al., 2008). A cohort of 20 CD patients suffering from diarrhea and abdominal pain took part in a randomized, double-blinded study to evaluate *S. boulardii* against a placebo control (Plein, 1993). A decreased frequency in bowel movements was observed in the intervention group for up to 10 weeks, however, relapse occurred in all patients past this point.

Unlike probiotics, prebiotics are not considered “live microorganisms”, however, they are selectively fermented nutrients that possess the ability to feed healthy bacteria of the GI tract. Two randomized controlled studies tested for clinical remission of CD with 2 different prebiotics, fructo-oligosaccharides (FOS) and lactulose, respectively. FOS has been shown to increase fecal *bifidobacteria* as well as attenuate pro-inflammatory cytokine production in a murine model (Yeh, 2014). In a study of 49 controls and 54 CD patients, CD patients were administered 15g/day FOS while controls received placebo

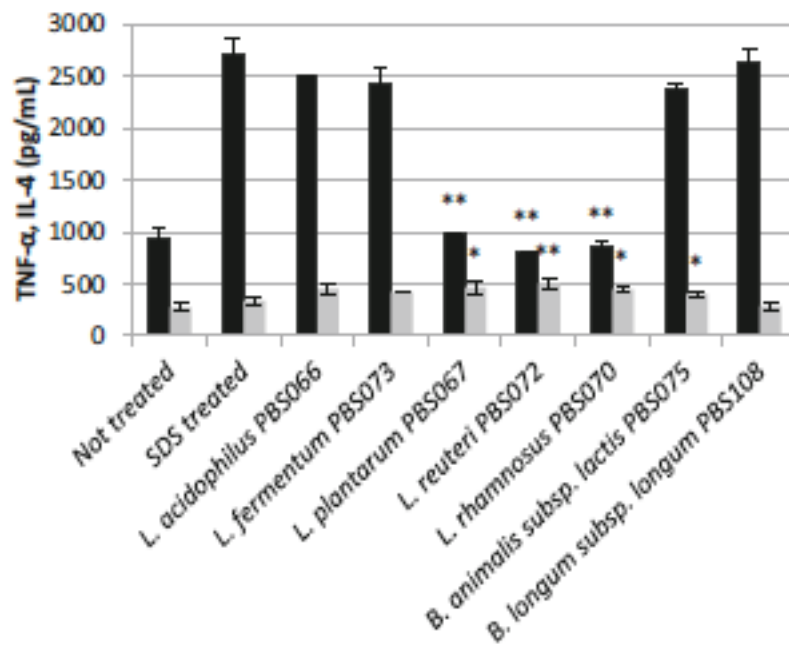


Figure 3: Effect of lactobacilli and bifidobacteria strains on TNF- α (black bar) and IL-4 (gray bar) secretion by gut fibroblast cells in the presence of sodium dodecyl sulfate (irritant) (Taken from Presti et al., 2015).

for 4 weeks with an endpoint of clinical remission determined by CDAI. No statistical difference in clinical remission was achieved despite improvement in CDAI scores (Benjamin et al., 2011). Also, fecal *bifidobacteria* levels were unchanged. Interleukin-6 positive dendritic cells of the *lamina propria* were demonstrated to be less abundant in the FOS treated group as suggested from previously published data (Yeh et al., 2014). Hafer et al. (2007) published experiences with a RCT in which lactulose was the experimental prebiotic for patients with CD. No significant difference was found between the control and experimental groups.

To date, trials studying the efficacy of pre- and probiotics for the treatment of CD have been predominantly unsuccessful. Although *in vitro* evidence in murine models suggests a beneficial role for probiotics, they have been inadequate at inducing clinical remission in CD patients. More research is required in order to discover bacterial species that confer a wide range of beneficial properties to the host.

Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) is not a revolutionary procedure. The concept for FMT in the United States dates back to 1958 when fecal enemas were used as an adjunctive therapy in order to treat pseudomembranous enterocolitis, a disease that had a 75% mortality rate at the time (Eiseman, 1958). Literature on the procedure did not reappear until 1984 when FMT was utilized to cure *Clostridium difficile* infection (Schwan, 1984). The mechanism of action of FMT is similar to that of probiotics in that healthy bacteria are transplanted into a recipient's GI tract in order to compete with

harmful, inflammation inducing bacteria for a place in the microbiotic niche. Unlike probiotics, which introduce one bacterial strain to a recipient's gut, FMT aims to transplant an immense population of healthy bacteria to the gut. The return of the microbiota to a healthy state is thought to promote an anti-inflammatory environment in the gut and hopefully, remission of clinical disease.

Fecal microbiota transplantation involves the introduction of potentially hazardous infectious material into a recipient. It is important, therein, that a rigorous screening process of both the donor and recipient be implemented and followed. Consensus among hospitals and centers across the country have detailed a list of potential threats to screen for including HIV type 1 and 2, hepatitis A, B and C, *Clostridium difficile*, and *Helicobacter pylori* amongst others (Brandt, 2013). Furthermore, ideal donors will not have been on an antibiotic course over the previous 3 months as effects on the microflora can persist during that time. Lastly, patients with a history of GI disorders such as IBD, Irritable Bowel Syndrome, chronic diarrhea, metabolic syndrome etc. should be precluded from donating stool for use in FMT (Mattila et al., 2012).

FMT Preparation and Administration

There is no universal protocol for the preparation of stool for fecal transplantation. Fresh stool is predominantly reconstituted in saline, water, or milk and administered within hours of collection. The standard minimum amount of fecal matter processed is 50 grams (Owens, 2013). Frozen fecal samples are usually processed in a similar fashion to the standard practice utilized at each individual center. In one study

attempting to distinguish a difference between a fresh and frozen sample, samples from unknown volunteers were frozen for 1-8 weeks and thawed for approximately 2-4 hours (Hamilton, 2012). Patients with recurrent CDI were administered either fresh or frozen samples by colonoscopy and showed no significant difference in remission rate (92% vs. 90%). Having a volunteer based collection of pre-screened, frozen FMT samples provides a beneficial means to treat time-sensitive cases.

Modes of administration of FMT have evolved from retention enema to colonoscopy and eventually nasogastric/nasojejunal tubes. Colonoscopy seems to be the preferred method for FMT. Randomized-controlled studies are necessary to determine not only the efficacy of the transplantation itself but the best method of administering FMT as well.

Fecal Microbiota Transplantation and Clostridium difficile Infection

Clostridium difficile infection affects nearly 500,000 Americans annually, killing approximately 29,000 (Lessa et al., 2015). Dysbiosis of the gut microbiome is a prerequisite for CDI, usually the result of prolonged antibiotic use for an unrelated infection (Theriot et al., 2014). Metagenomic analysis of 16S-RNA-encoding gene sequences show a decrease in phylogenetic diversity among the microbiome of CDI patients (Chang et al., 2008). Decreased diversity does not necessarily allow for *Clostridium difficile* invasion, but it is an indication of the dysfunction of the gut to resist colonization. This dysfunction allows for the invasion of *Clostridium difficile* to take up residence in the host. *Clostridium difficile* spores produce toxins that disrupt epithelial

integrity and stimulate host immune responses resulting in symptomatic CDI (Eckert et al., 2015). Symptoms of CDI are similar to that of CD including diarrhea, abdominal pain and inflammatory flare-ups. With a growing resistance rate and increased recurrence rate in antibiotic treated CDI cases (Martin, 2013), FMT has established itself as an efficacious and safe treatment.

In the only published RCT of CDI treated with FMT, 20 patients received a short regimen of vancomycin 3 times daily for 3 days followed by infusions of fecal suspension via colonoscopy (G. Cammarota et al., 2015). The control group consisted of 19 patients receiving vancomycin for 10 days followed by symptom dependent vancomycin dosing for 3 weeks. In the experimental group, 18 patients (90%) reported cessation of clinical diarrhea and resolution of CDI for up to 1 year. The control group consisted of only 5 of 19 (26%) patients resolving CDI with cessation of diarrhea. This study found a markedly increased clinical remission rate amongst those receiving FMT over the currently standard antibiotic treatment.

These results verify the findings of a meta-analysis completed in 2013 of 11 peer-reviewed and published articles attesting to the efficacious potential of FMT for CDI patients (Kassam, 2013). Figure 4 chronicles the results of this meta-analysis. Methods of administering FMT included colonoscopy (7 trials), nasogastric tube (2 trials), and naso-jejunal and enema (1 trial each). Of these trials, only 2 involved completely anonymous donors while the rest were patient selected, predominantly consisting of relatives. Results of the meta-analysis showed 245 out of 273 (89.7%) achieved clinical remission of CDI dependent on the criteria established by study author. Modality of

FMT exhibited a 10% difference in those administered to the upper GI tract (nasojejunal, nasogastric tubes) versus the lower GI tract (colonoscopy, enema) with the lower GI administration resulting in more clinical remission. There was no significant difference between patients receiving fecal transplants from anonymous donors versus known relatives. Adverse events were noted in 3 trials in which nasogastric/nasojejunal tubes were placed. Upper gastrointestinal bleeding was noted in one patient who underwent nasogastric tube placement (MacConnachie, 2009). Peritonitis (Aas, 2003) and enteritis (Polák et al., 2011) were also found in patients having received FMT via upper gastrointestinal means, however, these modalities could not be implicated in the cause of such events. More research is necessary in determining the safest mode of administration of FMT.

The termination of clinical symptoms, chiefly diarrhea, is an important endpoint for the treatment of CDI; however, the restoration of the microbiome is essential in preventing recurrence of infection. The microbiome of CDI patients post-FMT has indeed been shown to resemble that of the donor feces (Seekatz et al., 2014). Figure 5 depicts the shared species between that of a patient pre- and post-FMT, the patient post-FMT with donor, and the patient pre-FMT with donor. Both methods of analysis show significantly improved similarities in bacterial species between the recipient post-FMT and the donor.

Fecal microbiota transplantation has supplanted antibiotic therapy as a safer and more clinically successful means of resolving clinical symptoms of recurrent CDI while restoring a healthy host microbiome. A proof of principle for FMT as an efficacious

treatment for CD exists in its utilization against burgeoning CDI. It is now the “gold standard” for treatment of CDI in cases where antibiotics, such as vancomycin and metronidazole, have proven unsuccessful.

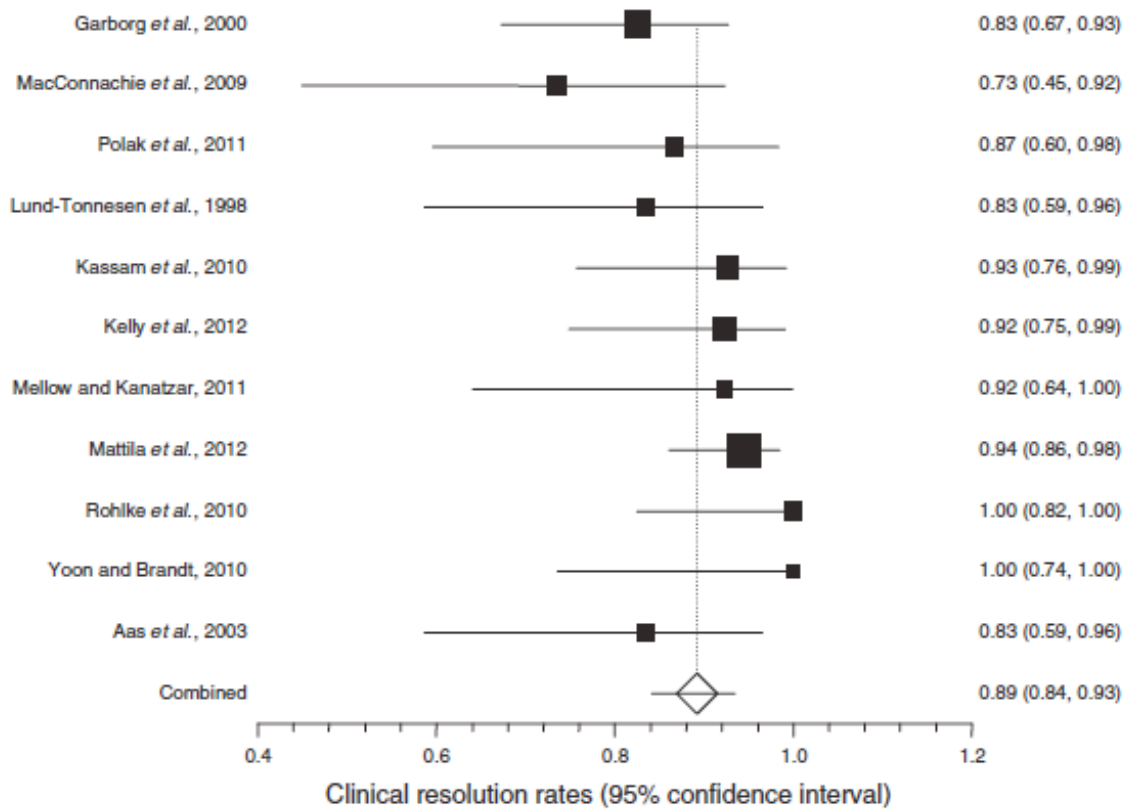


Figure 4: Results of meta-analysis of peer-reviewed trials using fecal microbiota transplantation to treat *Clostridium difficile* infection (Taken from Kassam *et al.*, 2013).

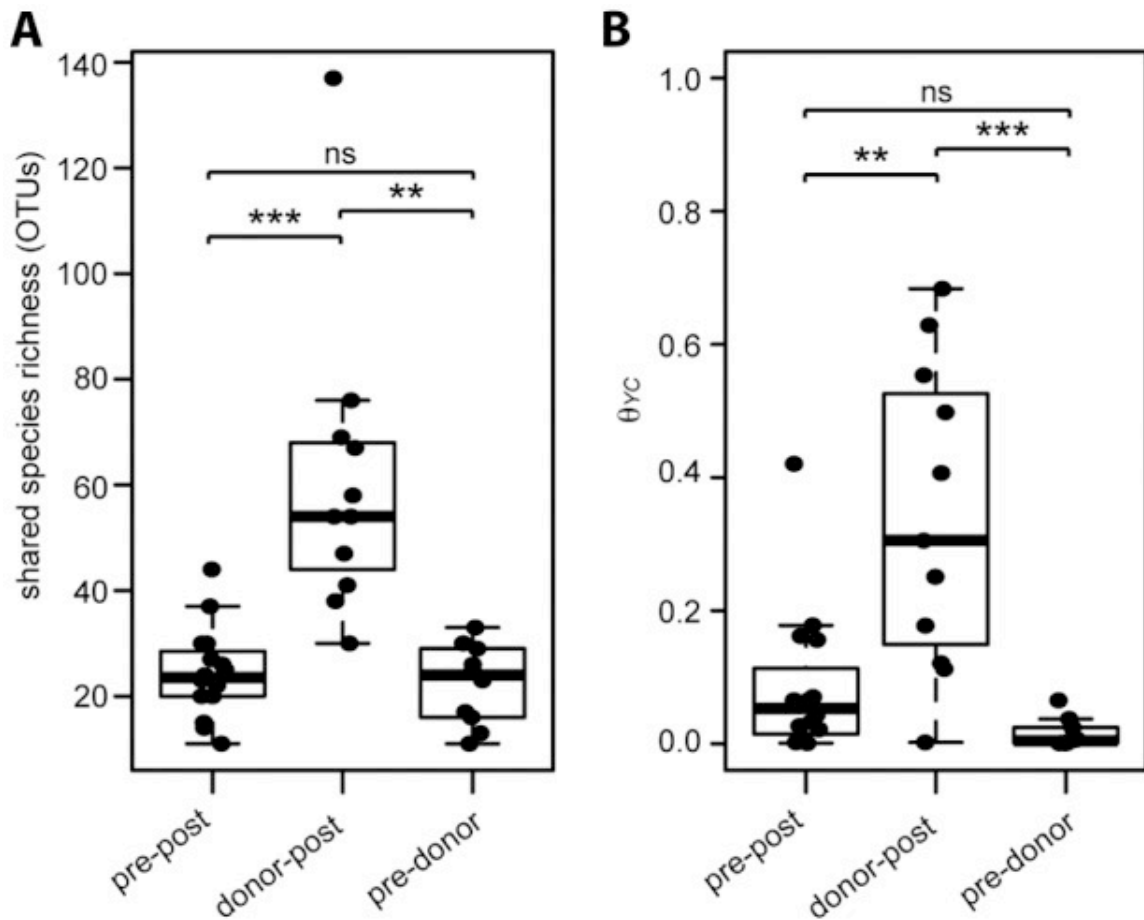


Figure 5: Microbiota species analysis of stool showing similarities between the patient post-FMT with donor as compared to the patient pre- and post-FMT as well as the patient pre-FMT with donor. Species richness determined by (A) operational taxonomic units (OTU's) and (B) the Yue-Clayton theta similarity (Taken from Seekatz et al., 2014).

Fecal Microbiota Transplantation and Crohn's Disease

Although the successes of FMT in treating CDI over the past decade are an exciting indicator of its potential value, there is very little literature on FMT for the treatment of Crohn's disease. There is a significant lack of randomized-controlled trials studying the efficacy of this treatment option. Cohort studies and case reports have found variable results in determining efficacy and present a range of limitations.

Case Studies

A case study from Borody et al. (1989) details the improvement of a patient after receiving FMT via retention enema. While the article indicates improvement of symptoms 4 months post-FMT without the need for additional medication, limitations on the study include unidentifiable parameters for clinical diagnosis and remission, mode and frequency of the administration, as well as stool preparation guidelines.

Another case report from the West Indies reports a 35-year old women presenting to a hospital with bloody diarrhea and abdominal cramps. Colonoscopy revealed Crohn's-like fistulae and chronic inflammation. Stool samples were positive for *Clostridium difficile* toxin A and B, and a diagnosis of Crohn's colitis complicated with CDI was made. The patient did not respond to oral vancomycin, metronidazole, mesalazine or steroidal treatment with prednisolone. FMT was administered via enema with only transient success for the first six days. Repeated FMT was administered when diarrhea returned with, still, only marginal success. A fourth FMT was administered via

nasoduodenal tube resulting in resolution of diarrhea and an increased appetite back to normal levels.

In yet another case report, a 26 year-old man presented to the emergency room on two occasions with a perianal fistula (Kao, 2014). Shortly after recovering with a regimen of antibiotics the man returned with a 1-month history of bloody diarrhea, weight loss and abdominal pain. Upon colonoscopy, chronic inflammation of the cecum and rectum were observed, and biopsies revealed moderate to severe Crohn's disease. After exhausting numerous treatment options including prednisone, mesalazine, and a combined antibiotic therapy of ciprofloxacin and metronidazole, symptoms persisted and fecal transplantation was opted for. The patient received FMT from an anonymous donor in a 400mL fecal suspension of 0.9% normal saline via colonoscopy. Figure 4 is a gross, anatomical view of the patient's cecum attained by colonoscopy pre- and post-FMT. Biopsies showed full recovery of mucosal integrity. Patient reported well-formed bowel movements and decrease in abdominal pain up to 4 weeks out from FMT. Calprotectin, an indication of intestinal inflammation, was reduced from 3490 μ g/g pre-FMT to a more normalized 189 μ g/g post-FMT.

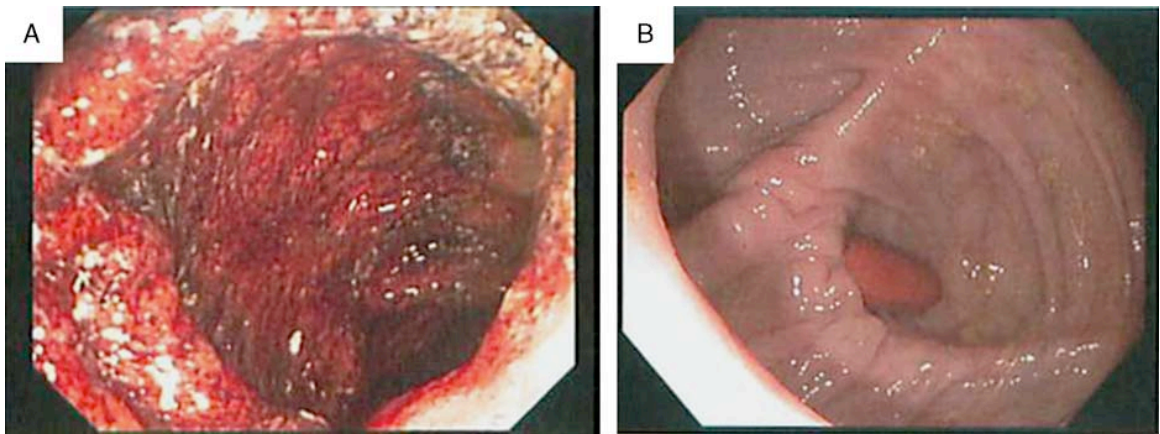


Figure 6: Cecal images before (A) and after (B) fecal microbiota transplantation (FMT) (Taken from Kao, 2014).

Cohort Studies

A cohort study of 49 patients with Crohn's disease who were refractory to all other treatment options used single FMT with an adjunctive therapy of mesalazine, a common anti-inflammatory drug that is localized to the gut (Cui et al., 2015). Endpoint goals of the study were a reduction in symptoms including diarrhea and abdominal pain, decrease in C-reactive protein, and change in Harvey-Bradshaw Index (HBI) of 3 with clinical remission indicated with HBI score less than 4. The HBI is a simplified version of the CDAI in which only clinical symptoms are determinants in the overall score and disease-state (Harvey & Bradshaw, 1980). These symptoms, including liquid bowel movements, abdominal pain, overall well-being, and bowel mass formation, are subject to gradation and scored. FMT was administered to the mid-gut of all patients via gastroscopy. Mesalazine regimen was started 2 weeks pre-FMT and maintained for 3 months post-FMT then tapered off. Donors were all relatives of patients and pre-screened. Nineteen patients were disregarded for analysis due to complications with *C. difficile*, undefined IBD, or inability to follow-up for 6 months. Table 4 displays the results of the study. Peak clinical improvement (86.7%) and clinical remission (76.7%) via the parameters stated above occurred at the 1-month mark. At 6 months, 20 of 30 (66.7%) patients still saw improved symptoms while only 18 of 30 (60%) had HBI scores of less than 4. As far out as 15 months post-FMT, 6 of 7 patients still had improved symptoms while 4 of 7 were in remission.

The results of this study show an initial positive efficacy of FMT in alleviating symptoms of CD. The decline in effectiveness could be attributed to the discontinuation of mesalazine following the 3-month follow-up. Despite the decrease in effectiveness this study provides a good look at the benefits of FMT. These patients were all unresponsive to traditional therapies including antibiotics, anti-inflammatories and steroids. Fecal transplantation to the mid-gut provided improvement of symptoms, if only transiently, to many of the patients in this study.

The latest prospective cohort study, conducted in January of 2015 at Seattle Children's Hospital, reported on the findings of 9 pediatric patients with Crohn's disease undergoing fecal microbiota transplantation (Suskind et al., 2015). Participants in the study ranged from 12 to 21-years old and all had pediatric CDAI (PCDAI) scores ranging from 10 to 29 indicating mild to moderate severity of disease (Turner et al., 2010). Of the 9 patients, all but one newly diagnosed individual were on treatments prior to FMT. These treatments included methotrexate (4 patients), azathioprine (1 patient), mesalazine (3 patients), and 5-mercaptopurine (1 patient). Study endpoints included clinical remission with a PCDAI score below 10 and decreased calprotectin and C-reactive protein (CRP) levels. In addition to clinical results, pre- and post-FMT microbiome was analyzed for similarity to donor stool samples. Results of this study can be found in Table 5. Clinical remission was observed at the 2-week follow-up in 7 of 9 patients. Mean baseline PCDAI score was 19.7, which dropped to 6.4 at the 2-week time-point and 8.6 at the 6-week time-point. Four patients required additional therapy before the study reached its endpoint. Two patients received standard treatment due to flare-ups.

FMT Results of Thirty CD Patients in Cohort Study

Table 2 Clinical response to FMT

Clinical response	Post-FMT	Clinical improvement (n)	Clinical remission (n)
Activity based HBI	1 week	83.3% (25/30)	60% (18/30)
	1 month	86.7% (26/30)	76.7% (23/30)
	3 month	80% (24/30)	70% (21/30)
	6 month	66.7% (20/30)	60% (18/30)
	9 month	57.1% (12/21)	52.3% (11/21)
	12 month	60% (9/15)	53.3% (8/15)
	15 month	85.7(6/7)	57.1(4/7)
Body weight (before vs post)	1 month	48.9 ± 10.1 vs 49.9 ± 10.2* (30)	/
	3 month	48.3 ± 9.9 vs 50.1 ± 11.3* (23)	/
	6 month	48.8 ± 9.9 vs 51.8 ± 10.9** (22)	/

Paired t-test, * $P < 0.05$, ** $P < 0.001$.

Table 3: Results of cohort study of 30 refractory CD patients receiving single FMT with adjunctive mesalazine regimen. Body weight (mean ± SD) (Taken from Cui et al., 2015).

Table 4: Results of 9 pediatric patients with CD undergoing FMT (Suskind et al., 2015)

Patient	Sample	PCDAI	CRP, mg/dL	Calprotectin, µg/g	Clinical Remission at 2 wk	Engraftment Score at 2 wk, %	Engraftment Type	Pre-FMT Similarity to Donor, %
1	Baseline	27.5	3.1	1380	Yes	10	Gradual	13
	2 wk	7.5	1.1	1287				
	6 wk	12.5	1.3	729				
	12 wk	10	<0.8	827				
2	Baseline	10	4.5	544	Yes	-15	Failure	50
	2 wk	2.5	1.7	353				
	6 wk	2.5	3.4	718				
	12 wk	10	7.4	791				
6	Baseline	15	<0.8	<16	No	41	Immediate	37
	2 wk	10	<0.8	<16				
	6 wk	10	<0.8	<16				
	12 wk	5	<0.8	<16				
7	Baseline	22.5	1.4	23	Yes	46	Immediate	42
	2 wk	5	<0.8	<16				
	6 wk	5	<0.8	<16				
	12 wk	2.5	1.5	<16				
8	Baseline	12.5	1.9	1456	Yes	11	Gradual	55
	2 wk	2.5	1.6	899				
	6 wk	2.5	2.3	672				
	12 wk	7.5	1.1	1186				
10	Baseline	27.5	4	1074	Yes	12	Gradual	32
	2 wk	2.5	2.7	799				
	6 wk	7.5	2.4	1349				
	12 wk	20	2.3	1320				
13	Baseline	22.5	2	596	Yes	14	Gradual	46
	2 wk	2.5	1.5	532				
	6 wk	15	3.9	1318				
	12 wk ^a	2.5 ^a	<0.8 ^a	434 ^a				
15	Baseline	12.5	1.5	>2500	Yes	22	Immediate	31
	2 wk	2.5	1.7	1231				
	6 wk ^a	7.5 ^a	1 ^a	>2500 ^a				
	12 wk ^a	0 ^a	<0.8 ^a	22 ^a				
18	Baseline	27.5	2.6	839	No	0	Failure	69
	2 wk	22.5	1.5	906				
	6 wk	15	1.1	1224				
	12 wk	22.5	2.1	1172				

One patient was placed on metronidazole and infliximab after the 6-week follow-up while yet another patient was started on methotrexate and prednisone. Mean CRP levels decreased from 2.4mg/dL to 1.5mg/dL at the 2-week endpoint and remained below baseline levels in 5 of 9 patients. Calprotectin levels saw an initial decline but rose to baseline levels by the end of the study in almost all 9 patients.

The results of the microbiome analysis showed immediate engraftment (measurement of post-FMT microbiome population similarity to donor) in 3 participants, gradual engraftment in 4 participants, and no engraftment in 2 participants. Patient 2 is an interesting case in that he achieved clinical remission by week 2 only to see his PCDAI score increase to baseline level by studies end. This patient was 1 of 2 who did not achieve donor engraftment. Patient 18, the other individual who did not achieve engraftment, never achieved clinical remission, and while her baseline PCDAI score improved over the 12-week study period she had the highest severity of disease at the beginning of the study. Furthermore, the individual with the greatest microbiome similarity to donor pre-FMT, patient 18, did not display a positive clinical course throughout and needed additional therapeutic intervention. This is in contrast to patient 1 who had the most dissimilar microbiome to donor. This patient's PCDAI dropped from 27.5 to 7.5 by week 2 before rising to 10 by the end of the study. This patient's CRP and calprotectin levels also dropped significantly throughout. This indicates a possible correlation between diversity of patient/donor microbiome and clinical outcome. Figure 5 represents 31 microbial species that were detected at greater than 0.1% abundance in patients post-FMT that were at less than 0.001% abundance pre-FMT. Notable bacterial

species that saw an uptick in abundance in patients with a positive clinical course in this study were of the genus *Bifidobacterium* and *Bacteroides* of the phylum *Actinobacteria* and *Bacteroidetes*, respectively. As stated above, these bacteria have been known to confer beneficial attributes to the gut.

The limited availability and reliability of studies reporting on FMT in a CD population lends caution to these results. Further research investigating the potential benefits of FMT for CD patients is needed in order to draw conclusions about its possible efficacy and safety.

Microbial Species Abundance Pre- and Post-FMT

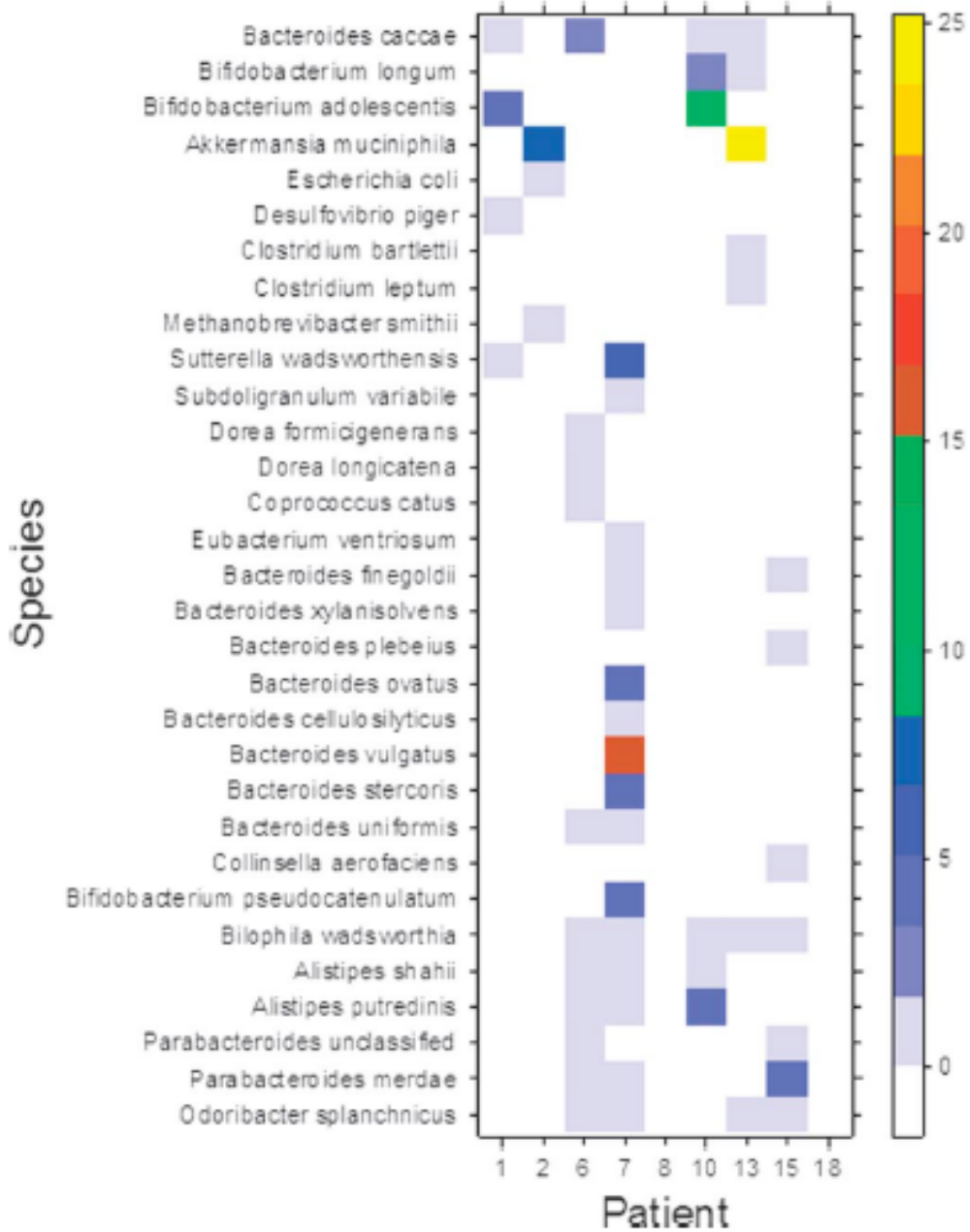


Figure 7: Thirty-one microbial species that were present at less than 0.001% in recipients pre-FMT that were found to be present at greater than 0.1% in recipients post-FMT (Taken from Suskind et al., 2015).

DISCUSSION

Crohn's disease presents difficulty for clinicians and researchers due to its unknown etiology and interplay between immunological and microbial inhabitants of the gut. A clear understanding of the principles and mechanisms underlying its manifestation are currently being researched and elucidated. Current management of CD targets the resolution of symptoms and the underlying mechanisms of the inflammatory response. Symptoms of CD overlap with other inflammatory disorders and infections of the gut including ulcerative colitis and CDI. In this regard, it has been commonplace to use similar treatment strategies in order to attain clinical remission of disease. Recent findings of genomic sequencing and genome-wide analysis studies have allowed for the cataloging of potential risk loci for CD. Although 31 of these have been noted as specific to CD, there are currently 109 other loci that are described as universal risk factors for IBD, particularly CD and UC (Jostins et al., 2012). Furthermore, other inflammatory disorders including psoriasis and ankylosing spondylitis have been implicated as having similar risk loci. These loci can code for mediators of the inflammatory response that can affect a wide range of organ systems. This identifies the phylogenic repercussions of a disrupted inflammatory response gene as a major detriment to almost all CD patients.

The popularity of TNF- α inhibitors in recent years is evidence of the need to suppress mediators of the inflammatory response in order to alleviate symptomatic CD. Although the meta-analysis by Stidham *et al.* above produced favorable results to standard-care controls, remission and response rates of individual RCTs were disappointing towards the ultimate goal of such therapies. The study that produced the

best results in terms of overall clinical remission and response rates was only able to induce remission in 35.5% and clinical response (reduction of CDAI score by 100) in 50% of patients (Hanauer et al., 2006). Also, all 10 of the RCTs evaluated in the meta-analysis utilized anti-TNF- α agents adjunctively with baseline medication regimens. They all differed in some regard but consisted of a combination therapy of immunosuppressants and antibiotics with only one trial administering additional probiotics (Sandborn et al., 2011). This lends to the thought that the antigenic stimulus of inflammation is being insufficiently cleared in the gut by the antibiotic regimen administered. Complications with anti-TNF- α agents have been documented in patients that require surgery (Zaghiyan, 2015). Reports have indicated higher post-operative infection rates in patients using infliximab. Also, there have been reports of patients gaining infliximab resistance over time (Nagata et al., 2015).

The ever-growing research dedicated to the classification of the microbiome suggests an environmental risk factor involved with CD. The dysbiotic environment usually decreases the number of beneficial bacteria in the gut allowing for the penetration of otherwise normal, commensal bacteria across intestinal epithelial cells. This event in genetically susceptible individuals can manifest chronic inflammation. While dysbiosis is a hallmark of CD and CDI alike, the mechanism by which each present symptoms are different. CDI disrupts the integrity of the epithelial cells of the gut via production and secretion of toxins, leading to its most prevalent symptom, diarrhea. In CD, however, unbalance of beneficial and harmful bacteria illicit unpredictable results, which in turn present with a myriad of symptoms from mild to a severe disease state. It makes sense,

therefore, that a treatment regimen dedicated not only to suppressing the inflammatory response but also bettering the overall well being of the microbiome be investigated.

Antibiotic and probiotic trials in CD patients have proven insufficient in resolving chronic inflammation. Antibiotics function to eliminate harmful bacterial species that could be stimulating an inflammatory response. They also have the capacity to elicit their antimicrobial effects on beneficial microbes of the host gut. In doing so, antibiotics can provide space for more harmful and pathogenic bacteria to colonize within the niche of the microbiota, which is often the case in CDI. Fecal microbiota transplantation provides an alternative method for re-establishing a healthy bacterial population within the gut. FMT is advantageous in achieving this goal because of its mechanism of infiltrating and engrafting within the recipient without providing opportunity for harmful bacteria to colonize. Probiotic use typically consists of the ingestion of large amounts of a single bacterial species that can be helpful to the gut microbiota. In this regard, the benefit of a probiotic supplement could be singular depending on its activity. Figure 3 showed the ability of some *lactobacilli* probiotic strains to modulate cytokine secretion, however, many strains of the same phyla were unsuccessful in inhibiting TNF- α secretion (Presti et al., 2015). Finding the appropriate bacterial strain to confer a wide range of healthy benefits for the host has been challenging. Moreover, probiotics must be ingested in large amounts daily in order to be effective in preserving a healthy gut, and there is a serious lack of evidence that suggests curative properties of probiotics (Parra, 2004). Successful FMT would be administered once, colonize the gut, and engraft a population of diverse, healthy gut flora that can provide all encompassing benefits.

The published literature on experiences with fecal microbiota transplantation for Crohn's disease is extremely limited. To date, 2 cohort studies and 3 case studies have been published. Suskind *et al.* were able to show a beneficial clinical course in 7 of 9 pediatric patients. FMT was well tolerated in the study and further provided evidence for the potential safety and efficacy of this treatment option. This study was not without limitations, however. As a prospective study with no placebo controlled group the possibility for participant bias is a factor. An aspect of the PCDAI score is subjective in nature, which could lead to bias on the part of the patient. Additionally, the small size of the study cannot allow for an accurate conclusion to be made from the results of FMT in a CD population. The cohort study by Cui *et al.* also demonstrated FMT as a positive therapeutic strategy in the treatment of a CD population of 30 individuals. Although clinical remission decreased within the cohort over time, more than 50% of patients who reported for the 15-month follow-up were in clinical remission without the need for further medication. One goal of the study was to determine the safety of the mid-gut administration of FMT via nasojejunal tube. No severe adverse events were recorded due to use of a gastroscope or endoscopy. However, this precluded the notion of administering FMT to the location of the gut most affected by CD in each individual patient. By this method, targeting the area of the gut most susceptible to chronic inflammation was not necessarily achieved. Again, this cohort study is subject to participation bias as all participants were knowingly receiving FMT, which could have had an effect on their HBI score. Long-term follow-up of patients receiving FMTs is also necessary in order to ascertain the effects of such a treatment on vital processes of the gut

including nutrient absorption and digestion. Safety regulation agencies are also concerned with the lack of long-term studies in determining the overall safety of the treatment. Despite these limitations, FMT has still been shown to be a safe and effective short-term treatment for CD patients and warrants further investigation.

There are several issues that have hampered the advancement of FMT research and clinical application. Firstly, there is no standard protocol for the handling and administration of human feces. To date, hospitals rely on in-house Institutional Review Boards (IRBs) to define and standardize their own protocols for the administration of FMT (Vyas, 2013). This includes standardizing donor-screening parameters as well as determining potential harms in transplanting possible infectious agents. More importantly, in 2012 The US Food and Drug Association (FDA) classified human feces as a biologic therapy and not human tissue, therefore requiring clinicians to file for an independent new drug (IND) application in order to treat patients (except for CDI) (Ratner, 2014). This was met with discontent amongst the patient and clinician-investigator community as the timeline for approval of an IND can take up to 1 month. The FDA asserts that due to the unknown long-term effects of such a treatment the IND application allows for a certain level of control over who receives an FMT. The re-classification of human feces from a drug to an organ would assist in the development of stool banks. Stool banks, such as OpenBiome in Cambridge, Massachusetts, would have the ability to provide donor-screened feces for transplantation to patients (Smith, 2014).

The promising results from trials utilizing FMT in the treatment of *Clostridium difficile* provides an exciting avenue to pursue for IBD researchers and clinicians.

Randomized-controlled trials are necessary in determining the efficacy of fecal microbiota transplantation as a suitable means for treating Crohn's disease. Preliminary data from limited resources suggest a beneficial approach to re-establishing a homeostatic, healthy environment within the gut of patients with CD. Additionally, the successful trials of anti-inflammatory medications, including TNF- α and alpha-4 integrin inhibitors, provides a possible adjunctive therapy to FMT in order to inhibit the inflammatory response. Targeting the microbiome as well as the immune-regulatory mechanisms within the gut has the potential to yield never before seen clinical remission and response rates within the CD population. Drug regimens and fecal transplantation may not be enough, however, as there is substantial evidence for the role of one's diet in sustaining a healthy microbiota. It is imperative that particular diet regimens be implemented and followed in order to maintain the positive effects that could result from any such treatment.

FUTURE STUDIES

The need for RCTs for the use of FMT to treat CD is evident. These trials have the capacity to elucidate preferential treatment methods, proper preparation dosages, standardized donor-screening techniques, and potential life-threatening side effects. Studies using placebo-controlled and standard care-controlled groups will have the ability to truly determine the advantages of using FMT. Furthermore, they can shed light on microbe-host interactions that can further help to understand the underlying mechanisms that induce chronic inflammation in CD patients. Genome-wide association studies continue to discover the genetic loci by which mutagenic activity can lead to induction of disease. Metagenomic sequencing will further our understanding of the microbes that inhabit our gut and their role in disease. The idea that Crohn's disease manifests itself differently within each patient suggests a need for personalized, patient specific treatment options. By understanding the mechanistic dysfunction in each patient, appropriate drugs and medications could be administered on a patient-to-patient basis. Suskind et al. alluded to the idea that recipient-host microbiome dissimilarity could correlate to positive FMT results and achieve clinical remission. In this regard, metagenomic analysis of patient and donor microbiomes could help to decide the best possible donor for each individual.

Drug development for IBD in other fields of study is extremely important also. Currently, studies investigating siRNA carrying nanoparticles and their ability to silence gene transcripts at the point of inflammation are underway (Nielsen et al., 2012).

CONCLUSION

Standard treatment of CD with immunosuppressants, steroids and antibiotics achieve clinical remission in only two-thirds of patients. Anti-inflammatory drugs such as infliximab and natalizumab have outperformed standard therapy in randomized trials but still fall short of desired remission rates. Complications involving infection and resistance have also been reported with these drugs. Fecal microbiota transplantation is now the “gold standard” treatment for patients with CDI who are refractory to antibiotics. Preliminary data from studies using FMT for the treatment of CD are promising but extremely limited. Further investigation of this modality is required in order to determine its possible efficacy for Crohn’s disease.

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CURRICULUM VITAE

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Education:

09/2013-Present Boston University, Boston, MA
Master of Science, Medical Sciences

09/2011-03/2012 Northeastern University, Boston, MA
College of Professional Studies

09/2004-05/2008 Marist College, Poughkeepsie, NY
Bachelor of Science, Biomedical Sciences
Minor: Mathematics

Professional:

8/2010-8/2013 Transplantation Biology Research Center
Massachusetts General Hospital, Boston MA
Research Technician I

02/2007-05/2007 St. Francis Hospital Poughkeepsie, NY
Internship, Emergency Room Shadowing

Honors/Awards:

2004-2008 Marist Collegiate Scholarship

Abstracts and Presentations (* denotes presenting author):

- 1) Leonard DA, Horner B, Randolph MA, Duran-Struuck R, Crepeau R, Matar A, **Mallard C**, Sach DH, *Cetrulo Jr. CL, Huang CA. *Induction of Vascularized Skin Tolerance by Establishment of Stable Mixed Chimerism across a Major Histocompatibility Barrier in Miniature Swine* (American Transplant Congress, 06/2012 Boston Massachusetts, USA)

- 2) *Albritton A, Leonard DA, Leto Barone AA, Torabi R, **Mallard C**, Kurtz JM, Sachs DH, Cetrulo, Jr CL. *Lack of Cross-sensitization Between α -1,3-galactosyltransferase Knockout Porcine and Allogeneic Skin Grafts Permits Serial Grafting* (American Transplant Congress, 06/2012 Boston Massachusetts, USA)
- 3) Leonard DA, Horner B, Randolph MA, Duran-Struuck R, Crepeau R, Matar A, Albritton A, **Mallard C**, Kurtz JM, Sachs DH, Huang CA, *Cetrulo Jr. CL. *Induction of Skin Tolerance by Establishment of Stable Mixed Chimerism across a Major Histocompatibility Barrier in Miniature Swine* (Plastic Surgery Research Council, 06/2012 Ann Arbor Michigan, USA)
- 4) Leonard DA, Albritton A, Leto Barone AA, Torabi R, **Mallard C**, Kurtz JM, Sachs DH, *Cetrulo, Jr. CL. *Lack of Cross-sensitization Between α -1,3-galactosyltransferase Knockout Porcine and Allogeneic Skin Grafts Permits Serial Grafting and Extended Temporary Coverage of Simulated Burn Wounds* (Plastic Surgery Research Council, 06/2012 Ann Arbor Michigan, USA)
- 5) Leonard DA, Torabi R, **Mallard C**, Albritton A, Randolph MA, Kurtz JM, Sachs DH, Cetrulo, Jr. CL. *Prolonged Vascularized Composite Allograft Survival and Multi-lineage Mixed Chimerism across a Full Major Histocompatibility Barrier in Swine* (Plastic Surgery Research Council, 06/2012 Ann Arbor Michigan, USA)
- 6) Torabi R, Leonard DA, Leto Barone AA, **Mallard C**, Randolph MA, Sachs DH, Yamada K, Cetrulo Jr. CL. *Renal Grafts and Vascularized Composite Allografts Pose Different Barriers to Tolerance Induction* (Plastic Surgery Research Council, 06/2012 Ann Arbor Michigan, USA)
- 7) Leonard DA, Torabi R, **Mallard C**, Albritton A, Randolph MA, Kurtz JM, Sachs DH, Cetrulo, Jr. CL. *Prolonged Vascularized Composite Allograft Survival and Multi-lineage Mixed Chimerism across a Full Major Histocompatibility Barrier in Swine* (American Association of Plastic Surgeons, 04/2012 San Francisco California, USA)
- 8) Leonard DA, Horner B, Randolph MA, Duran-Struuck R, Crepeau R, Matar A, Albritton A, ***Mallard C**, Kurtz JM, Sachs DH, Huang CA, Cetrulo Jr. CL. *Induction of Skin Tolerance by Establishment of Stable Mixed Chimerism across a Major Histocompatibility Barrier in Miniature Swine* (Scientific Advisory Committee, 04/2012 Boston Massachusetts, USA)
- 9) *Leto Barone AA, Torabi R, Randolph MA, Leonard D, **Mallard C**, Albritton A, Duran-Struuck R, Matar A, Crepeau R, Tang Y, Scalea JR, Wang Z, Tena A, Hawley RJ, Kurtz, MJ, Huang CA, Sachs DH, Cetrulo CL, Jr. *Vascularized Composite Allograft Transplantation and Mixed Hematopoietic Chimerism Across a Full MHC Barrier in Swine: Preliminary Results* (European Plastic Surgery Research Council, 08/2011 Hamburg, Germany)
- 10) *Leto Barone AA, Torabi R, Leonard DA, Weiner J, Albritton A, **Mallard C**, Tang Y, Hanekamp JS, Yamada K, Kurtz JM, Sachs DH, Cetrulo CL Jr. *Xenogeneic (GalT-KO) Skin followed by Allogeneic Skin as a Potential Strategy for Temporary Coverage of*

Severely Burned Patients (European Plastic Surgery Research Council, 08/2011 Hamburg, Germany)

- 11) *Leto Barone AA, Torabi R, Randolph MA, Duran-Struuck R, Hawley RJ, Horner BM, Matar A, Crepeau R, Albritton A, **Mallard C**, Tang Y, Tena A, Scalea JR, Kurtz J, Huang CA, Sachs DH, Cetrulo Jr. CL. *Composite Tissue Allotransplantation in Swine Across a Full MHC Barrier Using a Mixed Hematopoietic Chimerism Protocol* (American Association of Plastic Surgeons, 04/2011 Boca Raton Florida, USA)
- 12) Leto Barone AA, Torabi R, Weiner J, Albritton A, **Mallard C**, Tang Y, Hanekamp JS, Smith RN, Yamada K, Kurtz JM, Sachs DH, Cetrulo Jr. CL. *Absence of Cross-Sensitization Between GalT-KO Skin and Allogeneic Skin May Allow Prolonged Temporary Coverage of Severely Burned Patients* (New England Society of Plastic Surgeons, 06/2011 Brewster Massachusetts, USA)
- 13) *Leto Barone AA, Torabi R, Weiner J, Albritton A, **Mallard C**, Tang Y, Hanekamp JS, Smith RN, Yamada K, Kurtz JM, Sachs DH, Cetrulo Jr. CL. *Absence of Cross-Sensitization Between GalT-KO Skin and Allogeneic Skin May Allow Prolonged Temporary Coverage of Severely Burned Patients* (Plastic Surgery Research Council, 04/2011 Louisville Kentucky, USA)

Research Experience:

Transplantation Biology Research Center (TBRC)

Research Technician I

08/2010-08/2013

Involved in multiple projects investigating the potential for skin tolerance across xenogeneic and allogeneic barriers in a large animal model. Responsibilities include:

- Assisting in surgeries/procedures comprised of:
 - Vascularized Composite Allograft transplantation
 - Split-thickness skin grafting
 - Central line placements
 - Thymus and bone marrow biopsies
 - Bone Marrow Harvest
 - Leukapheresis
- Immediate pre and post-operative care for transplant recipients and donors
 - Administering immunosuppressive therapies, antibiotics, and analgesics
 - Daily maintenance and care of grafts and wounds
- Performing in vitro assays for monitoring pre- and post-operative cellular responses including
 - Mixed Lymphocyte Reaction
 - Cell-Mediated Lympholysis

Teaching/Mentorship

- Fall 2014 Teaching Assistant
- Biochemistry/Cell biology course in the Division of Graduate Medical Sciences
- Summer 2009-2010 Personal Academic Tutor
- Subjects included Pre-Calculus, Physics, Biology, and Chemistry

Volunteer Experience

- 12/2013-3/2014 Outreach Van Project, Boston University
- Provided clothing and food for less fortunate of East Boston
- 09/2006-05/2007 Harriet Tubman Academic Skills Program
- After-school mentoring program for students in grades 1-5