Influence of Chemotherapy on the Microbiome and Immunological Functioning of the Gut

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Abstract

In addition to the billions of human cells comprising the human body, parts of our bodies are actually home to large communities of microorganisms, known as microbiomes. The organisms present in these communities include bacteria, yeasts, protozoa, viruses and more. Unique microbiomes colonize the skin, the oral cavity, the gastrointestinal tract, and the genitourinary tract. The microorganisms usually are commensal or mutualistic bacteria that maintain homeostasis in our organ systems, prevent infection, and aid in necessary biological functions within the body - such as digestion.

The gut microbiome is influenced by many factors including: age, diet, lifestyle, environment, and intake of medications. Disturbances in the community of microorganisms within the gut (defined herein as referring to the intestines) has ramifications throughout the body and involves multiple other organ systems, including the digestive system, nervous system, and immune system. Chronic diseases may cause or develop due to microbial dysbiosis originating in the gut.

Chemotherapeutics are known to induce microbial gut dysbiosis and cause serious side effects, including intestinal mucositis–a painful, ulcerative inflammation of the intestinal epithelium. As a summer research intern in the laboratory of Dr. Nissan Yissachar, Bar Ilan University, I was involved in isolating bacteria from fecal samples of breast cancer patients, before and after chemotherapy treatment. These isolated bacteria will be used in an transepithelial electrical resistance (TEER) assay, to determine their influence on the epithelial lining of the gut. The goal is to identify those bacteria that colonize the gut subsequent to chemotherapy and that strengthen

the epithelial lining of the gut. The overall intent of this research is to utilize these bacteria to prevent dysbiosis-induced side effects, including chemotherapy-induced mucositis. The laboratory requested that, when authoring this Senior Project, I limit my description of the research and its findings.

I. Introduction

History of the gut and microbiome illness and treatment

Long before antibiotics, colonoscopies, and camera endoscopy capsules of our time, people were fascinated by the pathologies and inner workings of the gut. In ancient Egypt and Greece, the gut was seen as a host to dangerous "residues" that were presumed to be the cause of all disease (Brown, 2021). Though we now know this to be untrue, by examining the ancient perceptions of gut health and understanding their treatments, specifically ancient Jewish perceptions, we may be able to gain greater insight into the development of gastrointestinal health care and where the future of innovation is directed.

In the times of the Talmud, there were several different methods that were seemingly employed as therapeutic and diagnostic tools in assessing the gut. In the *Mishnah Shekalim* (4:2), Rabbi Natan suggested that Moshe sequestered himself in a cloud for six days to purge his body of all food and drink, so that he may be like the angels. While the context is not seemingly of medical importance, the mere mention of such a procedure implies the practice of "colon purging" was known to the rabbis of the Talmud. It is likely that this procedure was used as a form of treatment or prevention of disease, much like that practiced by the ancient Egyptians and Greeks. Today, physicians caution against such a procedure, for its false claims and potential to cause serious side effects, ranging from dehydration to bowel perforation. However, the common practice of this procedure indicates that people of that era knew that the fecal content of the intestines was critical to the development and functioning of the gut (Brown, 2021).

Pushing the same point, in the Talmud *Nedarim* (50b), Shmuel describes the *turmita* egg, a unique diagnostic tool that was used frequently in cases of gastrointestinal pathologies, which was tedious to prepare. The egg was shrunken using a series of hot then cold water baths until it could be swallowed whole. The residues on the egg were then examined by a doctor post excretion. Upon examination, the doctor could supposedly determine the type of medicine and treatment that the patient needed. The *turmita* egg is comparable to the diagnostic tools used today, such as analyses of stool samples or possibly gastrointestinal probes of today, like the capsule endoscopy (Brown, 2015).

The Pillcam is a capsule endoscopy that was invented in Israel. It is a small capsule with several cameras that, when swallowed, can capture high-quality imaging of the entirety of the intestines (Brown, 2015). This capsule endoscope allows for imaging of middle portions of the intestines that colonoscopies and endoscopies cannot reach. The images are wirelessly transmitted to a receiver which can then be viewed by a doctor. This device is incredibly useful for the diagnosis of certain conditions. However, in certain cases, the use of the capsule would have to be followed up with a colonoscopy or endoscopy and simultaneous biopsy for treatment (Wikipedia, 2022).

Much like today, however, the causes and treatments for certain gut pathologies remain unknown. The Talmud *Avoda Zara* (40b) mentioned that Rabbi Yehuda Hanasi suffered from a bowel disease. In Talmud *Bava Batra (*103b) there is a discussion about how the great extent of the pain he experienced was often accompanied by loud screams. Dvorjetski, in her paper on the ailments of Rabbi Yehuda Hanasi, suggests that Rabbi Yehuda likely had inflammatory bowel disease (IBD), a class of diseases associated with painful inflammation of the intestines. Much like today's patients with IBD, Rabbi Yehuda Hanasi

was careful about his diet, because he felt that it played a role in his illness. He scheduled his meals, making sure that if he ate in the day, then he would not eat at night (*Mishnah Pesachim* 9:1). He also always made sure to include cucumber, radish, and lettuce into his meals, as he believed they aided in digestion (*Avoda Zara* 11a). Rabbi Yehuda Hanasi also seemed to have trusted in certain homeopathic remedies, such as apple cider, as suggested to him by Rabbi Ishamel, son of Rabbi Jose (*Avoda Zara* 40b), to help in the temporary alleviation of his gastrological pains (Dvorjetski, 2002).

Despite the many advances in our understanding of the gut and the development of treatments, there is still so much that we do not know. This is particularly true of our knowledge of the gut microbiome and the role it has on the well-being and well-functioning of the gut. Today's patients with IBD often suffer a similar fate as Rabbi Yehuda Hanasi, being forced to turn to homeopathic remedies and diet changes, when modern medicine, such as anti-inflammatory medications, cannot provide significant relief to their symptoms (Cleveland Clinic, 2021). Many scientists believe that a possible key factor in the development of this disease has to do with the microorganisms that inhabit the intestines (Loh & Blaut, 2012). Gaining a greater understanding of the microbiome's role in the gut, as well as in gut-related diseases, is critical for developing future treatments of gastrointestinal diseases.

By examining the past through a Talmudic lens, we can not only see how much our understanding and treatment of gastrointestinal diseases have advanced, but also what has remained the same, and where we must look to advance. Despite the long history of gut diagnostics and treatments, there is still a lot that is still unknown about the gut -- specifically, as it relates to the gut microbiome.

II. Background

Layers of the gut and overview of the digestive system

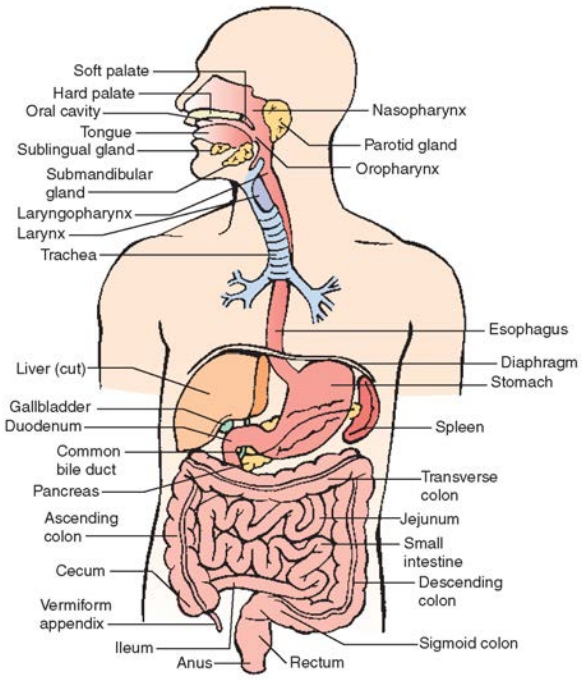

Figure 1: Diagram of the Digestive system (retrieved from the internet, source unknown).

The digestive system (Figure 1), the organ pathway that starts at the oral cavity and ends at the anus, is responsible for the breakdown of food, absorption of nutrients and water, and excretion of wastes. In the oral cavity, food is broken down mechanically with the teeth. The secretion of saliva introduces enzymes that begin the chemical digestion process and help lubricate the food so that it passes easily through the pharynx and esophagus. The food is pushed, with the help of the tongue, to the pharynx (back of the mouth) and then down into the esophagus. Peristalsis muscle contractions push the food through the esophagus, past the sphincter, and into the stomach (Reece & Campbell, 2016).

The stomach is the site of temporary food and fluid storage, as well as the site where gastric secretions are churned with the incoming food and fluid, to allow for further chemical breakdown, forming a mixture called chyme. Because of the acidic environment of the stomach, very few bacteria inhabit that organ. Peristalsis of the stomach muscles slowly

pushes the chyme through the second sphincter into the initial portion of the small intestine (Reece & Campbell, 2016).

The small intestine is a long tubular organ, with many folds, functioning as the main site of the chemical digestion and nutrient absorption. The small intestine is divided into three sections: the duodenum, the jejunum, and the ileum. The small intestine has finger-like projections, called villi, which increase the surface area and allow for enhanced absorption. The microvilli, microscopic projections from the surface of the cell membranes of the epithelial cells of villi, further increase the surface area of the small intestines. In the duodenal portion of the small intestine, chyme mixes with secretions from the liver, pancreas, and gallbladder to allow for breakdown of macromolecules. Peristalsis pushes the chyme through the remaining portions of the small intestine, first the jejunum and then the ileum. The chyme is propelled into the large intestine, which is a long, winding tube (Reece & Campbell, 2016).

The large intestine is divided into three sections: the cecum, colon, and rectum. The cecum, a bulging structure adjacent to the junction of the small and large intestine, is responsible for the fermentation of foods. The colon portion is further divided into three sections which include the ascending colon, the transverse colon, and the descending colon, the last of which leads into the rectum and anus (Reece & Campbell, 2016). The colon is responsible for the reabsorption of water into the extracellular fluid, and the solidification and excretion of the fecal waste. When the feces reach the final portion of the large intestines, known as the rectum, it triggers a reflex that causes the feces to be expelled through the anus (Unglaub Silverthorn, 2006).

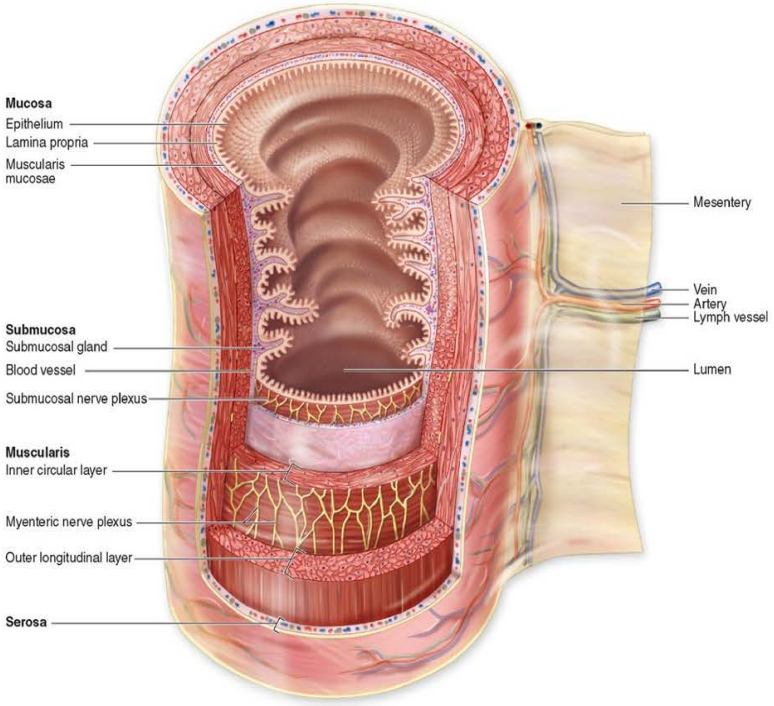

Figure 2: Labeled Diagram of the layers of the gut (here a section of the small intestine) (Mescher & Junqueira, 2010).

The gut refers to both the small and large intestines, which stretch from the duodenum to the anus. However, this paper will concentrate on the large intestine - as it was the focus of my research and is the home to the majority of bacteria in the body. The gut is a complex layered system (Figure 2), in which each layer has cells that carry out specific and different functions. I will focus on the three most central layers, as they most directly pertain to my research. At the center of the gut is the lumen, through which the chyme passes in the process of chemical digestion and excretion. The lumen is home to the gut's microbiome, which is discussed later in depth. Surrounding the lumen is the epithelium. Both in the small and large intestines the epithelial cells are constantly shed and replaced by cells that rise up from the crypts of Lieberkühn, in which multipotent stem cells generate the different types of epithelial cells, which perform various tasks, such as neuroendocrine functions, immunomodulating, and digestion (Mowat & Agace, 2014). Epithelial cells are held together by tight junctions, which limit the permeability of substances between cells and through tissue layers, and thereby serves as a

protective barrier from dangerous macromolecules and microorganisms (Ugalde-Silva *et al*., 2016).

Coating the inside of the lumen of the large intestine, above the epithelium, are two layers of mucus (Mowat & Agace, 2014). While the outer mucus layer provides a symbiotic environment for bacteria to live in, the inner-layer, under healthy conditions, does not contain bacteria (Johansson *et al*., 2011). These mucus layers prevent the penetration of bacteria into the epithelial layer by forming a physical and chemical barrier. The mucous layers are composed of mucin glycoproteins, substances toxic to many bacteria. The layers allow for the adhesion of antibodies and antimicrobial peptides, which regulate the bacterial populations within the lumen, using both specific and nonspecific immune defenses.

Beneath the epithelium lies the lamina propria, a layer of connective tissue, that houses blood, lymph and nervous networks (Mowat & Agace, 2014). If bacteria perforate the epithelial tissue, the consequences could be severe, due to the many connective networks located within the lamina propria. For example, there have been instances in which the invasion of bacteria, such as *Enterococcus gallinarum*, caused the development of hepatic pathologies, as well as disorders of the immune system. Additionally, the gut is connected to a series of other organ systems, and therefore, changes to the gut microbiome can affect the brain via the gut-brain axis. While these instances did not involve the transmission of viable bacteria to the brain, neurotransmitters, microbial metabolites, and other signals from the gut-brain axis were found to hinder microglia development in the central nervous system, and also potentially alter the nervous-immune response. Moreover, research has shown that the breach of bacteria into the epithelial surface of the gut puts individuals at greater risk for colon cancer, as well as for intestinal diseases, such as colitis (Mowat & Agace, 2014).

Microbiomes and their importance to the human body

The gut microbiome is but one of several microbiomes, each within its own particular niche in the body and its own specific function. In addition to the gut, microbiomes are found on the skin, oral cavity, and uro-genital tract. As these microbiomes have many commonalities with the gut microbiome, a discussion of a microbiome in general has applicability to that of the gut microbiome. The inhabitants of these microbiotic communities include eubacteria, archaea bacteria, fungi, yeast, protozoa, and viruses. However, each microbiome has its own unique microbiota (Matijašić e*t al*, 2020). Scientists estimated that the ratio of bacterial cells to human cells is approximately 1:1. This magnitude of the microbiome strongly suggests that such a large population of microorganisms is implicated in the overall health of the human body (Sender *et al*., 2016).

Perhaps, the main function of all microbiomes is to maintain homeostasis, thereby preventing disease and infection. For instance, the vaginal microbiome helps to maintain pH and prevent disease (Neugent, *et al*., 2020). For the skin, commensal microbes prevent infection and diseases through microbial competition, as well as in the production of antimicrobial products. These microbial activities on the skin essentially reinforce the skin as the first-line barrier of our immune defense (Boxberger *et al*., 2021). Certain oral bacteria hinder the development of dental caries and periodontitis (Yamashita & Takeshita, 2017). The commonality of all microbiomes within the body is the utilization of similar methods of microbial competition and antimicrobial secretions to maintain homeostasis and balanced communities of commensal microbes. Methods used by commensal and mutualistic

microorganisms to regulate growth include secretion of antimicrobial substances and microbial competition to prevent opportunistic pathogens from colonizing an undercultured niche.

Microbiome of the gut

Of all the microbial communities, the gut microbiome is the most studied - likely due to it being the largest community of microorganisms in the body. Specifically, the magnitude of bacteria within the colon is greater than all other organs of the body by an order of at least two (Sender *et al*., 2016). The process of microbial colonization of the human gut commences at birth. Prior to this, it is believed that an infant's gut is sterile or home to very few microbes. Colonization of the infants' digestive tract happens rapidly during the natural birth process, in which the fetus is exposed to the mother's vaginal microbiome. Children born by a C-section delivery displayed much delayed development of a gut microbiome, yet are exposed postnatally (Bull & Plummer 2014). Breast milk can be a good source of microbes to promote the development of a healthy microbiome, because it is high in bacteria, such as *Bifidobacteria* and *Lactobacillus* (Belizário & Faintuch, 2018).

The microorganisms in an individual's gut microbiome are highly variable, and differ between individuals. Factors such as age, disease status, diet, genetics, and the environment influence the species of microorganisms that comprise the gut microbiome. For instance, in one study, children with *Helicobacter pylori* infections, who consumed yogurt– which is high in commensal bacteria *Bifidobacterium* and *Lactobacillus* – experienced a reduction in *H. pylori* microbial content, a restoration of the optimal *Bifidobacterium/Escherichia coli* ratio, and positive effects on their immune system response. Additionally, in individuals who

consumed yogurt on a daily basis, there was an increase in overall intestinal microbial diversity (Lisko *et al.*, 2017). Conversely, factors such as age are generally associated with an overall decrease in gut microbial diversity (Deng *et al.*, 2019).

Despite variability between microbial gut compositions among individuals, there are some core functions shared among these populations, as highlighted by similar gene profiles in bacteria. Such common functions include the metabolism of carbohydrates and of amino acids, the latter is used in regulating digestion in the human gut (Lozupone *et al.*, 2012).

Microorganisms in the gut are implicated in almost every realm of our health, including digestion, weight regulation, immune modulation, and possibly neuro- and cognitive-social development. Additionally, improperly regulated or developed gut microbiomes are associated with the development of adverse health conditions and diseases, such as allergies, IBS, and frequent infections (Mohajeri *et al.,* 2018). One factor suggested as the key in regulating body-microbial interactions is the level diversity of microorganisms present in the gut microbiome. Decreased diversity of microorganisms is indicative of poor microbiome health, and is associated with the adverse health conditions (Deng *et al.*, 2019). The human body is incredibly sensitive to changes in the gut microbiome, and loss of a specific bacterial species from the gut microbiome can result in exaggerated immune responses or, conversely, in the inhibition of immune functioning (Belizário & Faintuch, 2018). Microbial diversity goes hand-in-hand with dysbiosis. Dysbiosis, an imbalance in the quantity of a microbial species within the microbiome, is indicative of an unhealthy microbiome. This imbalance is often caused by death of specific bacterial populations, and the subsequent proliferation of potentially pathogenic bacteria. Dysbiosis can allow for those bacteria, known as opportunistic pathogens, found as a small percentage of the total bacterial

population within a healthy gut microbiome, to overgrow and become the dominant population. Dysbiosis and microbial diversity are interrelated and many of the factors that can influence microbial diversity can also create dysbiosis. These factors (many were noted earlier) include age, stress, dietary changes, disease, medications, especially antibiotics, and exposure to other gut-altering substances, such as chemotherapeutics. Dysbiosis-induced health effects, can include increased gut permeability, gut inflammation, and chronic conditions - such as IBD, IBS, cancer, obesity, and diabetes (Belizário & Faintuch, 2018). The link between gut microbial dysbiosis and gut disease can be understood by examining a model of two bacterial species with different properties. *Faecalibacterium prausnitzii,* a bacterium present in healthy individuals, has anti-inflammatory properties. Conversely, other bacteria, such as Bacteroides and *Ruminococcus gnavus,* have pro-inflammatory properties. The balance between these two bacterial species may lead to positive or harmful health effects (Belizário & Faintuch, 2018).

Drugs affecting the diversity of the gut genome

Medications can also alter the state of the microbiome. Use of oral antibiotics for treatment of bacterial infections can result in disturbance of the homeostatic gut microbiome and induction of microbial imbalances. Most antibiotics have general mechanisms of action, targeting not only the pathogenic bacteria causing the infection, but also commensal and mutualistic bacteria needed for specific functions, including preventing overgrowth of pathogenic bacteria. Frequent use of antibiotics can be potentially harmful and induce infections, such as those by *Clostridium difficile*, more commonly known as "*C. diff*." Additionally, overuse of antibiotics can select for antibiotic-resistant bacteria in the gut

microbiome, creating a population of potentially pathogenic bacteria that is more difficult to eliminate from the body (Belizário & Faintuch, 2018). At times, unexpected results may result from medicinal intake and the gut microbiome. In a study that examined patients and the use of 28 different drugs as well as mixed drug combinations, researchers found that while many medications induced adverse effects to the gut microbiome, surprisingly, certain drugs had a beneficial impact on the gut microbiome. However, as expected, the repeated use of antibiotics was associated with a potentially unhealthy decrease in bacterial diversity within the gut microbiome. Use of drugs, such as proton pump inhibitors, also were associated with negative effects on the gut microbiome. On the other hand, a combination of loop diuretic medication and beta-blockers were linked with higher levels of *Roseburia* bacteria, known for their health bolstering influence within the body, including a reduction in inflammation. Additionally, patients with cardiovascular diseases taking statins, a common cholesterol medication, showed healthier diversity in their gut microbiomes (Mayer, 2021).

Virulence factors

Whereas alterations in the gut microbiome may cause negative health effects, other contributors, termed virulence factors, may lead to the establishment and subsequent proliferation of pathogenic bacteria in the gut microbiome. Virulence factors can be naturally selected for within a bacterial population because they provide characteristics that favor bacterial survival. Such factors are then transmitted to other bacteria, through various mechanisms (including transformation, transduction, and conjugation).

Chemotherapy-induced intestinal mucositis and other side effects

Intestinal mucositis is associated with inflammation of the epithelial lining (and other mucosal layers) of the gut, as seen in Figure 3. This condition can result in pain, ulcers, diarrhea, nausea, and bacterial infection that may lead to sepsis. Intestinal mucositis is a frequent side effect of chemotherapy, occurring in 40 to 100% of all patients receiving chemotherapeutic drugs (Mohajeri *et al*., 2018).

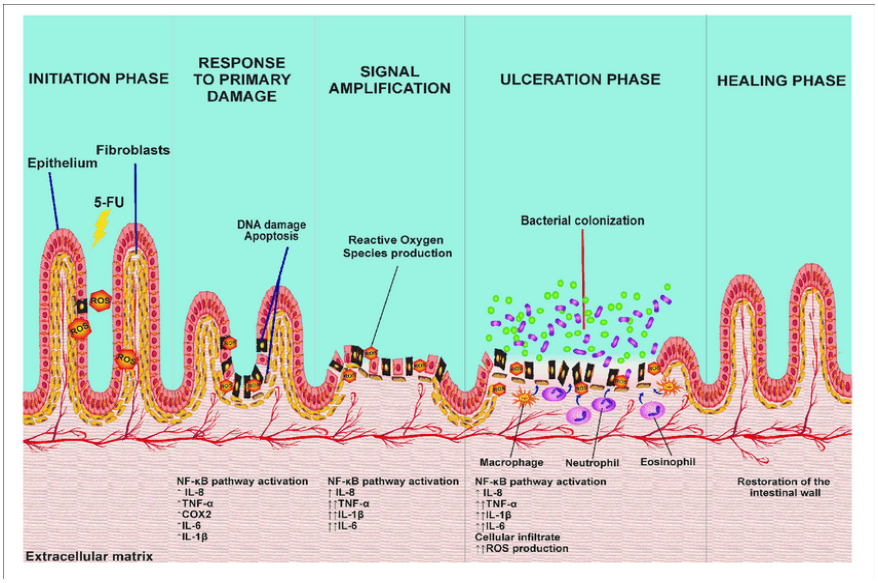

Figure 3: Depiction of the phases of intestinal mucositis. Toxic exposure casues DNA damage in epithelial cells. This triggers sudden death, known as apoptosis. Exagerated inflammatory response signals the ulceration phase, in which the intetsinal lining is further degraded and can be colonized by bacteria (Batista *et al.,* 2020).

In individuals experiencing chemotherapy-induced mucositis, quality of life is significantly diminished due to the painful side effects that accompany the condition, the increased need for hospital stays, and the possible need to obtain nutrition parenterally. Additionally, to mitigate the side effects, chemotherapeutic dosage is often reduced or is terminated completely, leading to shorter remission periods and increased mortality rates (Secombe *et al*., 2019).

Chemotherapy treatment targets rapidly dividing cancer cells, but can also inhibit the replenishment of other frequently dividing cell populations, including intestinal epithelial

cells. While there are many different types of chemotherapeutic treatments, many work by interfering with DNA of the target cancerous cells, preventing cell proliferation, and triggering apoptosis, cell-mediated death. Erosion of the epithelial layer allows for penetration of bacteria from the lumen of the gut into deeper layers of the gut, creating infection that furthering the already present inflammation (Dahlgren *et al.*, 2021). Such conditions can result in bacteremia, the spread of bacteria through the bloodstream (Mohajeri *et al*. 2018).

Additionally, chemotherapy treatment can also be toxic to the commensal bacteria residing in the gut, resulting in microbiome dysbiosis and the proliferation of harmful gut bacteria. Rat model studies noted chemotherapy-induced mucositis, with an overall decrease in microbial diversity and content in fecal samples, but an increase in Bacteroides species (Mohajeri *et al*. 2018). Additional studies with mouse models showed that in addition to decreasing the population of commensal bacteria and the protective qualities they provided, chemotherapy treatment resulted in increased pathogenic and pro-inflammatory Gram negative bacteria (Secombe *et al*., 2019).

The relationship between the gut microbiome and chemotherapy is quite complex. In a study performed on EL4 lymphoma tumor-bearing mice, germ-free mice, and mice with diminished gut microbiota demonstrated less cancer cell death in the presence of the chemotherapeutic agent, oxaliplatin. The effectiveness of the chemotherapeutic was, thereby, diminished. This indicated that there were certain ideal interactions between the gut microbiome and chemotherapeutics that influenced the effectiveness of the treatment. In addition to bacteria dictating the effectiveness of chemotherapeutics, chemotherapy, as indicated earlier, can induce changes in the microbial environment of the gut. In mouse

models, cyclophosphamide, a chemotherapeutic agent, assisted in the transport of Gram positive bacteria into secondary lymphoid organs, where they induced production of pathogenic immune cells (Temraz *et al*., 2019) .

Procedures to repopulate the gut with a "healthy" microbiota

There are several methods utilized in the treatment of chronic gut diseases that are related to microbial dysbiosis. Many of these procedures showed varying rates of success, were only useful in particular circumstances, or were not practical. Methods such as diet regulation have shown promising results in controlled settings, with the ability to restore eubiosis, *i.e*., the microbial balance within the body, and even possibly strengthen the epithelial cell barrier. However, the diet plan would need to be incredibly personalized to the specific patient. Secondly, the positive influence of diet on the microbiome quickly regressed to its original state when the diet was terminated. Additionally, diets, in general, showed a very low compliance rate and were difficult for individuals to follow (Ruff *et al*., 2020).

Similarly, non-individualized treatment, such as use of probiotics and the use of fecal transplants - a procedure in which a fecal sample from a healthy donor is introduced into the gastrointestinal (GI) tract of the patient - have shown varying results when not targeted to the specific gut microbiome of the patient (Ruff *et al.*, 2020). However, it should be noted that fecal transplants are 90% effective in treating patients with recurrent *C. difficile* infections (Le *et al,*, 2017). Despite the success in these patients, there is still concern with this procedure because it is recognized that along with the commensal bacteria received through donor fecal samples, potentially pathogenic viruses, archaea, eubacteria, and parasites can also be introduced into a patient's system (Belizário & Faintuch, 2018).

Another potential treatment for bacterial induced illnesses is phage therapy, in which virulent bacterial viruses are used to infect and kill specific types of bacteria. Despite the success in clinical trials of such bacteriophage "cocktails," there were very few countries that permitted the use of the phage therapy. The research surrounding the use of these drugs must be further investigated and tested clinically before introduction to the market. Additionally, another set back for this type of treatment is the potential for bacterial resistance to phages via immune regulating systems, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR has the potential to mark and target foreign DNA, such as that of the phage, for destruction by endonucleases (Belizário & Faintuch, 2018).

While there are many treatments intended for the use of regulating gut bacteria, there is no clear treatment that has shown consistent success. It is clear that improved therapies are needed to treat dysbiosis and bacterial-induced diseases. Scientists are looking towards bacteria as the new frontier for treatments for bacterial-induced illnesses. One instance of such innovation was reported in a paper titled, "An engineered live biotherapeutic for the prevention of antibiotic-induced dysbiosis." Scientists at MTA engineered *Lactococcus lactis*, a bacterium that secretes the enzyme, β-lactamase, which breaks down β-lactams, a class of antibiotics which include cephalosporins, penicillin, and carbapenems. The intent was to inactivate existing antibiotics already in the intestines. When delivered orally, these bacteria populated the gut microbiome and secreted enzymes to inactivate antibiotics present in the gut. In a mouse model system treated with antibiotics and the modified bacteria, the antibiotics were maintained circulating within the body, while not harming and diminishing the local gut microbiome. This shows potential as an adjuvant treatment for those taking antibiotics for non gut-related infections, thereby serving as a form of protection from

dysbiosis. Use of this as an adjuvant treatment may allow for prevention of secondary infections, which may lead to chronic conditions (as mentioned above). As the administered antibiotics do not kill the gut microbiota, there is no selection for antibiotic resistant strains within the gut microbiome. When the bioengineers designed this bacterium, they divided the gene encoding for β-lactamase into two coding sections, inserted far apart from each other within the *L. lactis* genome, thereby preventing gene transfer to other bacteria in the gut. Antibiotic resistance is a growing issue within the medical community. As more antibiotic resistant bacteria are selected and then perform horizontal gene transfer to other bacteria, bacterial infections become significantly more difficult to treat (Ktori, 2022).

III. Research

Testing bacteria altered by chemotherapy to find potential bacterial treatments

Similarly, the laboratory I interned in the summer, 2021 at Bar Ilan University also researched potential treatments for dysbiosis-induced side effects using engineered bacteria. Their studies compared the gut barrier functioning in patients pre-chemotherapy with those post-chemotherapy. Earlier findings in the laboratory found that the post-chemotherapy microbiota altered the gut barrier's functioning, compared with the pre-chemotherapy microbiota collected from the same mouse/patient. It was postulated that this difference resulted from some alterations in the indignous bacterial populations of the gut.

The aim of the experiment in which I participated was to isolate bacteria from breast cancer patients before and after chemotherapy, to analyze changes to their gut microbiome, and to assess their potential exacerbation or therapeutic influence of chemotherapy induced tissue damage (such as mucositis and increased gut permeability seen frequently in

chemotherapy patients) in the gut epithelium. To accomplish this, fecal samples were collected from breast cancer patients, before and after chemotherapy. Bacteria were cultured from one patient, known as patient 5. Patient 5 was treated with the following chemotherapeutics: adriamycin, cytoxan, perjeta, herceptin, and axol.

The bacteria were cultured and isolated, under both aerobic and anaerobic atmospheres, using selective media. A total of 121 bacterial colonies were selected, of which 59 were from pre-chemotherapy fecal samples and 62 were from post-chemotherapy samples. Of those in the pre-chemotherapy sample, 24 were isolated under anaerobic and 35 were isolated under aerobic conditions. From the post-chemotherapy samples, 38 colonies were anaerobic and 24 were aerobic.

The genetic material was extracted from the 121 bacterial cultures, amplified via PCR (polymerase chain reaction) using primers for bacterial 16s ribosomal RNA gene. The ribosomal 16s sequence in bacteria is a variable region of RNA, containing genetic information by which bacteria can be classified phylogenetically (Belizário & Faintuch, 2018). A series of agarose gel electrophoresis studies were run to assess the purity of the isolated samples and of the control, as well as to ensure that there was a high enough concentration of the genetic material in each sample, to be sent for genetic sequencing. Of the 121 isolated colonies, 95 were sequenced. The results of the sequencing are visualized in Figure 4, which displays the percentages and types of bacteria that were successfully isolated and subsequently sequenced from both the pre- and post-chemotherapy fecal samples. Following the sequencing of the 95 colonies, the data was analyzed to determine 11 specific samples of bacteria to be reisolated and used in further testing. Those in the 11 samples showed predominantly one sequence, with very little contamination, as displayed in Figure 5.

The laboratory will use these bacterial samples in the transepithelial electrical resistance (TEER) assay to assess the bacteria's altered influence on permeability of gut epithelial cells. In the TEER assay, bacteria are plated on intestinal epithelial cell lines. An electrical current is generated and transmitted through the culture in the presence of the isolated bacterial samples. The objective was to test whether the presence of bacteria changed the permeability of the gut, as reflected in overall transmission of electrical current on the other side of the culture.

Goal of experiment

The goal of the research was to isolate bacteria with gut-strengthening qualities that can be used to counteract the negative side effects of chemotherapy-induced tissue injury, such as mucositis and leaky gut, often experienced by chemotherapy patients. Simultaneously, the laboratory will continue to isolate more bacteria from the pre- and post-chemotherapy samples for genetic sequencing to test the effects of additional bacteria on the epithelial barrier. Research is still ongoing and the results are promising.

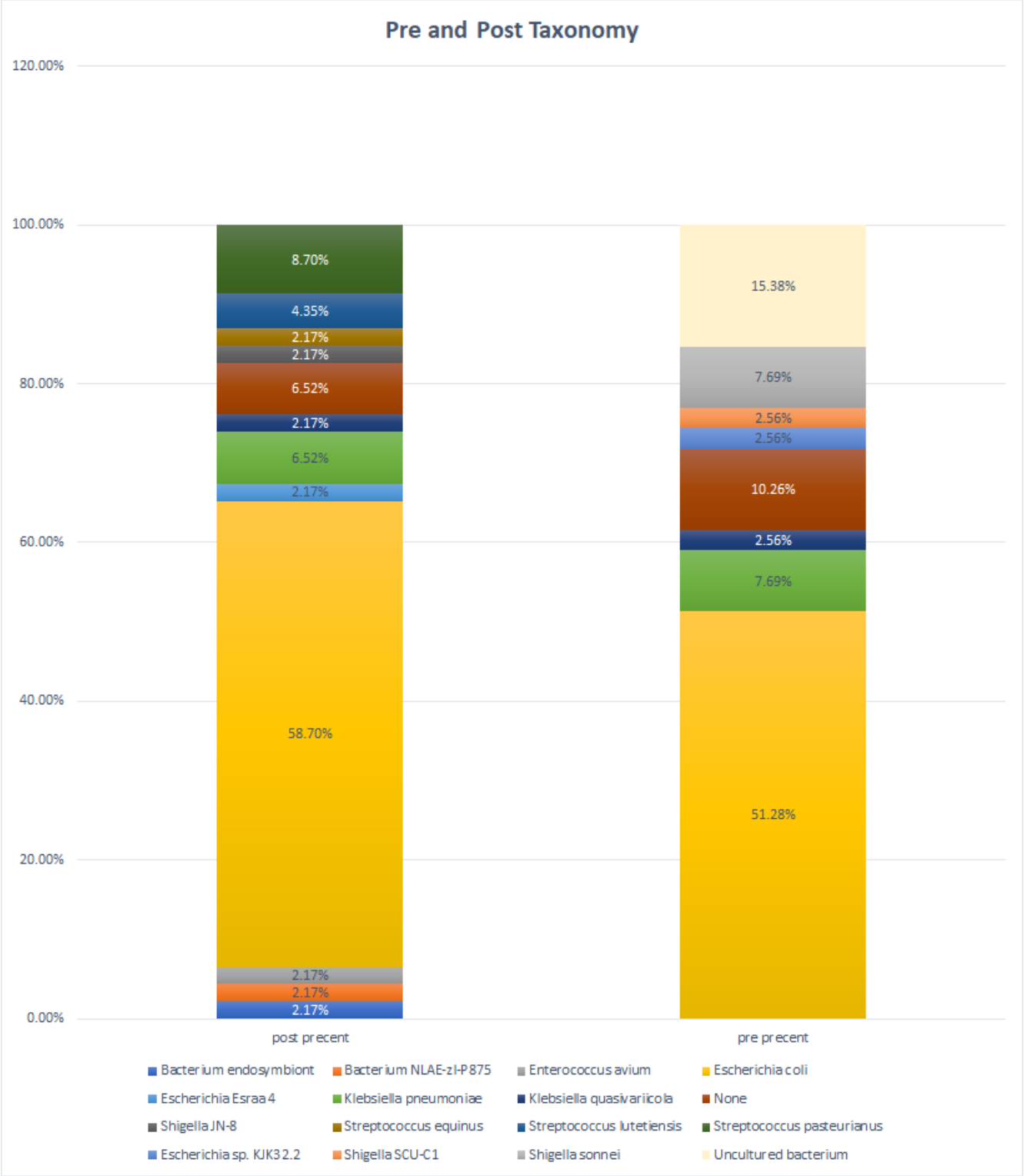

Figure 4: Result from first set of genetic sequencing shows the percentages and types of bacteria present in the pre and post chemotherapy treatment samples.

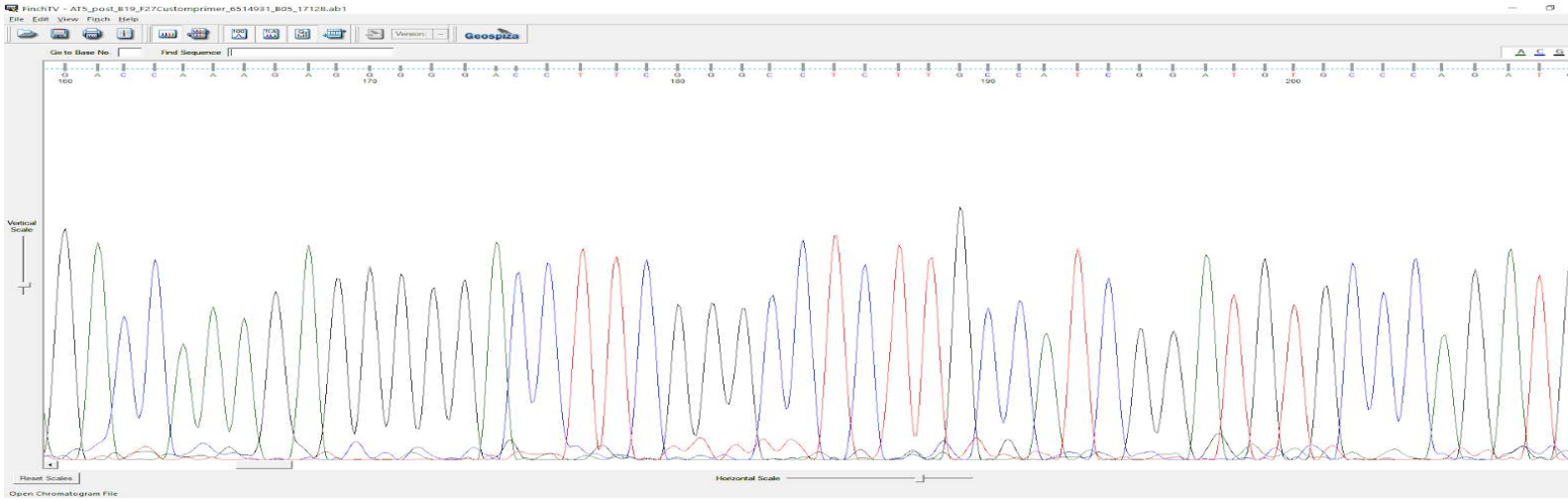

Figure 5: Successful results from genetic sequencing of a sample show a dominant genetic sequence with little contamination

IV. Discussion

Potential therapy and future research

Currently, the use of bacteria in therapeutic treatments is novel and is very limited. This research opens up the door to many opportunities for other studies into the use of bacteria as therapeutic treatments for medical conditions. It also brings into question how different drugs alter bacterial microbiomes. Finally, this research may provide insights as to better mechanisms of dosing chemotherapy treatments.

V. Conclusion

Though it has been known for centuries that fecal content is important to the functioning of the gut, only in the past few years has there been a boom in the understanding of the microbial impact on the functioning of many diverse organ systems in the body. While many different factors, such as age, genetics, and diet, change the composition of the gut microbiome, research has shown that the use of drugs, especially chemotherapeutics, has a significant effect not only on the gut microbiome, but on the gut itself. While the sensitive microbial environment of the gut poses many challenges and potential for disease, the same sensitivity allows us to alter the gut microbiome and find potential therapies for different ailments of the gut.

In our laboratory at Bar Ilan University, we studied the influence of chemotherapy on the gut microbiome. We isolated and cultured bacteria from pre- and post-chemotherapy patients, in order to assess the role of chemotherapy on specific bacteria and the microbiome at large. Additionally, we tested the response of the gut epithelial lining to these bacteria, in an attempt to identify those bacteria with healing properties. These latter experiments are

underway in Dr. Yissachar's laboratory. Future research likely would use almerioating bacteria in mouse models to assess their impact on the permeability and health of the gut. The research is innovative and exciting to report, but the laboratory requested that, when authoring this Senior Project, I limit my description of the research and its findings.

Overall, isolation of therapeutic bacteria may provide treatment for individuals experiencing the harmful side effects of chemotherapy and hopefully advance the field of bacteriotherapy.

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