David Banks

The Research Process

In 2011, when I first began my investigation into which undergraduate institution I would be calling home for the next four years, I visited UNCG and immediately recognized an atmosphere of academic momentum. An image of a library with nine floors and an endless number of volumes to explore became timeless in my mind; however, in no way could I have imagined the assistance and guidance it has given me over the past three years in terms of my research interests.

In developing this research synthesis, specifically, I found great support and guidance in the library staff and in resources that went far beyond the stacks of books I had been so eager to delve into once before. I have always been interested in literature review and analyses; this even deterred my ability to decide which major I would finally settle with. One day, however, I decided to migrate to the fifth floor and settle in for a late night of studying and found an entire collection tailored to biological and chemical science. I was floored, and it was here that I would stumble across a book, *Neuroimmunology,* that displayed an intersection between the brain and microbes I did not know existed. So, I took a Microbiology course and decided to contract it for Honors credit. Without this book, I am not sure if I would have been influenced in such a way as to change my projected research focus after obtaining my Bachelor's degree. This work, coupled with the encouragement and suggestions of Dr. Cannon of the Biology Department, fortified my decision to explore the human gut microbiome and how it affects the human body as a whole.

In the process of developing an Honors contract thesis, there were two databases made available to me, as a UNCG student, through Jackson library that helped me greatly in searching for primary literature on the topic at hand: Academic Search Complete and PubMed. It was

actually Prof. Kellam, a librarian I had taken a political science class with before that has been a great mentor and source of academic motivation, who revealed to me that these databases would likely assist the greatest in terms of *recent* literature. It was as if I had an actual memoir of scientific development at my fingertips. These databases, along with several members of the staff who helped me physically locate several books that were used for background information in the first several pages of my paper, enabled me to write a paper worthy of Honors credit.

With research, I have come to realize through this process, frustration is going to occur. The resources that provided me with the information necessary to formulate an argument and support its foundation also posed a challenge: sifting through what seemed like an endless number of candidate pieces. I was up to the task but, at times, it was difficult to decide which articles would coincide with my argument, while still keeping in mind the necessity to acknowledge and refute the claims of authors who may not have agreed with what I was trying to synthesize. When I began seeing this as an opportunity for virtual collaboration instead of a daunting task, it became much easier and more productive. In this way, the process enabled me to take a lesson with me in completing future projects: to view every resource as a candidate for interview and to choose the ones with which I would like to work in partnership in making this new product. Although challenges existed, they provided a learning experience I believe was necessary in order for academic and pre-professional maturation.

The Human Gut Microbiome: A Physiological System Approach

David Banks

16 November 2013

General Microbiology

Honors Contract Thesis

I. The Stage

Perhaps some of the most complex disorders that have been observed in humans are those that concern emotion and processes of the human brain. The pathway between stimulus and response in cases of depression and anxiety is one that can take many directions depending on the patient in question; for this reason, psychiatry has provided interesting modes by which pathology can be studied outside of a one-to-one causal relationship. Given the expected lifespan of the average individual and the decades through which different disorders can emerge and withdraw given changes in environment or situation, determining the cause of such pathologies as Chronic Fatigue Syndrome (CFS) or even Obsessive-Compulsive Disorder (OCD) can be daunting for any physician. How does one trace a pattern of feeling or behavior back to a causal event that may have occurred years before symptoms even emerged? In an age where Post-Traumatic Stress Disorder (PTSD) continues to gain relevance in light of a world at war, such questions must be asked. The lives of patients and their families depend on the ability of scientists to uncover pathways that can be manipulated to produce some treatment option or cure. After Dr. James Greenblatt was sought out by the parents of a young girl named Mary who had been diagnosed with a mixture of psychiatric disorders, including OCD and Attention Deficit Hyperactivity Disorder (ADHD), and treated with a range of psychotropic drugs that had remained largely unhelpful, his mode of thinking was able to change her quality of life (James 2013). He turned to her stomach as a possible root of the problem.

One may not expect a trip to the psychiatrist to include a survey of questions aimed at ascertaining his or her digestive health; however, Dr. Greenblatt employed this strategy and, because of his conclusion that the symptoms that were manifesting in the emotive regions of Mary's brain might actually originate from an imbalance in her gut microflora composition, was

able to prescribe a twice-daily dosage of probiotic that alleviated Mary's symptoms altogether. His assumption stemmed from an elevated level of metabolite HPHPA in her urine, a byproduct of the metabolic pathways of *Clostridium* species. Dr. Greenblatt's reasoning further developed from his ideas concerning the connection between the human gut and brain, an interface that had once been deemed a one-way street. By reversing this line of thinking and implementing a treatment plan that assumed a gut-to-brain avenue of communication, Dr. Greenblatt was able to treat Mary's symptoms, thereby continuing a thought that the gut microbiome plays more of a role in the human body than previously thought. While Dr. Greenblatt's treatment of Mary's condition constitutes an isolated case where an assumed role by the human gut microbiome existed, many clinical studies have also contributed to the belief behind this linkage.

The treatment of depression or anxiety as symptoms associated with some larger pathology has become a forefront of medicine and patient care. Chronic Fatigue Syndrome (CFS) is a functional somatic disorder marked by intermittent cycles of severe fatigue, often coupled with cognitive dysfunction and/or gastrointestinal disturbances. In fact, half of all CFS patients meet the criteria for Irritable Bowel Syndrome (IBS) diagnosis and a similar fraction meet the criteria for an anxiety disorder and/or major depressive disorder diagnosis (Rao 2009). The connection between the gut and brain are certainly noticeable in patients suffering from CFS. In a 2009 study, this connection was tested by defining some relationship between the introduction of a probiotic and emotional symptoms experienced by patients suffering from CFS.

In a pilot study, thirty-five patients, twenty-seven females and eight males, were scored and chosen based on scaled inclusion and exclusion principles outlined by the researchers and included such criteria as the ability of participants to withstand an eight-week study. At the beginning of the study, each patient was evaluated using the Beck Anxiety Inventory (BAI) and

Beck Depression Inventory (BDI), two qualitative psychiatric assessments for anxiety and depression respectively, and a fecal sample which was tested for total aerobe, anaerobe, *Lactobacillus* spp, and *Bifidobacteria* spp counts. In this way, despite a small sample size, the study employs by qualitative and quantitative modes of analysis. For an eight-week period, after each meal or three times daily, patients consumed the contents of an unmarked container, one type which was a placebo or the other which contained eight billion colony forming units (CFUs) of *Lactobacillus casei* strain Shirota (LcS). After the eight-week period was complete, patients were again assessed using BAI, BDI, and fecal analysis by the same methods. While 37.5% of patients in the control group exhibited an increase in *Bifidobacteria* and 43.8% in *Lactobacteria*, out of the patients receiving the probiotic, 73.7% of patients saw a significant increase in both. BAI results showed a significant decrease in anxiety symptoms in patients receiving the probiotic compared to those receiving the placebo, as supported by a significant statistical difference between the two groups (Rao 2009).

In this study, one can see a clear correlation between microbiome composition and symptoms associated with pathology, specifically one that connects neuropsychiatry and gastroenterology. However, complete causation cannot be concluded because the mode of symptom alleviation in CFS patients in this study may have diverged from a one-way-street type of thinking and the small sample of patients assayed leaves room for speculation. For instance, one could argue that the probiotic may have increased bowel function thereby alleviating symptoms associated with anxiety; however, this avenue between independent and dependent variable, or probiotic and symptom alleviation, was not investigated in this study specifically. The avenue between independent and dependent variable does not change the fact that one still has some resonant effect on the other, and such is the case in this instance. The role of the gut microbiome cannot

be ignored and because of this, understanding its composition and development is crucial to discovering new connections between this part of the body, perhaps a human physiological system on its own, and pathology.

II. Diversity and Development

Before one can begin to clearly understand how the gut microbiome is linked to human pathology through interaction with other human physiological systems, one must first ascertain the composition of the gastrointestinal (GI) tract: what constitutes this structure and the microorganisms residing within? The GI tract is a tube approximately eight meters long extending from the mouth to anus; therefore, this tubular passage is open to the external environment. Although the GI tract is composed largely of muscle and mucous membrane, its associated structures and organs include the esophagus, stomach, small and large intestine, pancreas, gallbladder, liver, appendix, colon, and rectum. By association with these organs alone, one can conclude that the GI tract plays a major role in the digestion and processing of materials consumed through one's mouth or that have entered by some other means, via gastronomy tube for instance. It is within the GI tract that ingested materials are converted into absorbable forms via mechanical digestion by chewing and chemical digestion by pH fluctuation and enzymatic activity (Mullen 2011). In this way, nutrients are utilized; without this step, these vital substances would simply pass through the body and be excreted because transport mechanisms do not exist for many larger molecules. Because of the presence of many enzymes targeted at degrading all types of macromolecules and a pH of approximately two because of its hydrochloric acid composition, the human stomach also serves as a bottleneck through which many microorganisms, besides those evolutionarily-tailored to this type of environment, cannot pass without degradation and death. Therefore, discussion of the gut microbiome often refers to

those microbes that inhabit the lower GI tract, specifically the colon, and small and large intestines.

What microorganisms constitute the gut microbiome and how did they come to reside here? The answers to these questions are much more involved than a simple list of microorganisms constructed by fecal sample analyses, especially when it is already known that the composition of the gut microbiome varies with environmental conditions. The composition of the gut microflora community of an individual from a developing country such as Somalia is likely far different than that of an individual from a developed country such America or Switzerland. In fact, such studies have proven just this. A 2011 study aimed at discerning core similarities and differences between gut microbiome populations between individuals did so by analyzing side-by-side DNA homology data via the Sanger method. Data obtained from twenty-two individuals of six different nationalities, including those from America, Spain, and Japan, were analyzed by these methods. The environment of the human gut microbiome is, in fact, the human; therefore, this study utilized quantitative metagenomic methods of data collection and analyses targeted at ascertaining commonalities of the following characteristics between gut microbiomes of individuals of different nationality: the number of different groups and the abundance of each group. While many themes were ascertained concerning the composition of the microbial communities of the gut, three main clusters of bacteria, or enterotypes, were developed that correlated strongly with metabolic activity. Differences did exist depending on nationality; for instance, individuals of Japanese origin hosted gut microbiomes consistent with the first enterotype categorized (Arumugam et al., 2011). However, the development of any enterotypes at all indicates the presence of a core population derived in each human GI tract, lending itself to

the idea that some homologue community exists among humans that has developed alongside the human species through evolution.

The existence of homologous structures has contributed to the understanding of how evolution has progressed over billions of years; in a similar way, a homologous gut microbiome reveals an understanding and confirmation about the processes we already associate with this area of the body. A core similarity between microbe populations in the human gut, across the globe even, is an indicator that the functions served by specific genus and species groups have arisen as a result of some specific function they perform, and due to interactions between microbe species and the larger human body. In essence then, the microbiome serves as vital a function as the pathways that allow for sight or hearing. Interactions between cells along a pathway, through neurotransmitters and other signaling molecules, allow for a response at the organismal level, guided by a distinct nervous system. The existence of this organization across all individuals of the human species, as a homologue, shows that these cells are present and vital to the function they serve. Without them, function would cease. Similarly, a homologous gut microbiome indicates that the functions served by this system are vital to the success of the organism through evolution. In other words, if the human gut microbiome has evolved as a homologue within the human body, its absence would serve a decrease in fitness that would lend any human "behind on the times" in terms of the evolutionary survival of the fittest outlined by Charles Darwin decades ago.

The idea that the microorganisms present during adulthood originate from the materials ingested through adolescence would fail to recognize that a distinct microbiome exists at infancy and develops from that point in a successional and coordinated fashion much like human cell differentiation occurs during gustation. Therefore, the differences in microbial composition

between individuals of American and Japanese origin noted above would not come as a result of different diets associated with each region alone. Another 2011 study, instead of ascertaining the similarities and differences among human gut microbiome composition across nationality, investigated the development of gut microbiome composition through time, starting at infancy (Koenig et al., 2011). Through this investigation, researchers were able to ascertain what changes in the gut microbiome occur through time and as a result of what events; if one is to understand the current state of any system in the body, defining events associated with the development of that system are crucial. Without understanding how red blood cells of the cardiovascular system originate in the bone marrow and develop through denucleation, for instance, the presence of nucleated blood cells in a fetal blood sample might not lead to a correct diagnosis of leukemia (Hermansen 2001). One can find an analogy in the homologous gut microbiome: without understanding how the community started and developed through time, how can one determine the reason for current microbiome composition in relation to metabolic changes associated with such pathologies as IBS, celiac disease, or Crohn's disease, digestive diseases associated with inflammation or infection of the GI tract wall? In 2004, digestive disease was determined to be the underlying cause of almost 250,000 deaths in the US alone (Everhart 2004). Because of the mortality rate due to pathologies associated with changes in the gut microbiome, analyzing how such a system changes and develops outside of pathology is vital to understanding what goes wrong when pathogenesis does occur.

In the 2011 study introduced above, sixty healthy infants were recruited for a study in which fecal samples were taken regularly over a 2.5-year timeframe. Fecal samples were analyzed by 454-pyrosequencing to determine microbial composition, which produced over 300,000 16S rRNA sequence profiles. Twelve fecal samples were further sequenced for DNA homology,

allowing the researchers to further identify the types of bacteria present. From these findings, researchers were able to conclude that the infant gut microbiome starts from exposure to the mother's vaginal and fecal microbiota during birth. During early infancy, *Bifidobacteria* are abundant, largely due to their ability to digest milk oligosaccharaides. However, before the introduction of solid foods, fecal sample analysis indicated the presence of functional genes involved in plant polysaccharide metabolism. As solid foods are introduced, the infant gut microbiome begins developing into its later adult composition via the following changes: a sustainable increase in *Bacteroidetes*, and an increase in functional genes coding for enzymes that contribute to carbohydrate metabolism, biosynthesis of vitamins, and degradation of xenobiotics. Additionally, this research included a case study of a young male infant through an equal 2.5-year timeframe, which indicated gut microbiome composition changed over time in coordination with life events such as fever, and that the microbes present were not randomly associated but interacted with each other as indicated by ecological tests such as the C-score and checkerboard measures. After referencing the OTU-based cluster analysis, researchers used the above statistical methods to conclude that species occupying the infant gut existed according to community assembly rules. High C-score and checkerboard values, which test if species exclude one another from shared niches and if species exist in the gut that never co-occur, indicated that the gut microbiome species exist through interaction rather than random association (Koenig 2011).

III. A Physiological System Approach

From the findings of this study, one can conclude several things about the human gut microbiome. A human gut microbiome exists during infancy even before solid foods are introduced, and development occurs through succession of microbial species and interaction with

their environment. Interestingly enough, these themes reside in other human physiological systems as well, including the nervous system. A nervous system exists long before the addition of reaction-producing stimuli and neurons within the brain increase in number and differentiate over time until the adult mode of brain function is reached (Tucker 2013). In this instance, we see a clear similarity between the human gut microbiome and a distinct physiological system. To go a step further and define the human gut microbiome as such, however, several more factors must be analyzed. Associated pathology via disruption of homeostatic function, including correction via transplantation, communication with other areas of the body through inter-systemic interaction, and coordinated communal action in response to stimuli, as outlined by a series of studies, are all marked characteristics that allow one to define the human gut microbiome as a unique human physiological system.

i. Homeostasis and Pathology

As already noted, the composition of the gut microbiome is associated with such conditions and pathologies as CFS. However, a distinct mode of thinking that connects the human gut microbiome with a disruption in homeostasis, indicating that it plays a usual role in maintaining it, and pathogenesis is necessary for one to define it as a human physiological system, as opposed to conclusion by mere correlation. A 2011 study attempted to discern such a connection between the gut microbiome of mice, a prime model organism for human homeostatic regulation, and the onset of autoimmune encephalomyelitis via impairment of normal immune functioning. All mice used were of a model exhibiting relapsing-remitting autoimmune encephalomyelitis, a pathology originating in $CD4^+$ T cells and myelin oligodendrocyte glycoprotein-autoantibody-producing B cells of the immune system. In this study, mice devoid of gut microbiota were compared to those who contained commensal flora isolated to prevent disruption of the study by

introduction of pathogenic species that could have produced an undocumented immune response leading to pathogenesis. Those mice devoid of gut flora exhibited no spontaneous generation of expected pathology, lending the researchers to conclude that the existence of commensal flora is necessary for the autoimmune response responsible for the pathogenesis outlined. Additionally, when mice devoid of gut microflora were recolonized with commensal microorganisms, the relapsing-remitting autoimmune encephalomyelitis pathology generated within several weeks, further indicating that the pathology is at least partly dependent on the presence of a commensal gut microbiome (Berer 2011). This pathology in mice, because of its mode of destruction of myelin in neurons, serves as a model of multiple sclerosis in humans, and indicates a clear dependence of homeostatic mechanisms in pathogenesis.

Other studies have also clearly linked the human gut microbiome with disruption of homeostasis and pathogenesis in areas throughout the human body; one such study investigates this criteria by analyzing the effectiveness of fecal transplantation in returning the human digestive system to its original state of function in the context of recurrent *Clostridium difficile* infection. The human GI tract can become invaded by the species *Clostridium difficile* if normal immune response is compromised or resistant strains enter the gut; such infections are common in hospitals and long-term care facilities. Antibiotic treatment of this infection may seem an obvious form of treatment and one might expect this treatment to be fully effective in eliminating infection; however, recurrence of infection arises in about half of all patients given this treatment option. Therefore, in 2012, researchers sought to elucidate the effectiveness of fecal transplantation in voiding the GI tract of reoccurring *Clostridium difficile* infections. In 70 patients, donor feces was inserted into the cecum during colonoscopy. In all patients, no immediate complications were observed, an important factor in determining the efficacy of any

new treatment option. Within 12 weeks, all patients not infected by strain 027 *Clostridium difficile* saw a complete alleviation of symptoms and 89% of patients with infections by strain 027 *Clostridium difficile* had similar results. In this instance, the fecal transplantation was not only effective at eliminating the recurrent infections, but even infections caused by a known virulent strain. Four patients did not respond, having previous comorbidity, and died of colitis. Within the first year, four patients whose symptoms were alleviated experienced relapse and were treated again, two by fecal transplantation and two by antibiotics; in all instances, eradication of the reoccurring infection was successful.

In this instance also, the gut microbiome is connected to a pathology associated with a disruption in homeostasis. Reoccurring infection by varying strains of *Clostridium difficile*, including those marked by virulence, serves as the example pathology in this case. In the study examining the role of the microbiome in pathogenesis of relapsing-remitting autoimmune encephalomyelitis in mice, a positive-trend relationship was observed between microflora and pathology. In the presence of gut microflora, pathogenesis occurred; however, in its absence, pathology remained absent. In discussing fecal transplantation in the context of categorizing the human gut microbiome as a unique physiological system, however, a negative-trend relationship is noted. Despite these differences in correlation, both cases still connect the gut microbiome with disruptions in homeostasis, either contributing or correcting this disruption. One could argue that the positive-trend observed between this gut flora community and pathology disallows its classification as a physiological system; however, it is the red blood cells of the circulatory and respiratory system, altered by inheritable genetic mutation, that serve as agents of symptoms caused by sickle cell anemia, despite the usual vital function they perform (Ravindra et al., 2012). If cells of a physiological system serve as agents of pathology, their role in homeostatic

disruption is even more obvious; this quality does not exclude them from the physiological system within which they function. Similarly, classification of the human gut microbiome as a physiological system is only confirmed by having both of the studies discussed above side-by-side, revealing that this system has an intimate relationship with pathologensis at both ends of the spectrum.

ii. Inter-systemic Communication

How is it that the presence or absence of commensal microflora in the gut can contribute to a disruption of a homeostasis leading to pathogenesis in an area of the body as far away as the central nervous system, as in the case of relapsing-remitting autoimmune encephalomyelitis? Some form of communication must occur between this area of the body, possibly mediated by some other systemic response, and the central nervous system: a conclusion that was made by Dr. Greenblatt in his treatment of Mary's symptoms and the researchers that tested the connection between the introduction of a probiotic and alleviation of symptoms associated with emotion in CFS patients. Inter-systemic communication producing some response as a result of a stimulus is a hallmark of physiological systems within the human body, and is a characteristic shared by the gut microbiome. Such connection and communication is obvious when effects on the adrenal gland, a landmark of the human endocrine system, are observed in patients suffering from sepsis. In these cases where toxins are introduced into the human body by pathogenic microbes, dysfunction of the adrenal gland occurs by multiple modes, including inflammation and cell death. In a 2013 study, LPS-induced inflammation was set in mice and, as a result, overexpression of cytokines and chemokines and a mass recruitment of lymphocytes into the adrenal gland were all observed, leading to changes in an entirely different physiological system (Kanczkowski et al., 2013). In this instance, a communication scheme is offered, connecting the

immune and endocrine systems through pathogenesis. Such a line of communication also seems to exist between the gut microbiome and the nervous system, via interaction with the immune system, as indicated by the study examining autoimmune encephalomyelitis in mice outlined above.

The means of communication by which the gut microbiome could lead to pathogenesis in the nervous system lies in the proximity between and integration of the GI tract and immune system. The physical interface between gut and immunity lies in the gut-associated lymphoid tissue (GALT). This system of lymphoid tissue is physically integrated into the wall of the GI tract, specifically around the intestine. Some may argue that the primary mode of immune response occurs via the human circulatory system. Such an argument is feasible given the fact that cells of the immune system responsible for targeting and destroying foreign particulate matter and pathogenic species travel to their targets through blood vessels spanning the entire human body. However, the storage house of these immune cells, including B and T lymphocytes, is the GALT (Honjo 2006). The simple proximity between the gut and cells of the immune system is enough for one to point to a connection between these systems; however, a simple connection does not conclude a specific line of communication associated with inter-systemic interaction between physiological complexes. This gap in reasoning is closed when one references such studies as that linking microbiota presence and pathogenesis of relapsing-remitting autoimmune encephalomyelitis in mice. In fact, the dynamics surrounding communication between the gut microbiome and immune system investigated by Berer is only one case of inter-systemic communication between the human gut microbiome and other physiological systems; such a link also exists between the flora in the human gut and the production of sex hormones, species of the

endocrine system, which constitute a layer of context surrounding the pathogenesis of autoimmune disorders.

A 2013 study investigated the linkage between the human gut microbiome and the origin of autoimmunity, which coincides with the focus of that performed by Berer in 2001; however, the mode by which this linkage occurs requires communication via sex hormones according to Markle. In this study, nonobese diabetic mice were employed that host spontantaneous, immune-mediated annihilation of pancreatic β cells, which causes type 1 diabetes. Male mice were protected from pathogenesis when colonized with commensal flora, marked by an increase in systemic testosterone levels. Like in humans, a disparity was obvious between the male and female mice in terms of type 1 diabetes pathogenesis. However, when maturing female mice were colonized with flora from male mice, the microflora composition of their gut was altered in a way that exhibited that of male mice; in turn, systemic testosterone levels increased as expected. However, something more interesting occurred. In female mice treated with the flora taken from male mice where no type 1 diabetes was observed, autoantibody generation by the immune system and inflammation of the pancreatic islet decreased, thereby protecting these treated female mice from type 1 diabetes. Not only does this study confirm that transplantation of gut microbiota can alter the course of pathogenesis, but it brings into a play a new series of communication mechanisms: sex hormones. When microflora composition was altered, sex hormone secretion changed in a way that decreased an autoimmunity response required for type 1 diabetes to emerge. Also tested was the dependence of this phenomenon on the presence of androgen receptors, which were required for the effects observed (Markle et al., 2013).

In other words, the presence of sex hormones alone could not alter pathogenesis; the receptors for these molecules were also required, lending to the argument that communication

definitely occurred between multiple inter-systemic complexes: the gut microbiome, sex hormones, and the immune system. One could argue that not all of these players were required for the disease state that emerged in these mice; however, without any one of these components, even in the presence of the other two factors, pathogenesis failed to occur according this study. By this line of thinking, a signaling pathway has been mapped that is disrupted when one component, either signaler or receptor, is taken out of the equation; therefore, the conclusion that the gut microbiome is involved in inter-systemic communication pathways is clearly supported.

iii. Communal Response

One criteria of life is the ability of an organism to sense and respond to changes in its external environment, thereby maintaining homeostasis. To say that the system remains unchanged in the context of an environment fluctuating in growth factors from second to second, however, would be false. Instead, it is the responsibility of the organism and its component regulatory mechanisms to sense and respond to these changes via signaling pathways in order to keep the organism within internal constraints required for survival. For instance, in vertebrates, when the wall of a blood vessel becomes mechanically damaged, some mode of clotting is required in order to prevent hemorrhage. The process involved requires a series of different proteins in the blood stream that interact with one another in a cascading fashion, eventually leading to a clot comprised of platelet and fibrin components. The pathway between wall damage and clot, however, requires changes in gene expression that allow for the synthesis of a plethora of molecules and complexes, and the ability of these species to react in a cooperative fashion. These species include the endothelium, coagulation factors, platelets, and leukocytes, to

name a few. The ability of these molecules to sense one another and respond to a previous step in the coagulation pathway is a hallmark of the circulatory system and its regulatory mechanisms. However, as stated, homeostasis does not denote a constant state; in other words, the formation of clots is not the only necessary step in allowing for the survival of the organism. With fibrinolytic factors, these clots would remain indefinitely and increase at an exponential rate near the site of vessel damage (Gailani 2007). The homeostasis loop, then, requires a communal response by a series of proteins and other structures that allows for the fluctuation between clot formation and clot degradation, without which the organism could not survive.

Classification of the human gut microbiome as its own physiological system requires a communal response by its components to an outside stimulus, a determining factor exhibited by the coagulation pathways that exist in the circulatory system. A signal, the breaking of a vessel wall, leads to an intrinsic communication pathway mediated by different coagulation and fibrinolytic factors that ends homeostatic maintenance achieved by clot formation and eventual degradation. Does such a mode of stimuli and cooperative response exist in the gut microbiome? While the specific mechanisms of the circulatory system's hallmark coagulation cascade are not replicated by the microbes in our gut, pathways connecting stimuli and cooperative signaling and response involving changes in gene expression do exist, according to recent students, contributing to the argument that this area of the body can, in fact, be classified as a distinct physiological system. One such instance where the gut microbiome collectively receives a stimulus, employs some signaling pathway involving changes in gene expression to produce some end response involves the gut reaction to xenobiotics, as indicated by a 2013 study. First researchers categorized microbes in the gut as inactive or active, and identified Firmicutes as the largest cohort of active microbes, using a combination of 16S rRNA sequencing,

metatranscriptomics, and flow cytometry. This allowed for the characterization of cells in the gut prior to manipulation by the introduction of xenobiotics, including eight antobiotics and sex-host targeted drugs. After the addition of these xenobiotics, fecal sample analysis showed an increase in the damaged cell cohort via incomplete membrane lysis. In this instance, one can observe a signaling pathway in its most basic form: a drug stimulus, pathways for cell lysis, and a response of cell damage (Maurice et al. 2013).

The study went further in analyzing other changes that took place within the gut microbiome after the addition of these substances. Researchers, after organizing the metagenomic data into gene clusters, recognized a statistically significant increase in the expression of genes that call for drug metabolism, antibiotic resistance, and phage induction across all active microbes. In this instance, the gut microbiome has entered into a phase known as a "stress response," a series of changes in the cellular machinery and metabolism in response to substances or environmental factors that threaten cell integrity and survivability (Maurice et al. 2013). In this study, a clear relationship between independent variable, xenobiotic addition, and dependent variable, changes in microbe dynamics via physiology and gene expression, can be clearly observed. However, some may argue that the results of the study reported thus far, despite a noted collective response of the entire microbiome in terms of changes in cell integrity and gene expression, do not contribute to the gut microbiome's classification as a unique human physiological system due to the fact that no specific genetic-molecular pathway between stimulus and response was charted to the extent of other response pathways like the coagulation cascade of the circulatory system. Because the researchers were able to identify the specific genes that were being activated or overexpressed, specific pathways were actually reported.

One specific drug administered in this study was Sulfasalazine, a compound that decreases bowel inflammation in patients suffering from ulcerative colitis and rheumatoid arthritis. Because of the linkages already noted between the gut microbiome and digestive diseases marked by inflammation of the GI wall, this drug is a primer in discussing the ability of the gut microbiome to respond to some stimulus in a communal manner. The addition of this drug led to an immediate increase in the expression of genes encoding for thioredoxins, protein involved in oxidation-reduction reactions that is conserved across many species, and nitrate reductases, which are enzymes that reduce nitrate to nitrite. These molecules are prime examples of species involved specifically in the metabolism of drug products to decrease toxicity or convert them to absorbable forms necessary for efficacy (Maurice et al. 2013). In characterizing the specific pathway associated with Sulfasalazine addition to the gut microbiome, researchers were able to further identify instances where the gut microbiome responds collectively to a stimulus. Whether the stimulus was antibiotic or host-targeted drug, the existence of any collective response, as generic as partial cell lysis or specific as Sulasalazine metabolism, in the microbiome contributes to the argument that this area of the body is a physiological system, rather than a collection of individual microbial species surviving and interacting with the larger human host without causing any observable effects on homeostasis, exhibiting communication with other physiological systems of the human body, or acting collectively to respond to different stimuli.

One might argue that the organisms within the gut merely respond individually, leading to a directed response; however, the fact that the organisms that reside in the gut constitute thousands of different genus and species is enough to refute this argument. The diversity of metabolic pathways and molecule secretion mechanisms between even two species would likely

prevent the end products of two different pathways from combining into some joint response; therefore, the microbes found in the gut survive and interact cooperatively in order to produce a systemic response to changes in the outside environment, modeled by the manipulation and addition of different stimuli. In fact, this was confirmed by high C-score and checkerboard numbers reported by Koenig in 2011 whose contribution has already been discussed in the context of gut microbiome development in infancy, indicating that the microorganisms in the gut do not exist randomly and independently of one another but actually interact in a commensal way, leading to the observable microbial compositions and metabolic characteristics present.

IV. Conclusions and Clinical Implication

The criteria for categorizing any grouping or complex of different cell types as a unique physiological system of the human body, alongside such college course giants as the circulatory and nervous systems, were separated into three larger thematic motifs:

(1) An intimate relationship with homeostatic maintenance, as characterized by the disruptions associated with pathology and corrections facilitated by transplantation of component cells

(2) Communication with other areas of the body and physiological systems through inter-systemic interaction, either directly or indirectly through a mediating molecules like hormones of the endocrine system

(3) Collective or majority response to stimuli, be it a specific molecule or change in some environmental factor, characterized by a specific pathway involving both genes and proteins

These three criteria were all met by the human gut microbiome, a conclusion supported by the results of different research reports from the last four years. For this reason, despite the various counterarguments proposed, it is more than called for that scientists begin referring to the human gut microbiome as a unique physiological system of the human species. The organization of this microbial community under such a title will allow new modes of thinking that will guide research in a new direction. If this area of the body is classified as such, studies examining more specific instances of inter-systemic communication will allow new insights into pathogenesis. Boundaries between the systems will be drawn more clearly and pathways of communication mapped with far more detail than before; as a result, changes or disruption in these newly-discovered genetic and molecular pathways can be manipulated more specifically in the laboratory, even down to the specific amino acid sequence of a receptor on a gut microbe, allowing for the characterization of pathogenesis unlike before.

Bibliography

Arumugam, Manimozhiyan et al., Enterotypes of the human gut microbiome. *Nature.* **473**, 174-180 (2011).

Berer, Kerstin et al., Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination, *Nature*. **479,** 538-541 (2011).

Everhart, James E., The Burden of Digestive Diseases in the United States. *United States Department of Health and Human Services, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases,* http://www3.niddk.nih.gov/Burden_of_Digestive_Diseases/ (2008).

Gailani, David and Renne, Thomas, Intrinsic Pathway of Coagulation and Arterial Thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. **27**, 2507-2513 (2007).

Hermansen, M., Nucleated red blood cells in the fetus and newborn. *Archives of Disease in Childhood: Fetal and Neonatal Edition.* **84**(3), F211-F215 (2001).

Honjo, T. and Melchers, F., *Gut associated lymphoid tissue*. (Springer, New York, 2006).

James, Susan D., Anxiety In Your Head Could Come From Your Gut. *ABC News*, http://abcnews.go.com/Health/anxiety-head-gut/story?id=20229136 (2013).

Kanczkowski, Waldemar et al., Characterization of the LPS-induced inflammation of the adrenal gland in mice. *Molecular and Cellular Endocrinology*. **371**(1-2), 228-235 (2013).

Koenig, Jeremy E. et al., Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America.* **108** (1), 4578-4585 (2011).

Markle, Janet G.M., Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity. *Science.* **339** (6123), 1084-1088 (2013).

Maurice et al., Xenobiotics Shape the Physiology and Gene Expression of the Active Human Gut Microbiome. *Cell.* **152**(1), 39-50 (2013).

Mullen, Gerard E. and Swift, Kathie M., *The Inside Tract: Your Good Gut Guide to Great Digestive Health*. (Rodale, New York, 2011), pp. 30-48.

Rao AV., A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathology.* **1**(1), 6 (2009).

Ravindra, Kumar et al., Sickle Cell Anemia—Molecular Diagnosis and Prenatal Counseling: SGPGI Experience. *The Indian Journal of Pediatrics.* **79**(1), 68-74 (2012).

Tucker, Kerry L. and Caspary, Tamara J., *Cilia and Nervous System Development and Function.* (Springer, New York, 2013).