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The Microbiome and Medicine: The Relationship between Early-life Colonization and Health Outcomes such as Asthma and Allergies

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Abstract:

Bacteria inhabit all surfaces of our bodies, and this symbiotic relationship is critical for our survival. The human gut, is colonized by bacteria who aide in digestion and other functions within our immune system. A healthy gut environment is characterized by a rich and diverse microbial ecosystem. The microbiome is fundamental in the proper functioning of our immune system and can influence many health outcomes. The gut microbiome has an essential role in infant health and development. In recent years, this long established relationship between beneficial bacteria and humans has been disrupted by environmental factors, antibiotics, maternal diet, delivery mode, breastfeeding. Early-life colonization is critically important for the establishment of diverse communities that impact short-term and long-term health outcomes. The immune system specifically is tied to the gut microbiome, and there are strong associations between immune development facilitated by microbes, and asthma and allergies.

Introduction:

Many researchers have attempted to understand the role of the microbiome and the symbionts found in our bodies. The microbiome is comprised of trillions of bacteria that inhabit all surfaces of the human body, including the gut (Yatsunenko et al. 2012). These organisms, their genes, metabolites, and interactions with one another, as well as with their host collectively, represent our microbiome. These microbes aide in digestion, outcompete and provide protection from harmful microbial species, produce vitamins and metabolites, and modulate the immune system. The study of phylogenomics delves into evolutionary adaptations and explores the functions of genes in complex relationships between multiple species. The knowledge of microbial genome and what they encode is severely limited. There are limitations in exploring

which species are critical for certain functions in our systems, and there are limited records of what species have been important over time. It is also difficult to culture certain bacteria that live in extreme conditions or have very specific niche growth requirements which limits the ability to observe these systems in-vitro. Studies often rely on comparisons of fecal samples to observe prevalence of populations (Bureello et al. 2018). The composition of the gut microbiome has been shifting, and keystone species may not be present to produce critical substrates and metabolites our bodies need. The diversity and microbial composition in our bodies is fundamental for our health and is impacted by the environment exposures (Blaser 2016).

Microbial Perturbations

Epidemiology studies and analyzes the distribution and causes of health and disease conditions in populations. In western civilization, medicine and antibiotics have been changing disease outcomes in these populations (Ferrer et al. 2017). Sanitation, antiseptics, vaccines, and antibiotics have drastically reduced the prevalence of the major illnesses of a hundred years ago (Blaser 2016). Modern illness is not polio, tuberculosis, or typhoid fever. These diseases that are so devastating in the past have been essentially wiped out. Antibiotics are life-saving drugs that have revolutionized our world. Antibiotics are able to target and inhibit components of bacterial cells, and kill the microbes in our system (Ferrer et al. 2017). These drugs are not targeted to attack the harmful microbe, but rather, eliminate all bacteria (Blaser 2016). Since Penicillin was discovered, prescriptions of antibiotics have been handed out for every cold and inconvenience. They have become so prevalent that most children receive multiple courses of antibiotics in their first two years of life (Bailey et al. 2014). Antibiotics are the most common medication for infants, with over 69% of children exposed and a median of 2.3 treatments by age two (Bailey et

al. 2014). This has been occurring for the past three generations, which also coincides with a rise in health conditions that were previously minimal issues in the population. Specifically, obesity, allergies, asthma, and atopic diseases have been rising in the general population and occurring in younger children.

Perturbation of microbial communities most often occurs by antibiotic use. These life-saving drugs have become so common-place in our society that most people who go to the doctor with a minor issue walk away with a prescription. Antibiotics kill the microbes causing the illness, but they also decimate populations of beneficial, or mutualistic, bacteria (Blaser 2016). The microbiome is a complex system that functions essentially as an organ. The interactions between species depend on colonization timing, population numbers, and the diversity of the system (Blaser 2016). *Clostridium difficile* infections are an example of what can go wrong when you eliminate mass populations of good bacteria. Patients experience extreme intestinal inflammation and gut microbiota changes as their community becomes dominated by one bacterial species (Milani et al. 2016). Other microbes also play critical roles in our bodies and when we eliminate them, our health is negatively impacted.

Studies have indicated that caesarian sections can lead to short-term and long-term health effects for women and their children (Sandall et al. 2018). This procedure, while often medically necessary, has increased dramatically in prevalence based on the convenience provided. Short-term risks for infants include altered immune development, reduced gut microbiome diversity and an increased chance of asthma and allergies (Sandall et al. 2018). There are long-term risks associated with C-sections as well, but longitudinal studies are less common and more difficult to implement. Longitudinal studies have immense value in studying the effects of early microbiome establishment and characterization of communities over time. One longitudinal

study looked into the impact of multiple antibiotic courses and the impact of delivery mode. That study described how the gut microbiome of the vaginally delivered children were dominated by Bacteroides (Yassour et al. 2016). They also discovered that the microbiota for the cohort of antibiotic children was, "less diverse in terms of both bacterial species and strains, with some species often dominated by single strains" (Yassour et al. 2016). Some recent findings have found an association between C-section infants and childhood obesity and asthma (Sandall et al. 2018). The developing neonatal microbiome's role in these outcomes is not well understood and more research into the mechanism leading of the development of these health issues needs more research.

Other than caesarean sections, other factors like maternal diet can impact the composition of bacteria in infants (Lundgren et al. 2018). The study examined the composition of species, determined by 16S RNA analysis on fecal samples, based on delivery mode and maternal diet. The microbial community structure of infants born vaginally was characterized by different species than infants delivered by C-section (Lundgren et al. 2018). Maternal diet that featured increased dairy intake was also associated with an increased chance of infants born by C-section having higher abundances of Clostridium (Lundgren et al. 2018). This study implicates maternal diet as a factor that can alter infant gut microbial communities, and the effects of diet are different based on the delivery mode of the infant.

Health Outcomes

Food allergies are a public health burden with prevalence increasing drastically in recent decades. In developed countries, the prevalence is estimated at 5-10% (Savage and Johns

2015). Food allergies have dramatically increased in prevalence is in the US and incidence is as high as 10% among 1-year-olds (Osborne et al. 2011). Specific mutualistic bacterial such as Bacteroides and Lactobacillus have been associated with decreased risk of atopic and allergic disease in model organisms like mice (Enomoto et al, 2014). Another study revealed that mice on antibiotic courses early in life had a higher chance of becoming sensitized to peanuts, and suggested that Clostridium was able to attenuate the occurrence of allergic disease and block allergic proteins from reaching the bloodstream (Stefka et al. 2014). These findings can have garnered support for the hypothesis that antibiotics may be an environmental exposure associated with the increase in allergy prevalence in children.

Allergic disease is strongly associated with environmental factors and perturbations of the microbiome and the immune system. The human microbiome is an essential mediator in the induction of oral tolerance and food allergy. Studies have explored the impact of gut imbalances in the early lives of infants and an increased risk of developing food allergies. Fecal samples of infants have been sequenced and showed less intestinal gut microbiota richness among infants who later developed allergic and atopic disease (Ismail et al. 2012). Other studies have examined the prevalence of microbiota diversity and milk allergies, and found microbiome enrichment can influence the likelihood of a child growing out of the allergy (Bunyavanich et al. 2016). While association does not prove causation, or identify specific mechanisms, these trends are significant to note that in general the microbiome contributes in some way to the development of asthma and allergies.

Other studies have explored intestinal barrier immaturity in preterm neonates, otherwise known as “leaky gut” (Ma et al. 2018). In this longitudinal study utilizing fecal sample microbiota analyses, increasing abundance in Clostridiales and Bifidobacterium were found to be

associated with decreased intestinal barrier function in preterm infants (Ma et al. 2018). Neonatal factors that positively affect newborn gut microbiota like breastfeeding, and reduced antibiotic exposure were associated with early colonization by Clostridiales and overall improved gut barrier maturity in infants (Ma et al. 2018). These findings point to the necessity of certain species to colonize infants at critical points in development in order for the infants to have optimized health. Another study looked at pre-term infants and health outcomes like sepsis (Wandro et al. 2018). These infants face abnormal colonization based on earlier exposure to bacteria and increased use of antibiotics. Fecal samples of 32 infants were collected and bacterial composition was characterized by 16S RNA sequencing and GC mass spectrometry. The results found that preterm infants were dominated by Enterobacteriaceae, Enterococcus, and Staphylococcus organisms based on their exposures to antibiotics. These infants lacked Bifidobacterium, a highly beneficial microbe. The study was unable to match bacterial signals to any one specific bacterial composition but noted that the profile of preterm infants was altered from that of a typical full-term infant who would experience different colonization (Wandro et al. 2018). This suggests that there is not one microbe causing the symptoms, but rather a difference in community structure overall that alters the host.

Immune System:

The gut is the barrier between your bloodstream and your environment. The mucosal lining of the gut, or Gut-associated lymphoid tissue (GALT), comprises almost 70% of the immune system. This represents a significant localized area that is home to complex immune development and interactions between external factors coming into contact with the gut-barrier. While there are many complicated features to the immune system, one important facet are T

cells. They are responsible for seeking foreign invaders and killing infected cells. T cells also contribute to oral tolerance of allergens (Mucida et al. 2005). Oral tolerance occurs in the gastrointestinal tract and is a response to the body ingesting antigens. The GALT have an important role in decreasing excess inflammation in response to commensal bacteria and allergens (Chinthrajah et al. 2016). The gut barrier is important in immune development and has strong associations with allergic outcomes for the host.

In most autoimmune diseases such as multiple sclerosis and diabetes, T cells have been highly associated with disease development. They have also been related to allergic reactions where food intolerance is based on recognizing self and foreign particles. The gut microbiome promotes oral tolerance to Immunoglobin E (IgE) and mediates allergies. The induction of food tolerance takes place in the gut when the immune cells encounter food antigens (Chinthrajah et al. 2016). Humans mainly acquire tolerance to food through dendritic cells, gut-barrier epithelial cells, and the gut microbiome (Mazzini et al. 2014). T cells also reduce the number of effector cells, which help develop oral tolerance to allergens. B regulatory cells suppress effector T cells and contribute immune tolerance of allergens (Rosser et al. 2015). Certain bacterial strains such as, *Bifidobacterium longum*, assist in the maturation of dendritic cells and the development of T cells, suppressing the expression of food allergy (Lyons et al. 2010). Specific microbial signals have been deemed necessary for proper education of regulatory T cells and invariant natural killer T (iNKT) cells which induce or suppress immune responses the immune tolerance to food allergens. Overall, there are many types of immune cells involved in developing oral tolerance, and the microbiome is highly associated with key interactions with the immune cells.

The overall community structure and balance within is very important for human health, but certain species can have profound effects on community diversity and interactions. The

composition and diversity of the gastrointestinal microbiota is measurably affected by perinatal (the time immediately before and after birth) *Heliobacter pylori* exposure (Kyburz et al. 2018). H. pylori, ancestral gut inhabitant is inversely associated with the prevalence of asthma in children. There is a protective factor conferred by the presence of certain bacteria, or the interactions between them and the community (Konieczna et al. 2015). Loss of keystone species can prevent immune system maturation and differentiation of T cells and B cells (Rosser et al. 2015). Maturation of the intestinal immune system is contingent on parallel development of the gut microbiome.

Mouse models have allowed researchers to investigate the role of the microbiome in relationship to health outcomes. Germ-free mice have significant immunological defects and improper development of immune cells (Rivas et al. 2013). Another study found a relationship between gut colonization by microbes in mice and increased protection against allergies and oral sensitivity (Rodriguez et al. 2011). These gut tissues require interaction with microbes for differentiation and specification and development of adaptive immunity (Konieczna et al. 2015). The associations between a lack of a microbial community and increased asthma and allergies points towards the significance of microbial communities for their host.

The Necessity of Bacterial Diversity

The microbiome is complex and diverse. The gut alone is comprised of 40,000 different species that compete and cooperate with one another (Kim et al. 2017). These interactions are intrinsically complicated. The various ways species interact and produce by-products contribute to host health in profound ways. The number of species and evenness of communities ultimately

results in a homeostatic gut. The diversity of gut communities has been cited by many researchers who observe the richness and evenness in association with certain trends or health outcomes. Over 200 health outcomes have been associated with improper balance of the microbiome. Humans need a high total number of bacteria, of numerous species in certain proportions to maintain balance and optimal health.

As interest in the microbiome has increased in recent years, the prevalence of birth cohort studies, and publications with data observed over longer frames of time have increased. A large cross-sectional survey of young children with milk allergy showed that greater gut microbiota diversity and enrichment of Clostridia and Firmicutes phyla during early infancy is associated with greater likelihood of out-growing milk allergy by eight years of age (Bunyavanich et al. 2016). Without bacterial diversity, significant health trends have been observed. Another study found that antibiotics given to neonatal mice reduced the abundance of Clostridia and as a result induced food allergies (Stefka et al. 2014). Clostridia colonization is important for stimulating IL-22 production to prevent food antigens from crossing the gut epithelium (Stefka et al. 2014). It is clear that there is a relationship between the presence of mutualistic bacteria and allergic disease.

Early-life colonization is fundamental to establish communities of microbes within the body. From the moment children are born, they are constantly exposed to various bacteria. The delivery mode introduces specific bacteria to the infant. As the infant passes through the birth canal, the nose, face, and body are coated with bacteria from the mother's vagina. These are some of the first species to colonize the infant, and are commonly Lactobacillus which will aide in digestion of the infant's first meal. A study on the difference between delivery mode and abundance of bacteria in infants reports that, "vaginally born infants have a higher abundance of

the Bifidobacterium genus compared with those born by C-section, as observed during the first week of life," (Jakobsson et al. 2014). The bacteria that colonize infants in their first moments of life are often keystone species that establish important relationships early on. C-section deliveries that have been shown to have an alteration of microbial composition and lower bacterial diversity, have also been associated with processes of immune system maturation, imbalances, and related diseases including asthma and allergic disorders (Bokulich et al. 2017). Studies have shown a difference in the composition of infant guts based on the delivery mode, but this discrepancy has also been cited as related to the antibiotics used in C-sections before and during the surgery and by the difficulty of breastfeeding during the first hour of life of those infants," (Bokulich et al. 2017). Infants born by C-section also had a "delayed colonization of the Bacteroidetes phylum, and specifically of the Bacteroides genera, during the first month of life," Nagpal et al. 2016). Decreased gut diversity and delayed colonization were both associated with reduced Th1 immune responses, which would lead to increased Th2 response to antigens and increased allergic outcomes.

Bacterial diversity and evenness in species abundance, is fundamental for optimal health. Intestinal microbes produce metabolites and nutrients that help the host gain establish resistance to infection. Gut homeostasis is dependent on the composition of the microbial community to maintain the balance between inflammatory mechanisms. One study found that regulatory T cells of the immune system express a transcription factor that has a central role in decreasing inflammatory pathways in the intestine (Arpaia et al. 2013). The results of this study suggest that the metabolites produced by microbes, including short-chain fatty acids, mediate communication between the immune system and the microbiome. The metabolites act as signals to the immune system and reveal insight into the way these by-products influence the balance of anti-

inflammatory cells and pro-inflammatory cells (Arpaia et al. 2013). This study is significant in revealing the association between mutualistic microbiota and how they are able to communicate with and influence the immune system through production of regulatory T cells. The complicated interactions between the immune cells and bacterial communities are difficult to detangle, but it is apparent that there is an association between the mutualists and immune development, which has a host of cascading responses on human health.

Challenges and the Future of Medicine:

It is clear that perturbations to microbial communities can occur through antibiotic use, maternal diet, and delivery mode of the infant. The dynamic nature of complex interactions between species and the host development during colonization is implicated in many health outcomes. How can this research feed into useful applications for patients with medical concerns? Where does the field of medicine go from here and can you detangle a culture based on quick-fixes and last-minute solutions rather than prevention? The medical field has close ties to big pharmaceutical companies and insurance companies who have immense investments depending on the prescription of expensive drugs to patients. Patients often go to the doctor once symptoms are unbearable. Physicians face the challenge of wanting to help their patients and giving them antibiotics is often rationalized as an option that "can't hurt."

Pre and probiotics are not in a place where they can target the specificity of unique microbial communities in each individual. Lately there has been a rise in use of probiotics, and while introducing bacterial cultures after a course of antibiotics can be beneficial, in most cases the effects are unknown. The FDA has yet to approve use of any probiotic on the market. It is

extremely difficult to recommend a product that could have wildly different outcomes based on the individual. Each person has such varied microbial communities, what is helpful to one person may be disruptive and damaging to another.

Fecal Microbiota transfers have had much success in *Clostridium difficile* infections. The transfer of healthy microbes into a person experiencing extreme inflammation issues has had much success in restoring the community balance to the individual. More data and samples of people worldwide should be taken for analysis of trends and patterns of populations. This data could give more insight into disease outcomes. Screening infant stool for microbial community structure could potentially be a way to detect future risks and issues. More research into specific mechanisms and pathways could elucidate how microbial community interactions lead to health benefits and could help identify what goes wrong in these interactions for people with negative health outcomes. Healthcare practices should be implemented now to change the way medicine diagnoses conditions and focus more on long term health. Reducing antibiotic use would certainly feed into this, if doctors were able to feel less pressure from patients to just give them a prescription, antibiotic resistance and the negative impacts to microbial communities could be lessened.

Our society is a long way off from individualized medicine, but as the function of our genome and the ways genetics impact our health become more common, the microbiome will certainly need to be factored in as well. Each person has a unique set of genes, and also a unique microbial make-up that contributes to their overall functioning and health. It is apparent that the composition and diversity of the microbiome has powerful effects on human health. The profound impact of our bacterial symbionts will hopefully be integrated and considered in future medical practices.

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