

REVIEW ARTICLE

Human Health and the Microbiota: Interactions Between Gut Microbes, Hygiene, and The Immune System

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Keywords:

Helper T cell (Th): Type of lymphocyte that via cytokines activates parts of adaptive immune system

Cytokine: proteins secreted by immune cells

Interleukin (IL): Type of Cytokine

T regulatory cell (Treg): Type of T cell which regulate immune responses

Xenobiotics: unnatural substance found in an organism

Eosinophil: Type of immune cell that targets parasites and contributes to allergic disease

Dendritic Cells: Antigen presenting cell

Antigens: Substance that may induce an immune response

Abstract

Background: In the past, much of the scientific research on microbes focused on mechanisms of infection and disease. This was not in vain, as we gained valuable knowledge about our immune system, as well as the ability to develop vaccines and antibiotics. However, the relationship between humans and microbes is complex. These species have been co-evolving since multicellular organisms evolved on Earth.

Summary: Recently, it is beginning to be appreciated that the majority of relationships between humans and microbes are beneficial. From this follows an understanding that beneficial microbes are vital to the normal physiological development of our gut and immune system. This beneficial relationship between the human host and the multitude of microbial communities is well established. However, currently in the developed world epidemiological studies are showing dramatic increases in autoimmunity, allergies, and obesity. It is thus suggested that within westernized societies hygiene is altering the relationship between the gut and the human host in a way that makes humans susceptible to conditions not seen in less developed countries. This understanding advanced the “hygiene hypothesis,” and more recently the, “old friends hypothesis” and “disappearing microbiota hypothesis” as possible explanations for the observed epidemiological phenomena. What follows is a review of the relationship between gut microbes and the host’s immune system, with a focus on how hygiene (antibiotics, chlorination of water, etc.) is beginning to alter this relationship. This review concludes that a further understanding of how hygiene affects the relationship between humans and microbes will be crucial for developing effective therapies considerate of our microbial friends.

Introduction

Bacterial life inhabited earth billions of years before humans evolved. Therefore, it is well accepted that during the evolution of complex multi-cellular organisms, the microbial world played a role in influencing the structure and function of humans (1,2). This proposition is further supported by the evidence that there are an order of magnitude more microbial cells than somatic cells in and on the human body (3,4). For these reasons, it is common to view the relationship between the host and most microbes as mutually symbiotic (2,5,6). The recognized ecologic definition of symbiosis put forth by Anton de Bary is a prolonged association between two or more organisms of different species. Mutualistic if both organism benefit and parasitic

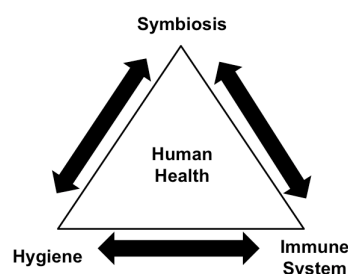


Fig. 1

This review will explore how the symbiotic microbes in our gut, hygiene (e.g., increased use of hand sanitizer, cleaning products, antibiotics and vaccinations), and the immune system interact with each other to contribute to human health.

if one is harmed while the other benefits. The relationship is mutually symbiotic in the sense that the microbiota access nutrients not attainable through host digestive mechanisms and help to guide the proper development of the host's immune system (2,3,4,5). In return the collaborating microbiota gain a safe, nutrient rich ecosystem (2).

Much of the microbiota that consists of bacteria, viruses, fungi, and other microeukaryotes live symbiotically within the human gastrointestinal tract (2,3,5). Thus, the gut serves as a useful setting to understand the benefits of this symbiotic relationship. In order to maintain this safe environment it is likely that the gut microbiota evolved to function closely with our own immune system, which would selectively destroy pathogenic agents and maintain the non-pathogenic populations (2).

Additionally, the gut microbiota play a vital role in immune homeostasis, or the maintenance of a stable immune system that neither overreacts or is too passive (5). However, this cooperation between species can be delicate due to the extreme microbial load present in the gut. The massive amount of microbes is a constant threat to the host systemically (7). If a usually symbiotic microbe's cost of cooperating becomes higher than the benefit it receives from the host, it has the potential to become pathogenic and invade systemically (7). Thus, those with immunodeficient disorders are at risk of infection from usually symbiotic bacteria, such as, *Enterococcus faecalis* and *Bacteroides fragilis* (7). For this reason the immune system must be able to maintain the balance of many different microbes while not knocking them out completely (7). The responsibility to differentiate between pathogens and commensal microbes, and to organize and maintain the location, in which these microbes inhabit their host, falls to the host's immune system (8, 9). The innate immune system seems to be primarily responsible for this intricate control of the mucosal environment (9). It must both protect against many pathogens as well as cooperate with innumerable amounts of symbionts. It accomplishes this by isolating all the microbes to specific locations within the host, as the symbiotic bacteria are often only symbiotic in the location in which they harbor, such as the gut, vagina, or skin to name a few (10). The innate immune system performs this task by sampling at the molecular level and can be induced by the components of microbial cell walls alone (11). For example, lipopolysaccharides, and peptidoglycans or microbial-associated molecular patterns (MAMPS), are ligands for pattern recognition receptors (PRR) on innate immune cells, such as, macrophages, and include toll-like receptors and mannose receptors (9, 11, 12, 13). More specifically gram-positive bacteria express lipopolysaccharides, and gram negative bacteria express teichoic acids (9). Through the recognition of specific MAMPS it is possible for the innate defence to differentiate between friendly MAMPS (symbionts) and pathogenic MAMPS or PAMPs (Pathogen associated molecular patterns) (9). If cells of the innate immune system (Antigen presenting cells) are activated via MAMPS interacting with PRRs, it is possible for an adaptive immune response to be activated (9).

Therefore, understanding the symbiotic relationship that the immune system shares with the microbiota may be a powerful tool to understand and improve human health. However, this relationship can be frustrated and strengthened with the addition of hygiene (Increased use of hand sanitizers, cleaning products, antibiotics etc.). This review will begin with an overview of the relationship between the host and its symbiotic microbes and conclude with a discussion on how hygiene fits into this relationship.

Evidence for symbiosis

The 20th century focus on pathogenic microbes led to an understanding of immune response to pathogens and medical advances in vaccinations and antibiotics. However, it is now being appreciated that most interactions humans have with microbes are either commensal (one organism benefits, while the other receives no benefit, but remains unharmed) or mutually symbiotic, where both organisms benefit in some way (8, 14, 15).

Within a successful host-microbe relationship, microbes may benefit the host by releasing molecules that inhibit pathologic microbes (8). This means that in addition to the chemical (e.g., intestinal mucosa containing immunoglobulins, cytokines, chemokines and antimicrobial peptides) and physical (e.g., gastrointestinal epithelium) barriers offered by the host immune system, the symbiotic microbes also assist the immune system in this defense (12). Microbes contribute to the gastrointestinal physical barrier defense by creating competition for nutrients against the pathogens (8,12), and also by modulating cytokine production (12).

Microbes may also attain nutrients for the host. Examples include the digestion of plant material (cellulose or host-derived mucin) by microbes required for their own growth, which are delivered to the microbes via the host's diet (8, 16). In return, the fermentation products can act as an energy source (e.g. butyrate as energy for colonocytes), gene expression regulators, and as inflammatory mediators to the host (8, 16). Other non-digestible materials such as inulin and fructo-oligosaccharides ingested by the host can act as energy sources (prebiotics) for the healthy microbes in our gut as well (16). Most interactions with microbes are not pathogenic, instead they are mutually symbiotic.

Evolution of normal host Immunity

Host immunity evolved in the presence of the microbiota. Due to a technique known as 16S ribosomal RNA gene sequencing much more accurate records of gut microbes can now be established when compared to only using culture based studies (8, 11, 16). 16S ribosomal RNA gene sequencing is beneficial taxonomically because it is found within most bacteria, has a conserved function and is large enough

to be detected (17). From 16S ribosomal RNA gene sequencing, it has been shown that microbial communities consist of a few phyla (8, 11, 16). Examples include Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Chlamydiae, Cyanobacteria, Defferribacteres, Deinococcus, Fusobacteria, Spirochaetes, Verrucomicrobia (8, 11). Although, at first glance this may not seem like a lot of variation, at a species and strain level there is much diversity (8). This is thought to be due the many years of co-evolution between microbes and humans, resulting in only the successful symbionts persisting and evolving within the human gastrointestinal tract ecosystem (11).

In order to elucidate the interactions between the microbiota and the host, a technique called gnotobiology is commonly used (11). Gnotobiology is the study of germ-free animals while observing the consequences when re-colonized with certain microbes (11, 17). A germ free animal is one aseptically derived such that it has never had a microbiota. These studies have shown that the microbiota tremendously influence the host's immune system, intestinal epithelial cells, metabolism of materials present in gut, absorption of nutrients and even endocrine function (11). One example of the microbiota's effects on the intestinal epithelial cells comes from studies involving *Bacteroides thetaiotamicron*. When germ-free mice were populated with only these microbes and transcriptional responses were observed with DNA microarrays, complement reactive protein-ductin receptor expression was observed (11). This receptor is known to aid in epithelial repair (11). However, it is important to remember that mono-colonization studies are limited in their interpretation because it is the net function of microbial communities that drives host function.

Without the microbiota present in the gut the host organism suffers in the way of undeveloped tissues, underdeveloped immune function, malnutrition, and a serious vulnerability to infection (11). As demonstrated by germ-free animal experiments, a reduction in IgA antibodies, smaller Peyer's patches, and a reduced number of T cells has been found, all important contributors to a normal immune system (17). Furthermore, without the continuous presence of microbes in the gut, the antimicrobial substances usually ubiquitously present that act as part of the innate defense to control not only pathogens but all microbial populations are drastically reduced (17). All these changes culminate to produce an underdeveloped, immature immune system. This is demonstrated by evidence showing that with germ free mice, secondary immune organs (i.e. spleen and lymph nodes) also contain underdeveloped lymphoid follicles and reduced B and T cell populations (17, 10). Thus, the host immune system requires the presence of microbes in the gut to develop properly.

Where does hygiene fit in?

Now that the importance of the host-microbe relationship has been established, how hygiene gets involved can be explored. In 1989, Dav-

id Strachan observed a huge increase in the amount of allergies such as hay fever and could not explain this huge increase with genetics alone, and thus, the hygiene hypothesis was born (19,21,26). If genetics alone could not explain this increase, then the environment must also be playing a role (21). He suggested that although increased hygiene (e.g. increased antibiotic use, vaccinations, and better cleaning procedures such as, pasteurization, sewage treatment and chlorination) has made great contributions to human health, it also altered our immune system such that we are more susceptible to the development of allergy and autoimmune disease (26). This "hygiene hypothesis" was advanced because such increases in allergies were not seen in undeveloped countries where hygiene was less controlled (22). Additionally, epidemiological studies in 1998 showed that atopic diseases such as asthma afflicted one in five children, with the numbers reaching epidemic levels today (22). Furthermore, autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, and multiple sclerosis have also had an increased prevalence in developed countries as compared to undeveloped countries (22). In other words, the hygiene hypothesis suggests that the increased hygiene has altered the symbiotic relationship between the host and the microbiota to such an extent that the host's immune system becomes reactive to the host and harmless antigens.

It is important to note that the interactions occurring are complex, and with so many compounding variables it is difficult to say with certainty that the rise in allergy and autoimmune diseases is truly a result of increased antibiotic use and hygienic practices. For example, opponents to the hygiene hypothesis suggest that perhaps the increase in autoimmune diseases may be simply due to better techniques for diagnosing such conditions in the developed world (22). Nevertheless, with epidemiological studies showing a difference between prevalence of allergies and autoimmune diseases between developed and undeveloped countries, combined with the clear difference in hygiene and medical practices, the hygiene hypothesis is worth considering (26).

One of the first mechanisms put forward to explain the observation of increased allergies and autoimmunity in Westernized societies is an imbalance between Th1 and Th2 cells, to key immune cells known to play a role in both autoimmunity and allergy respectfully (26). This hypothesis was explained by the knowledge that most allergies are associated with an increase of the immunoglobulin E (IgE) antibody, which is secreted by B cells via assistance from Th2 cells (22). In undeveloped countries where early childhood infection is common, antigen-presenting cells such as dendritic cells promote Th1 differentiation through secretion of IL-12 and IL-18 (20). However, in developed countries where early childhood infection is low, Th1 differentiation is also low. Because Th1 and Th2 cells can inhibit each other's differentiation, a loss of Th1 cells may lead to a skewed Th2 population of cells in the body resulting in inappropriate production of IgE and development of allergic disease (22).

This hypothesis may be too simple, as there are many paradoxes that cannot be explained. For example, in undeveloped countries there are also high levels of parasitic infection as well as bacterial and viral infections. Parasites such as intestinal helminths, or worms, elicit a potent Th2 response. Another paradox also presents itself with the increased prevalence of autoimmune diseases observed in developed countries. This is because autoimmune diseases are known to be associated with an inappropriate Th1 response, and if there are truly a lower number of Th1 cells due to less infection, there should not be an increase in autoimmune diseases (20, 21, 22).

Due to the above conflicts, researchers began to explore the possibility that T regulatory (Treg) cells, an immune cell responsible for the down regulation of inflammation, may be responsible for the protection observed in undeveloped countries (20, 21, 25, 26). Perhaps helminths do increase the Th2 response and also induce Treg cells to secrete interleukin-10, a potent anti-inflammatory cytokine to assist in their own survival within the host (20, 25). However, the confusion only deepens with more conflicting evidence. With some groups suggesting findings that helminths offer protection from allergies with increased interleukin-10 production (20, 25), while others groups have reported no difference in cytokine production (23). To further convolute the investigation, a report done in the United States surveying approximately 20,000 Americans on physician diagnosed autoimmune and allergic disorders found that an increase in allergy was positively associated with an increase in autoimmune disease (24). This data weakens the hypothesis that an imbalance in Th1 and Th2 cells provokes allergy and autoimmune disease (21). Although this explanation is now out of date, it was one of the first attempts made to explain the phenomena of the hygiene hypothesis.

Since then other suggestions have been put forward with an updated understanding of the hygiene hypothesis. Currently it is defined as the idea that the increased incidence of allergic and autoimmune diseases in developed countries may be linked to a lack of exposure to microbes in early childhood. This allowed for the old friends hypothesis, another possible explanation for the increased prevalence of autoimmunity and allergy in developed countries (21, 25, 26). The old friends hypothesis suggests that in undeveloped countries, chronic exposure to pathogens, including parasites that can establish themselves in the host, induces a chronic and harmful aggressive immune response that can damage healthy host tissue (21, 25). The immune system has adapted by up regulating the number of dendritic cells able to sample antigens from these chronic pathogens (21, 25, 26). As a result of this increased sampling, dendritic cells enhance the differentiation and/or function of Treg cells to these pathogens (i.e., "Old Friends"). These Tregs are constantly present, and thus, prevent damaging immune responses (21, 25, 26). Quite coincidentally, with increased number of dendritic cells sampling antigens, they also sample self-antigens and allergens more often as well, and similar to their response to the continuous exposure to "Old Friends" there is a corresponding up regulation of Treg cells towards self-antigens

and allergens as well (21, 25). Hence, the lower prevalence of autoimmune disease and allergies in undeveloped countries as compared to developed countries can be explained by dendritic cells increased presentation of self-antigens (21). Therefore it has been suggested that increased prevalence of allergy and autoimmunity may be the result of humans being able to adapt to their changing environment with technology much faster than our genetics are able to keep up (21). However, this hypothesis is not widely accepted on its own. To make this explanation more plausible it must be explained with the disappearing microbiota hypothesis (27, 28). In this hypothesis it is suggested that although antibiotics are a powerful tool we have to fight infection they also destroy the host's symbiotic microbial communities (27). This collateral damage to the host's symbiotic microbes is then hindered in its recovery by chlorination and pasteurization etc. that not only destroy pathogenic microbes but symbiotic ones in our environment as well (27). This lack of symbiotic microbes in the environment makes it difficult for the host to repopulate its beneficial microbial communities (27, 28). One example of a disappearing microbe is *Helicobacter pylori*, a microbe found in our stomachs (27, 28). Thus, this pattern of collateral damage to our microbiota and lack of symbiotic microbes in our environment slowly depletes our stores of healthy microbes (Hunter). The consequences of this are not only allergy and autoimmunity but obesity as well (27, 28). A key difference between the disappearing microbiota hypothesis and the previous two explanations put forward is that in the disappearing microbiota hypothesis emphasis is put on the lack of exposure to healthy microbes while the other two put emphasis on the lack of exposure to infectious disease (28).

These three hypotheses together prove that a complete mechanism to explain the epidemiological observation of increased autoimmunity and allergy in developed countries is not simple. However, they clearly outline the fact our increased hygiene in developed countries is altering the host's relationship with symbiotic microbes. Moreover, this altered relationship is affecting the host's immune system. I do not want to suggest that the use of antibiotics and hygienic practices such as chlorination of water are negative and thus should be stopped. The purpose here is to call attention and emphasize that the majority of our interactions with microbes is not pathogenic, and that we should be cautious and considerate of our microbial friends.

Conclusion

Over billions of years, we have co-evolved with the microbes that call us home. The gastrointestinal microbiota, a complex and dynamic ecosystem, is home to trillions of harmless microbes. These microbes are essential to the normal development and function of our immune system, and pivotal in absorbing nutrients and vitamins. Gnotobiotic animal studies have demonstrated the devastating effects on the host's ability to defend itself against various pathogens when the microbial community composition is altered.

It is clear that there is still much to learn. The mechanisms of the three hypotheses have proven to be elaborate, representing a challenge to find solid experimental evidence to explain the epidemiological patterns being observed, especially with regard to allergy and autoimmunity. Despite the complexity, it is clear that with a better understanding of our symbiotic relationship with microbes and our hygiene may affect the function of our immune system, it will be possible to incorporate better therapies for various ailments, and improve the quality of life for residents in both developing and developed countries.

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