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Detection of Microbiome Pathogens by the Immune System

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Abstract

In the past few years, interest in the human microbiome has grown, and its role in disease prevention and development is just now becoming evident. The oral microbiome is of particular interest to scientists due to the presence of pathogens in a highly vascular cavity. The oral pathogens produce no antibody response by the immune system until an inflammatory event spurs the immune system to react. How is it that pathogenic microbes reside in the oral cavity without being targeted by the immune system? It is common for people to have *Streptococcal mutans,* and other pathogens as part of the oral microbiota. There are those who "carry" *S mutans,* or *Staphylococcus aureus,* and other pathogenic microbes, and do not seem to get sick; although they are able to infect others. How is it that their immune system tolerates those pathogenic microbes? A hypothesis that might explain immune 'tolerance,' is that the immune system labels the microbes as 'self' instead of 'foreign.' Many are familiar with this concept in the realm of cancer cells. The immune system labels cancer cells as 'self,' and is unable to identify and destroy them. Exposure to pathogenic microbes may elicit an immune response but possibly people who 'carry' pathogenic microbes as part of their natural microbiota do so because their immune system identifies those microbe as 'self.' They could still become sick by this pathogen, if for example, there is a microbial imbalance and *S. Mutans* invaded the tonsils. If other non-pathogenic microbiota replicated enough to dominate the area, the infection would resolve. The immune system would not be able to kill it, if it could not identify it as 'self.' If this were the case, the microbiome would be more influential in the progression, or halting of the disease process.

Research studies show that many bacteria have developed the capacity to evade the immune system. Virulence factors help in the evasion process, and are many and varied.

However, the hypothesis explored in this paper looks into another possible explanation for pathogenic microbes not being detected by the immune system. It is hypothesized that some pathogens of the oral and skin microbiota are identified as 'self' by the immune system. Implications of oral pathogens being identified as 'self,' could be; uninhibited access to the body. Their toxins could produce an antibody response, but the bacteria would not be identified and eliminated. This could result in chronic, unresolved inflammation, plaque build-up and tissue destruction, especially in the case of periodontitis which will be examined in greater detail in this paper.

Atherosclerosis, rheumatoid arthritis, dementia, periodontitis, and other chronic inflammatory diseases have been linked to pathogens of the oral microbiome. Studies need to be done to determine if the pathogens, or bacterium that are implicated in those diseases elicit an immune response. Certain research studies show that toxins produced from certain microbes produce an antibody response, but the bacterium itself does not. If the body only responds to the toxins and not to the bacteria, this would create a situation of chronic inflammation. Antibodies would be sent to destroy the toxins, and the bacteria would continue to replicate and produce more toxins, which would lead to increased pain, swelling, and tissue destruction for the affected individual. More studies need to be done to determine if the immune system does not respond to the bacterium involved in chronic inflammatory conditions.

Detection of Microbiome Pathogens by the Immune System

The term microbiome has been defined as a community of commensal, symbiotic, and pathogenic micro-organisms that inhabit our bodies (Duzgunes, N., 2016). Nobel Laureate Joshua Lederberg helped define microbiome, and stated that the microorganisms of the microbiome "have been all but ignored as determinants of health and disease" (Duzgunes, N. 2016, p. 151). The oral microbiome has been identified as a source of pathogens that cause disease in other areas of the body. Endocarditis, atherosclerosis, rheumatoid arthritis and other diseases have been linked to oral pathogens (Gehrig, & Willman, 2016).

The Human Microbiome

Bacteria and other microorganisms colonize the mucous membranes of the respiratory tract, the oral cavity, the digestive tract and the skin surface. Bacteria and other organisms that are found in these areas and are not causing infection or disease, are known as normal flora. Normal flora may include commensal bacteria and pathogenic bacteria, fungi and even viruses (VanMeter, VanMeter, & Hubert, 2010). Microbes inhabiting the body will migrate to areas that favor their survival and replication. In the case of oral microflora, it is likely that there are over 700 common oral microbial species (Duzgunes, N. 2016). The normal flora protects the body against disease-causing pathogens by preventing their attachment to the host. Having a diverse microbiome has been associated with a state of health. This is due to the fact that the microbes on the body are competing for nutrients. The greater the variety of microbes, the less likely it is that a pathogenic microbe will out-compete the others for nutrients and replicate to the extent that it will initiate disease in the host. The fewer the microbes that a host carries, the more likely it is that a pathogenic microbe forming part of the normal flora, or encountered in the environment, will attach, replicate and cause disease. The pathogen that forms part of the normal

microbiota and causes disease under certain conditions is known as an opportunistic pathogen (VanMeter, VanMeter, & Hubert, 2010).

The microbiota of the body has resident flora, which are microbes that remain with the person their entire lifespan. *Escherichia coli* is one of those microbes. The body benefits from the presence of *E. coli* in the large intestine since it helps to breakdown nutrients to release vitamins. (Gehrig, & Willman, 2016). *Strep sanguines*, is a bacterium which helps prevent caries, while *Propionibacterium acnes* aids in the production of cobalamin and short chain fatty acids (Jimenez, et al., 2005). Other microbes are not beneficial, such as *Strep mutans,* which has been implicated in caries and heart disease, and *Aggregatibacter actinomycetecomitans,* which can damage gingival tissues and is present in many cases of periodontal disease (Gehrig, & Willman, 2016). Transient flora, are temporary residents that may survive only a few hours or months. Any new microbe that attaches to the host will have to compete with other microbes for nutrients, and will have to deal with the immune system's response if detected. The new microbe might also be eliminated if the environment changes in a chemical or physical way and is no longer conducive to survival. (Gehrig, & Willman, 2016.)

Acquisition of the Microbiome

There is no clear answer as to how we initially acquire our oral microbiota. It has been suggested that we acquire it after birth from our mothers, first as we pass through the vaginal canal, and second, through skin to skin contact, and skin to mouth transmission through feeding (VanMeter, K., VanMeter, W., & Hubert, 2010). Newborns are also exposed to microbes in the air. As infants breathe, microbes enter the mucous membranes of the lungs. Transfer of *S. salivar*

ius, S. mutans, and other microbes have been shown to have been passed through saliva, from mother to child, and through contact within family groups (Marsh & Martin, 2009). Some studies indicate that babies are exposed to bacteria even before birth (Jimenez, et al, 2005).

As microbes invade a newborn baby's body, it is hypothesized that the immune system is immature, and is not able to distinguish 'self' from 'non-self' cells. Another explanation would be that since mother and baby were one, the microbiome shared with mom is labeled as 'self.' In any case, microbes that are part of the initial invasion, may be labeled as 'self,' by the immature immune system. More studies need to be done regarding this hypothesis.

Pathogens of the microbiome are involved in producing hormones and vitamins that are needed to survive, but they are also linked to diseases involving chronic inflammation such as periodontal disease, atherosclerosis, rheumatoid arthritis, and many others (Gehrig & Willman, 2016).

The Oral Microbiota and Periodontal Disease

Periodontal disease is not curable. It is the destruction of the gingiva, periodontal fibers and alveolar bone of the periodontium. The bacteria that cause it can be reduced through use of antibiotics or mechanical removal. The current theory on periodontal disease proposes that the presence of plaque biofilm is necessary for disease initiation. This theory puts forth the idea that initial inflammation to plaque causes gingivitis, and the host response to the bacteria causes progression from gingivitis to periodontitis. It proposes that the inflammation within the subgingival tissues is caused by the host response which drives the changes in the microbial composition of the biofilm (Gehrig, & Willman, 2016). It is hypothesized that the process for developing periodontal disease may be as follows. Inadequate oral hygiene, which results in accumulation of plaque and calculus due to bacteria proliferation. When cells die they release

cytokines which attract neutrophils. Neutrophils migrate to the area damaged by periodontal infection and try to clean up the damaged cell area, the acidic waste products, and the bacteria. However, the biofilm composition and other factors make it difficult for the immune system to destroy the bacteria. Neutrophils may cause additional damage because after phagocytosing the damaged tissue and toxins, they die, and the lysosomal enzymes they carry are released, causing additional tissue damage. (Ibsen, & Anderson-Phelan, 2018) Neutrophils at the site decrease following acute inflammation, and macrophage, lymphocytes, and plasma cells increase. Macrophages remove tissue debris, and can also present on the surface the cause of the inflammation. Macrophages present the antigen to T lymphocyte cells and B lymphocyte cells. Dendritic cells in the oral mucosa also play a role in processing antigens and displaying them on their surface to T cells and B cells. The B-memory and T-memory lymphatic cells have the ability to 'remember' the antigen. They duplicate themselves many times over in a process called clonal selection. The next time they encounter the antigen they alert the immune system and the plasma cells to release a copy of a protein that is made to respond to the bacteria. The proteins released are antibodies which, when circulating in the blood, are also known as immunoglobulins. In the case of inflammation, an antibody titer can be drawn to determine how many antibodies are circulating in the bloodstream. Elevated levels of antibodies indicate the immune system is fighting against identified antigens. (Ibsen, & Anderson-Phelan, 2018) If the immune response were unable to identify and target the bacterium that was producing toxins, it could target the toxins and try to destroy them. The bacterium itself could continue to grow and reproduce while the immune system targeted the wrong 'invader'.

Destruction of the Periodontium

Periodontal disease that is not controlled, will result in destruction of the gingiva, periodontal fibers, and alveolar bone, which will result in tooth loss. Gram negative bacteria are part of mature plaque biofilm. Besides toxic waste products that can change the pH of a microenvironment, these gram-negative bacteria have an endotoxin called lipopolysaccharide, (LPS) found on the outer membrane, that could be responsible for initiating inflammation in gingival tissues. LPS is released if the cell is lysed or through vesicles released into the surrounding periplasm. LPS plays a major role in provoking disease states, and is able to elicit fever and apoptosis. Pathogenic bacteria also have the ability to produce enzymes, such as collagenase which can break down collagen and proteases which can break down proteins that are part of the periodontal structure. (Gehrig, & Willman, 2016). The immune system will also contribute to periodontal destruction. Cytokines, prostaglandins and matrix metalloproteinases (MMPs) are biochemical mediators that activate the immune response, and have the potential to initiate tissue destruction, in an effort to rid the body of the pathogenic agent. Periodontal disease causes a local inflammatory effect, and a systemic inflammatory effect (Noble & Papapanou, 2013). In chronic inflammatory disease, cytokines are known to initiate tissue destruction; prostaglandins initiate most of the alveolar bone destruction, and MMPs may cause extensive collagen damage. (Gehrig, & Willman, 2016).

Porphyromonas Gingivalis and the Link to Periodontal Disease and Rheumatoid Arthritis

Bacterial pathogens produce acidic waste products and other virulence factors that help them evade detection by the immune system. *Porphyromonas gingivalis* is a gram-negative bacterium, commonly found in periodontal infections. *P. gingivalis* is able to produce many virulence factors, lipopolysaccharide (LPS), and proteolytic enzymes, which cause cell damage

and provoke inflammation, cytokine response, and immune system activation. *P gingivalis* also produces an enzyme called peptidylarginine deiminase or PPAD. This enzyme produces citrullinated epitopes (proteins) (Potempa, Mydel, & Koziel, 2017). An epitope is the part of the cell that is recognized by the immune system as 'self' or 'foreign'. If identified as 'foreign', the antibody will bind to the epitope, and start the immune system removal process (VanMeter, VanMeter, & Hubert, 2010). Citrullination is the conversion of arginine, an amino acid, into citrulline, which is recognized as an antigen, by antibodies in the immune system. Only PPAD is able to deiminate C-terminal arginine proteins. Therefore, the production of the enzyme indicates the presence of *P gingivalis*. It also demonstrates how *P gingivalis* may provoke the immune system into attacking otherwise healthy cells. It is also of interest to note that PPAD autocitrullinates itself, converts itself into a target, and provokes an augmented response from the immune system, which helps create an inflammatory environment that benefits *P. Gingivalis*. The destruction of otherwise healthy tissue by the immune system is common in auto-immune diseases. Along with periodontal disease, other diseases such as rheumatoid arthritis, Alzheimer's, psoriasis, nephropathy, and multiple sclerosis have been associated with citrullination of proteins (Potempa, Mydel, & Koziel, 2017). ACPA was found to bind to receptors in the joints and was specific to the *P. gingivalis* bacterium involved in rheumatoid arthritis. Serum levels of ACPAs have been found to co-relate with periodontitis activity in otherwise healthy adults. (Potempa, Mydel, & Koziel, 2017). The presence of the enzyme produced by the bacteria, PPAD, indicate that the initiator of the antibody immune response is the product of *P. gingivalis*. The immune response seems to be directed against the enzyme produced, not the bacterium itself. This is important to the hypothesis that individuals with

chronic inflammation, may have bacteria that are identified by the immune system as 'self'. More studies need to be done to determine if this is the case.

Anti-citrullinated antibodies or ACPAs were found in 70% of people with rheumatoid arthritis (Potempa, Mydel, & Koziel, 2014). Once the cells have been affected by this enzyme, the immune system will target them for removal (Araújo, M., Melo, I., & Lima, V., 2015) It was found that ACPA in the gingiva precedes rheumatoid involvement by sometimes up to ten years. Rheumatoid arthritis involves chronic inflammation, and the study by Potempa, Mydel and Koziel (2017), reported that serum levels of ACPA from *P. gingivalis* in rheumatoid arthritis, were elevated. Rheumatoid factor and the resulting immune complexes, inflammatory cytokine production, and macrophage and dendritic activation, resulted in tissue damage. ACPA was not found in other degenerative diseases of the joints. Just as in periodontal disease, the chronic inflammation of rheumatoid arthritis resulted in progressive bone destruction (Potempa, Mydel, & Koziel, 2017). Rheumatoid arthritis is considered an inflammatory autoimmune disease, while periodontal disease is considered an immunoinflammatory disease of bacterial origin (Araujo, Melo & Lima, 2015).

A cohort study of almost 700 patients, found that patients with rheumatoid arthritis did not have higher anti-*P gingivalis* antibody titers than controls without rheumatoid arthritis. In the study, an anti-*P gingivalis* assay that detected antibodies to *P gingivalis* LPS was used. LPS is a toxin produced by *P gingivalis*, and the author noted the inadequacy of the test. It was mentioned that there are many tests for *P gingivalis*, but none of those methods, including the one used, was able to detect current *P gingivalis* infection. According to the author, the only method that would be able to prove *P. gingivalis* infection, would be the direct detection of the bacteria (Seror, et al., 2015). In another study, patients with rheumatoid arthritis were tested for

exposure to *P. Gingivalis* and were found to have a 78% exposure rate to the bacterium. The healthy control group was also tested for exposure to *P. Gingivalis* and they had an 83% exposure rate. (Scher, et al, 2012) Many tests used to determine the presence of bacteria, or past encounters with bacteria, test for serologic immune reactions to different toxins produced by bacteria. The method of detection of bacterial infection is important, because it may show a clear antibody response to toxins of bacteria, but not to the bacterium itself.

In the article, *The Link Between Periodontal Disease and Rheumatoid Arthritis: An Updated Review*, by Joanna Koziel & Piotr Mydel & Jan Potempa, it was stated, 'Similar to infection with P. intermedia, inoculation with either heat killed P. gingivalis or the purified cell membrane fraction from the same organism had no effect on either the rate or the severity of CIA (collagen-induced arthritis model). Taken together, these findings indicate the requirement for live *P. gingivalis*, which releases bacterial factors exerting a direct or indirect (via stimulation of host systems) effect on the host that ultimately triggers an autoimmune reaction' (Potempa, Mydel, & Koziel, 2014). The article itself seems to be stating that the immune system does not respond to the lysed bacterium, *P. Gingivalis.* It is interesting to note that many vaccines work by introducing a lysed or attenuated bacterium into the body along with an adjuvant. Usually, the lysed bacterium alone is not enough to produce an immune response. The bacterium must also be accompanied by a substance that causes inflammation or irritation. This substance is the adjuvant and the irritation it produces, is what causes an immune response. A vaccine is effective when the body identifies the bacterium with the inflammatory substance and produces antibodies to the bacterium (Centers for Disease Control and Prevention, *Understanding How Vaccines Work*, 2013, *Vaccine Adjuvants*).

According to the book, *Oral Pathology for the Dental Hygienist* (2018), the toxins produced by bacteria, may be labeled as antigens by the immune system. Pathogens are normally identified as foreign but in some diseases the body mistakenly identifies as 'self,' things that are not (Ibsen, & Anderson-Phelan, 2018). Studies need to be done to investigate the possibility that some microbes, possibly those from our initial microbiome, don't provoke an immune response.

The Oral Microbiota and Heart Disease

Strep mutans, commonly found in the oral cavity, is another one of the pathogens that can cause endocarditis, and has been linked to atheromatous plaques in the heart valves and arteries. In a study from the *Journal of Clinical Microbiology*, researchers tested 35 heart valves and 27 atheromatous plaque specimens and compared them to those in dental plaque samples from the same subjects. In the heart valve tissues, streptococcal species were found in 77.8% of the subjects, and they accounted for 59.4% of the bacterial population. In the atheromatous plaque specimens,' streptococcal species were detected at 88.9% frequency, with an 81.1% quantity. *Strep mutans* dominated the species and was found to have a 77.8% frequency and to form 48.1% quantity of the atheromatous plaque specimens. The study speculates that *S. mutans* could be a causative agent for cardiovascular disease. It related that several studies had documented the presence of periodontal related bacteria in atheromatous samples. *S. mutans* proliferation in the oral cavity resulted in increased plaque and calculus formation, and in the arteries and heart it was linked to atheromatous plaque build-up (Nakano, K., et al., 2006).

In the 2011 study by Koren, et al. (p.4592-4598) researchers studied human oral, gut and plaque microbiota in patients with atherosclerosis. Biopsies of atheromas removed from arteries showed the presence of *Streptococcus* and *Veillonella*. Those were also seen in oral microbiota, and the combination of the two have been shown to contribute to plaque and calculus formation.

Researchers hypothesized that the "pathobiology of the atherosclerotic plaque may be similar to that of dental plaque" (Koren, et al., 2011, p.4595). This study also identified the presence of *Chryseomonas* bacteria in all of the atheromas along with other types of bacteria. The report concluded that their findings supported the hypothesis that the oral cavity and the gut microbiota could be sources for bacteria associated with atherosclerotic plaque. They identified many bacteria common in the oral and gut cavity that were also present in atheromatous plaques. The study however, did not attribute microbial bacteria with causing plaque accumulation in the arteries. It hypothesized that microbes entered the plaques after they had formed (Koren, et al., 2011). More studies need to be done regarding the possibility that some of the oral or gut microbiota pathogens may be identified as 'self' by the immune system, and therefore spread undetected to other areas of the body to cause chronic inflammation and disease.

Pathogens Identified as 'Self" by the Immune System

Some of the oral pathogens that create plaque and calculus do not cause an immune response. According to Nejat Duzgunes, PhD, in the book *Medical Microbiology and Immunology for Dentistry* (2016), two oral pathogens, *Streptococcal sanguinis* and *Streptococcal gordonii* 'may appear to the immune system as "self"' (Duzgunes, p.152). *S sanguinis* produces an immunoglobulin A protease while *S gordonii* binds a-amylase which is the enzyme in the saliva that breaks down starch. These two pathogens are commonly seen in endocarditis infections. Research revealed involvement of putative lipoprotein PpiA which helped *Streptococcus gordonii* in evasion of phagocytosis by macrophages (Cho, K., Arimoto, T., Igarashi, T. and Yamamoto, M., 2013). Evasion of the immune system by identification as 'self' or other means, allows the pathogens to proliferate, enter the bloodstream, and take up residence in the heart valve and cause infective endocarditis. The author did not show how this pathogen

was identified as ‘self’ by the body. (Duzgunes, 2016). Research should be done to determine if *S gordonii* and *S. Sanguinis* are able to evade the immune response through identification by the body as ‘self’ and not just through virulence factors or production of toxins (Gehrig, &Willman, 2016)

Conclusion

Research studies show that virulence factors for *P. Gingivalis* and many other microbes are impressive in the ability that they have to evade detection by the immune system. The purpose of this paper is not to deny that this is the case. Rather, it is to propose that it is possible that a person may carry microbes that have been labeled by the immune system as ‘self’. In the case of cancer, it has been proven that viruses can enter the body and produce cancerous tumors that the immune system identifies as ‘self’. Scientists should consider the possibility that certain microbes may not be identified as ‘foreign’ by the immune system, which could lead to disease.

Research studies have shown cases where the bacteria have not been identified as antigens that elicit an immune response. Rather, the effect of the product or toxins produced by the bacteria were what produced an immune response. If the body directs its immune response toward the product of the bacteria, and not the bacteria itself, this could help explain chronic inflammation, and lack of eradication of the pathogen by the body. More studies should be done to determine if pathogens of the oral microbiome are identified as ‘self’ by the immature immune system. Implications of oral pathogens being identified as ‘self,’ could be uninhibited access to the body, resulting in chronic inflammation, and disease.

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