Periodontics

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Microbiologic Specificity of Periodontal Diseases

Traditional Nonspecific Plaque Hypothesis

In the mid1900s, periodontal diseases were believed to result from an accumulation of plaque over time, eventually in conjunction with a diminished host response and increased host susceptibility with age. The NSPH are part of a controversy that took place for over a century. At the end of the nineteenth century the most common idea about dental infections was that they were caused by the non-specific over-growth of all bacteria in dental plaque. This idea is referred to as the “Non-specific plaque hypothesis” (NSPH) and was based on the work of researchers such as Black (1884) and Miller (1890).

The nonspecific plaque hypothesis maintains that periodontal noxious products by the entire plaque flora are responsible in a proportional way to the severity of the gingival inflammation. According to this thinking, when only small amounts of plaque are present, the noxious products are neutralized by the host. Similarly, large amounts of plaque would produce large amounts of noxious products, which would essentially overwhelm the host's defenses. The NSPH have focused the quantity of plaque that determined the pathogenicity without discriminating between the levels of virulence of bacteria. Believing this, the host would have a threshold capacity to detoxify bacterial products (e.g., saliva neutralizing acid) and disease would only develop if this threshold was surpassed and the virulence factors could no longer be neutralized. The conclusion was that if any plaque has an equal potential to cause disease, the best way of disease prevention would be non-specific mechanical removal of as much plaque as possible by e.g., tooth brushing or tooth picking.

Several observations contradicted these conclusions. First, some individuals with considerable amounts of plaque and calculus, as well as gingivitis, never developed destructive periodontitis. Furthermore, individuals who did present with periodontitis demonstrated considerable site specificity in the pattern of disease. Some sites were unaffected, whereas advanced disease was found in adjacent sites. In the presence of a uniform host response, these findings were inconsistent with the concept that all plaque was equally pathogenic. Recognition of the differences in plaque at sites of different clinical status (i.e., disease versus health) led to a renewed search for specific pathogens in periodontal diseases and a conceptual transition from the nonspecific to the specific plaque hypothesis. In addition, the improvement of techniques to isolate and identify bacteria in the mid-20th century led to the abandoning of the NSPH. Although the nonspecific plaque hypothesis has been discarded in favor of the specific plaque hypothesis or the ecologic plaque hypothesis, much clinical treatment is still based on the nonspecific plaque hypothesis through mechanical plaque removal that represents the most efficient way of preventing disease.

Specific Plaque Hypothesis

The specific plaque hypothesis states that only certain plaque is pathogenic, and its pathogenicity depends on the presence of or increase in specific microorganisms. This concept predicts that plaque harboring specific bacterial pathogens results in a periodontal disease because these organisms produce substances that mediate the destruction of host tissues. Acceptance of the specific plaque hypothesis was spurred by the recognition of A. actinomycetemcomitans as a pathogen in localized aggressive periodontitis.

In the 1970s, culture-based techniques and microscopy allowed discrimination of specific bacterial species and opened the hunt for disease-related micro-organisms. It was noticed that the antibiotic kanamycin was particularly effective against cariogenic species such as oral

streptococci and reduced caries formation. This suggested that removing cariogenic bacteria from the oral cavity using antibiotics could prevent caries. In 1976, Walter J. Loesche announced the “Specific Plaque Hypothesis” (SPH), postulating that dental caries was an infection with specific bacteria in the dental plaque of which the most relevant were “mutans streptococci” (main species: Streptococcus mutans and Streptococcus sobrinus) and lactobacilli.

This hypothesis proposed that use of antibiotics against specific bacterial species could cure and prevent caries. However, results from clinical studies, then and today, are not very promising. For instance, even though the use of kanamycin resulted in an overall reduction of caries, at some surfaces the caries rate increased. This indicates that kanamycin failed to penetrate certain niches allowing cariogenic species to have a selective advantage and accu mulate there. Furthermore antibiotics reduced the abundance of cariogenic bacteria but failed to eliminate them thus as soon as the treatment was stopped, abundance increased, while a long period of treatment leads to antibiotic resistance. These suggested “specific-pathogens” are part of the indigenous microflora and unlike foreign pathogens cannot be eliminated from the oral cavity.

The development of the anaerobic hood in the 1970s for the first time allowed cultivation of the strict anaerobic species. This extended the SPH to periodontal diseases which were proposed to be inflammations caused by specific periopathogens and antibiotic treatment would be effective. However, in line with the use of antibiotics in caries treatment, recent clinical studies evaluating the effectiveness of antibiotics as adjunct in periodontal therapy have not booked significant success either. A Cochrane review stated that the use of chlorhexidine after scaling and root planing in patients with chronic periodontitis had only a modest positive effect, and concluded that the extensive use of chlorhexidine may be questioned.

In the decade after the SPH was introduced, potential periopathogens included: protozoa, spirochetes, streptococci, and actinomyces. In addition, Gram-negative, anaerobic rods including black-pigmented Bacteriodes such as Bacteriodes melaninogenicus (renamed to Prevotela melaninogenica) and others from the genus Wolinella (re-classified as Campylobacter) and facultative anaerobic, Gram-negative rods of the genera Capnocytophaga, Eikenella and Actinobacillus identified as periopathogens. However, these findings were limited due to the large number of uncultivable species (~50%) and the bias toward easily cultivable species. The finding of different species related to periodontal disease led to the idea that oral disease could be initiated by a number of specific pathogens. This idea was further investigated over the next decades and led to the famous Socransky-complexes which include bacterial clusters based on their association with periodontal disease.

Updated Nonspecific Plaque Hypothesis

Theilade also noticed that the "specific-pathogens" from the SPH were indigenous bacteria and sometimes common bacteria in health, which led to an updated NSPH in 1986 focusing on periodontal disease. At this time most researchers seemed to agree that gingivitis was a nonspecific inflammatory reaction to a complex indigenous microbiota. However, the updated NSPH took into consideration that some indigenous subgingival bacteria can be more virulent than others and that plaque composition changes from health to disease.

Nevertheless, it stated that all bacteria in plaque contribute to the virulence of the microflora by having a role in either colonization, evasion of the defense mechanism, and/or provocation of inflammation and tissue destruction. Theilade's statement that "any microbial colonization of sufficient quantity in the gingival crevice causes at least gingivitis" was supported by the fact that a non-pathogenic plaque (i.e., not causing gingivitis in the absence of oral hygiene) had never been observed. Additionally, it was considered that some people have gingivitis for a lifetime without tissue and bone destruction, while others encounter rapid progression into periodontitis. Unlike the classic NSPH, the updated NSPH could explain this by taking into

account that differences in the plaque microbial composition could lead to differences in pathogenic potential.

Ecologic Plaque Hypothesis

In 1994 Philip D. Marsh proposed a hypothesis that combined key concepts of the earlier hypotheses. In his "Ecological Plaque Hypothesis" (EPH), disease is the result of an imbalance in the total microflora due to ecological stress, resulting in an enrichment of some "oral pathogens" or disease-related micro-organisms. This idea was not entirely new since Theilade, in the review proposing the U-NSPH concluded that "increased virulence of plaque (leading to disease) is due to a plaque ecology unfavorable to the host and favorable for overgrowth by some of the indigenous bacteria having a pathogenic potential". Importantly, Marsh expanded this theory and related the changes in microbial composition to changes in ecological factors such as the presence of nutrients and essential cofactors, pH and redox potential (Marsh, 1994, 2003). For example, frequent exposure to a low pH, for instance as the result of sugar fermentation, leads to a relative increase of acid-tolerant species. The thought arose that disease could be prevented by interfering with processes that break down homeostasis and change composition. For example, non-fermentable sweeteners could be used to replace sugar and thus prevent acidification.

Marsh provided and collected convincing evidence to support his hypothesis, and it is still generally accepted that the composition of dental plaque depends on the environment. Thus, the classical "everything is everywhere, but, the environment selects" was successfully applied to dentistry. Marsh also considered the reverse: the bacteria in dental plaque affect the

environment. For instance, early colonizers of supragingival dental surfaces, are usually facultative anaerobic bacteria that use the oxygen, producing carbon dioxide and hydrogen. This lowers the redox potential giving strict anaerobes a chance to settle and multiply in the biofilm. Bacterial growth is dictated by the environment, which in turn is influenced by bacterial metabolism, leading to mutual dependencies in health but also a chain of events that lead to diseases.

The importance of the host-dependent environment in selection of bacterial species that colonize should not be neglected. It has been shown that even though there is a continuous passage of bacteria from saliva to the gut, only 29 out of over 500 taxa found in the mouth were recovered in fecal samples. However, like the other hypotheses, the traditional EPH does not address the role of genetic factors of the host that significantly contribute to the composition of dental plaque and to susceptibility to disease.

Keystone Pathogen Hypothesis

The concept of keystone species is derived from basic ecological studies. Certain species have an effect on their environment that is disproportional relative to their overall abundance. In 2012, George Hajishengallis and colleagues applied this concept to oral microbiology by proposing “The Keystone-Pathogen Hypothesis” (KPH). The KPH indicates that certain low abundance microbial pathogens can cause inflammatory disease by increasing the quantity of the normal microbiota and by changing its composition. For instance, Porphyromonas gingivalis is shown to be able to manipulate the native immune system of the host. By doing so it was hypothesized that it does not only facilitate its own survival and multiplication, but of the entire microbial community. In contrast to dominant species that can influence inflammation by their abundant presence, keystone pathogens can trigger inflammation when they are present in low numbers. When disease develops and advanced stages are reached, the keystone pathogen are detected in higher numbers. Importantly, even though their absolute number increases, keystone pathogens can decrease in levels compared to the total bacterial load which increases as plaque accumulates in periodontitis.

The KPH was developed by observing the properties of the “red complex” bacterium P. gingivalis. Studies in mouse models showed that very low presence of P. gingivalis (<0.01% of the total bacterial count in plaque) could alter the plaque composition, leading to periodontitis. In germ-free mice, P. gingivalis was able to colonize by itself, but was not able to trigger disease without the presence of other bacterial species. This indicates that (some of) the commensal microbiota is essential in the disease process. Evidence of P. gingivalis acting as a key stone pathogen was also obtained in rabbit models and non-human primates.

The role of the host-immune system is critical in the KPH. At health, periodontal tissue contains a wall of neutrophils, between the plaque and the epithelial surface, residing just outside the epithelial cells. Expression of mediators such as interleukin 8 (IL-8), intercellular adhesion molecule (ICAM) and E-selectin is required to form this neutrophil wall. E-selectin is required for neutrophil migration from the highly vascularized gingival tissue, IL-8 is a key neutrophil chemo- attractant produced by epithelial cells, and ICAM facilitates adhesion of neutrophils to the tissue allowing formation of this wall. Furthermore, the epithelium expresses low levels of a wide range of toll-like receptors (TLR's), including TLR1-TLR9 that mediate the response to a broad range of microorganisms. The array of different TLRs in combination with the multitude of bacterial species lead to a large variety of cytokines that are expressed at health.

Studies in germ-free mice show that there are low levels of innate host mediators, such as IL -1B, present in the periodontal tissue. This indicates that a basic level of cytokine expression is genetically programmed without bacterial challenge. The composition and amount of bacteria in plaque modifies cytokine expression further.

Evidence was found of three major KPH mechanisms of P. gingivalis that could impair the above mentioned host defenses: (1) Toll-like receptor (TLR) response manipulation, (2) interleukin 8 (IL-8) subversion and (3) the corruption of the complement system.

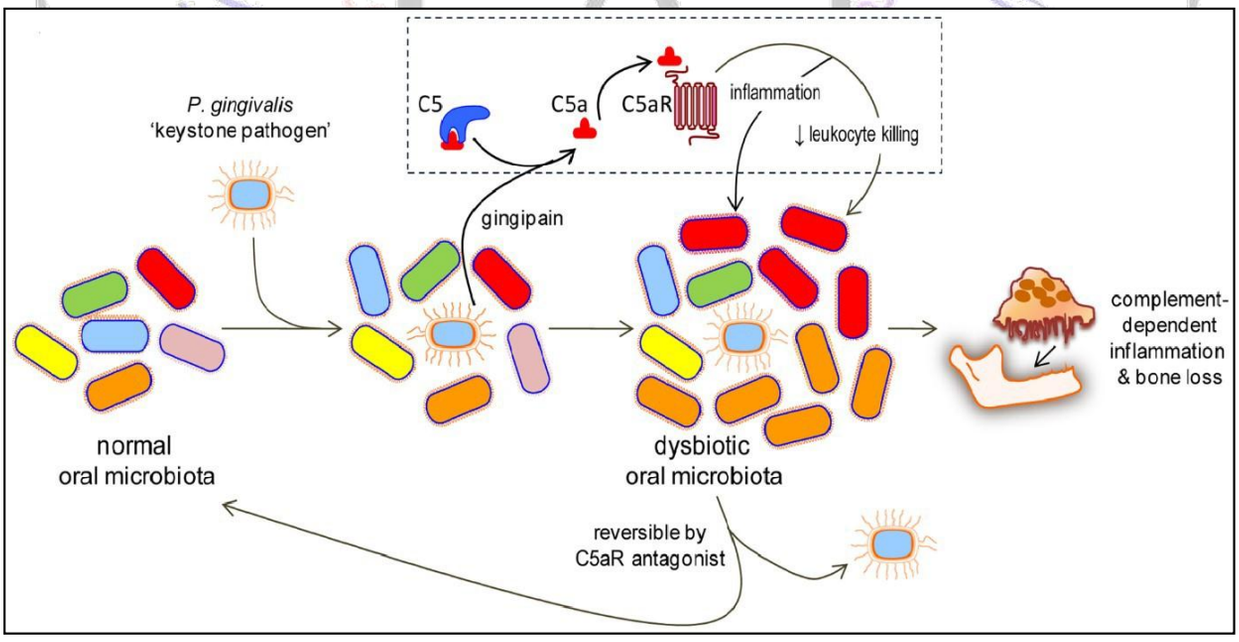
In vitro, the TLR response is manipulated by P. gingivalis with the help of two types of lipopolysaccharides (LPS) with different lipidA structures Pg LPS (type I) and Pg LPS (type II). Type I is a TLR4 agonist thus activating the immune system, while Type II is aTLR4 antagonist inhibiting the immune response to P. gingivalis. The concentration of iron determines which type of LPS is expressed. In the oral cavity, the main source of iron is hemin, found in the gingival crevicular fluid (GCF). During inflammatory process, GCF increases stimulating P. gingivalis type II LPS expression, thus reduces the TLR4 response. It was proposed that this could facilitate survival and multiplication of the entire microbial community.

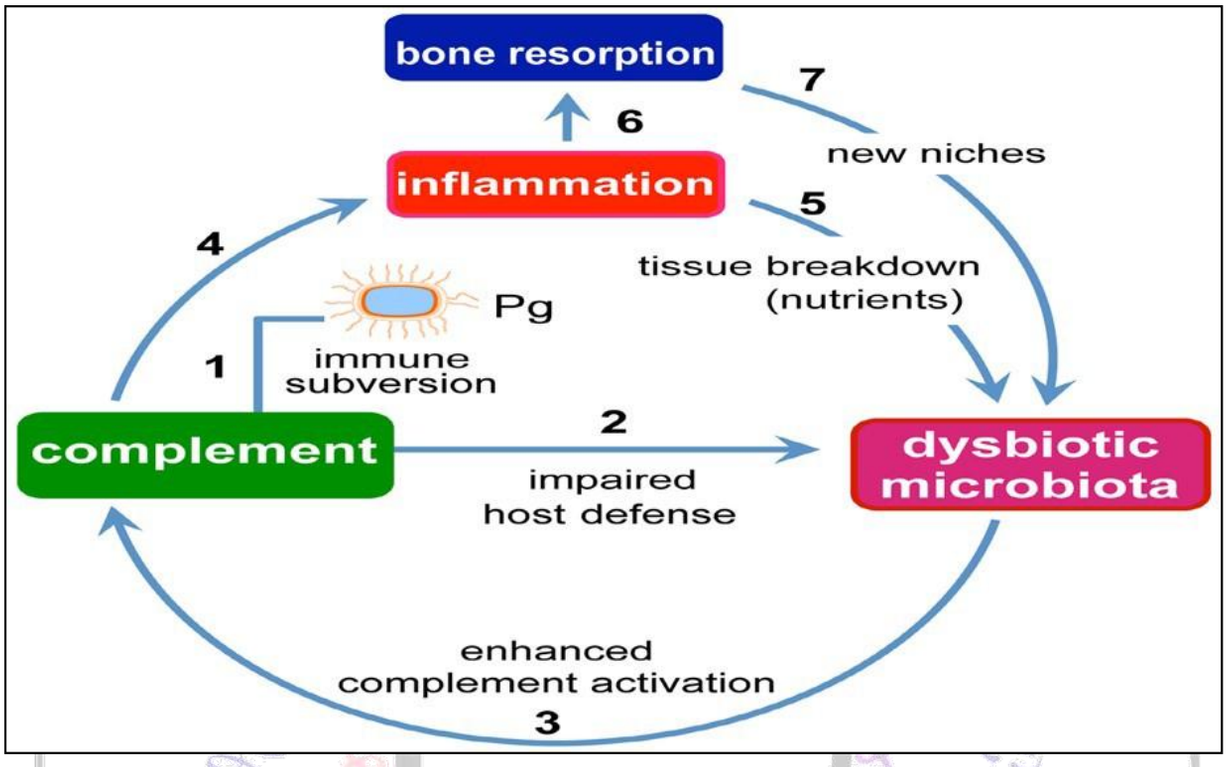
Porphyromonas gingivalis can block production of IL-8 in vitro, which is produced by gingival epithelial cells in response to other bacteria, by secreting a serine phosphatase that inhibits the synthesis of IL-8. This process is called “local chemokine paralysis” and delays the recruitment of neutrophils preventing proper neutrophil wall formation, of which was proposed that it could facilitate initial microbial colonization of the periodontium. Other “red complex” bacteria such as T. denticola, are also able to manipulate the IL-8 response of the host however the mechanism(s) involved is not understood.

The third and best in vivo documented key stone pathogen mechanism is the interference with the complement system. The complement system is a major component of the innate immune response involved in recognizing and destroying microorganisms with complex roles in homeostasis and disease. To be a successful pathogen in humans (and any other mammal) a microorganism needs to be able to avoid complement-mediated detection and killing. Again, the best-studied example in the oral cavity is P. gingivalis that produces membrane bound and soluble arginine-specific cysteine proteinases called “gingipains”. Gingipains can cleave complement factors C3 and C5 into active fragments C5a (cell activator) and C3b (phagocytosis enhancer). These fragments can be further degraded by gingipains resulting in loss of their function. However, this takes up to1h when adding purified compounds together in

vitro. More relevant is that in the presence of gingipains the levels of the inflammatory mediator C5a increase within seconds. This leads to an increased activation of the C5a receptor (C5aR) on leukocytes. C5aR is involved in cross talk with TLR2, which is activated in parallel by P. gingivalis (and other bacterial) surface ligands. While this crosstalk leads to increased inflammation, it impairs the killing capacity for leukocytes. In mouse models this mechanism has a major role in accelerating periodontitis development and bone loss. A P. gingivalis strain that lacks gingipains failed to change the oral microbiota and induce bone loss. Additionally, periodontitis did not develop in mice lacking one of the two involved receptors C5aR or TLR2. This provides clear evidence that in mice the dysbiosis caused by P. gingivalis is mainly due to complement subversion.

In conclusion, it was proposed that currently known and unknown keystone pathogens use a combination of these and presently unknown mechanisms to manipulate the innate defense system leading to destructive periodontitis.

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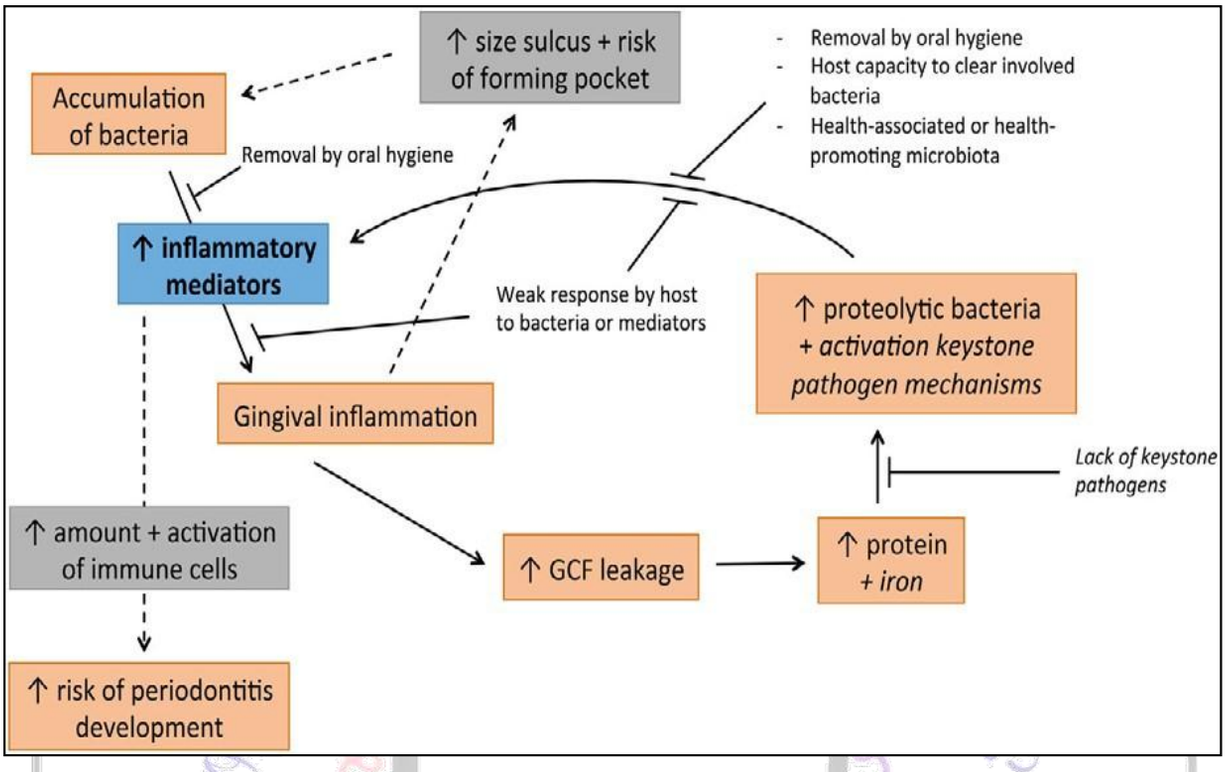

Cycle of disease involved in development of periodontal diseases, jointly supported by nonspecific, ecological and keystone pathogen hypotheses.