THE ETIO-PATHOGENESIS OF PERIODONTAL DISEASE

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ABSTRACT

The etiology of polymicrobial disease such as periodontitis is likely to be more complex than suggested by the traditional paradigm of disease involving a single virulent organism which up to now has been believed. This review limits its discussion to the other subgingival microbiota which is not yet cultivable, however it is suggested be implicated with the severity of periodontal disease. The intricate interactions between viruses and bacteria within periodontal pockets as a co-infection process reveal its role in the etiopathogenesis of periodontal disease. Also Archaea domain participate in syntrophic relationship with the microbiota life members in the subgingival crevice, promote colonization by special bacterial group during periodontitis. It is clear that periodontal diseases are not monoinfections.

Key words: polymicrobial disease; co-infection process; syntrophic relationship; severe periodontal disease; etio-pathogenesis mechanism

INTRODUCTION

It has been known that few subgingival microbiota associated with periodontal infections. Isolating and growing putative periodontal pathogens on artificial media in the laboratory has led to recognition of a widely accepted group of cultivable periodontal pathogens. In the current genomic area of exploration, it is now possible to detect and study the ‘not yet cultivable’ components of the subgingival microbiota. Accordingly, the list of putative pathogens is growing, and now includes a very diverse group of organisms in both the Bacteria and Archaea domains. It is becoming increasingly apparent that periodontal infections are caused by a much more diverse microbiota than merely Gram-negative anaerobes.[1] Therefore, members of the oral microbiota that are in the ‘not-yet-cultivated’ group, approximately 50%, were not included as etiological candidates.[2]

Archaea have been found in the mouth of patients with periodontitis. It establishes correlations between the presence of disease and the presence of Archaeal DNA, the severity of periodontal disease and the relative abundance of Archaeal DNA in subgingival plaque, and between disease resolution and diminished Archaeal DNA abundance.[3]

It has now been established that human viruses are part of the micro-ecosystem of the oral cavity. Evidence supports a co-infection in which the development and progression of periodontal disease is associated with infection by certain human viruses in conjunction with an increase in opportunistic pathogenic bacteria in subgingival microbiota.[1] This review limits its discussion to the other subgingival microbiota which is ‘not-yet-cultivable’, however it is suggesting be implicated with the severity of periodontal disease, and the etiopathogenesis of it.

PERIODONTITIS AS AN INFECTIOUS DISEASE

Periodontitis represents a specific inflammatory response to microbial residents of the subgingival biofilm. There is considerable variability in terms of clinical manifestation and disease progression rates. This variability may be attributed to differences in the composition of the subgingival microbial flora.[4]

In the case of periodontal infections, this is not a simple task, because disease onset and progression is not related merely to the presence of a specific microorganism, but most importantly, to the imbalance between levels and proportions of periodontal pathogens and beneficial species in different sites of the mouth.[5]

Oral microbiota is an enormously complex and dynamic entity that is profoundly affected by perpetually changing local environments and host-mediated selective pressures. These microorganisms live in hard-to-study biofilms
comprising organized polymicrobial communities that are
elegantly adapted to thriving and surviving in the multiple
micro-ecosystem of the oral cavity.\textsuperscript{[2]}

The consistent finding that putative periodontal
pathogens are often found in periodontally healthy people
for long periods without doing any harm, supports the
idea that these microorganisms are part of the normal
oral microbiota as commensal opportunistic pathogens.
Periodontal disease occurs when there is a disruption in the
host-microbe homeostasis associated with health.\textsuperscript{[3]}

Microbiological culture and culture-independent
molecular studies have identified more than 1,200 bacterial
species and 19,000 phylotypes in the oral cavity.\textsuperscript{[4]}
Only a limited number of bacterial species, from more than 500
identified in the subgingival biofilm, which have been
associated with periodontitis.\textsuperscript{[5]}
But fewer than 20 species
are considered to be major periodontal pathogens.\textsuperscript{[6]}
Although only a few dozen types of microorganisms
have been strongly implicated as etiological agents for
periodontitis, they do not act independently of their
neighbors. The disease-producing abilities of periodontal
pathogens are clearly modified by their interactions
with other members of the biofilm community. In some
instances, their virulence is enhanced, whereas in other
cases it is inhibited by the influence of neighboring
microorganisms within the biofilm.\textsuperscript{[2]}

Bilichodmath et al. (2009)\textsuperscript{[7]}
reported that the
etiopathogenesis of periodontal disease is a complex
process, involving the multifarious interaction between
microbial and host factors, a variety of disease-modulating
environmental factors, and a minor extent on yeasts and
parasites. Bacterial etiology alone has not been able to
substantiate various aspects such as 1) rapid periodontal
tissue breakdown with minimal plaque, 2) phase of disease
activity and quiescence, 3) site specificity in periodontal
disease, 4) progression to advanced periodontal destruction
which occurs in a fraction of a given population.

An interesting finding from culture-independent studies
is the association of microorganisms from the Archaea
domain with periodontal disease. Their association with
periodontitis is quite striking as they appear in progressively
greater numbers in the subgingival microbiota as a
function of disease severity (i.e. probing depth and clinical
attachment loss).\textsuperscript{[2]}

It has now been established that human viruses
(hvs) are part of the micro-ecosystem of the oral cavity.
Evidence from a variety of sources supports a co-infection
hypothesis in which development and progression of
periodontal disease is associated with dual infection by
certain human viruses (e.g. Epstein-Barr virus /E-BV and
cytomegalovirus/CMV) in conjunction with an increase
in opportunistic pathogenic bacteria residing in the
endogenous subgingival microbiota.\textsuperscript{[1]}

**SUBGINGIVAL MICROBIOTA**

Most of the destruction found in all cases of periodontitis is
mediated by host inflammatory and immunological
responses to subgingival biofilms.\textsuperscript{[1]}
These infections are caused by an extremely diverse consortium
of microorganisms that are part of the endogenous microbiota
of most people. The pathogens do not act alone but are part of
complex microbial communities called biofilms. Biofilms
are populations of microorganisms that are concentrated on
a surface and surrounded by an extracellular slime matrix
of microbial origin known as the glycocalyx.\textsuperscript{[2]}

It is well established that the biochemical and metabolic
properties of free-floating or planktonic microorganisms
are quite different when these same microbes become part
of an adherent biofilm community. For example, some
bacteria appear to sense when they touch a solid surface.
This contact triggers activation of genes that facilitate
irreversible attachment of the bacteria to the solid surface.
On the other hand, genes for certain virulence factors
are up-regulated in some bacteria once they become
members of a biofilm. This is important from an etiological
perspective.\textsuperscript{[2]}

An important feature of microbial communities in
biofilm is increased synthesis of quorum-sensing molecules
that promote coordinated reactions of the entire biofilm to
external stimuli. Indeed, biofilm tend to behave like
multicellular organisms when challenged with significant
environmental changes.\textsuperscript{[2]}
Finally, horizontal gene transfer
(HGT) between similar and dissimilar microorganisms
promotes the preservation of genes that enhance survival
in highly organized and competitive biofilm ecosystems. It
has been observed that HGT events play an important role in
the adaptation of microbes to new environments.\textsuperscript{[2,9]}
It has been detected that *Archaea* might be linked to virulence as
donors of virulence-promoting genes to pathogenic bacteria
through the process of HGT.\textsuperscript{[9]}

The development of 16S rRNA phylogenetic
methodology has widened the scope of detectable
microorganisms to include uncultivable organisms that may
play significant, as yet undefined, roles in pathogenesis.
Members of the domain *Archaea* are found in greater
abundance in dental plaque from sites with periodontal
disease than in plaque from non-diseased sites. It can
depend on the environment.\textsuperscript{[10]}
*Archaea* have been isolated from the human oral cavity, gut, colon, and
vagina;\textsuperscript{[3,8]}
but have not been established as causes of human disease.\textsuperscript{[3]}

It has been detected that interactions between viruses
and bacteria within periodontal pockets is a process of
etiopathogenesis of periodontal diseases.\textsuperscript{[8]}
Armitage et al. (2010)\textsuperscript{[11]}
wrote that human viruses are part of the
micro-ecosystem of the oral cavity of periodontitis patients.
Even Slots (2010)\textsuperscript{[6]}
stated that healthy periodontal site of
periodontitis patients may harbor more herpesviruses than healthy periodontal site of individuals with a generally healthy periodontium.

CO-INFECTION BY VIRUSES

Periodontal health is associated with median genome detection rates of 8% for E-BV and for CMV. Healthy peri- implant site have demonstrated an absence of CMV. The observation of few or no herpesvirus genomes in the healthy periodontium is in accordance with a herpesvirus (hv) infection of periodontal inflammatory cells. Herpesvirus-infected periodontal healthy and gingivitis sites typically harbor the viruses in a nontranscriptional state.[6] Bilichodmath et al. (2009) also wrote that herpesviruses are usually present in the body in inactive state.

Herpesviruses may cause direct cytopathic effects on keratinocytes, fibroblasts, endothelial cells and inflammatory cells. Some researchers have detected CMV in monocytes/macrophages and T lymphocytes, E-BV 1 in B lymphocytes, and herpes simplex viruses (HSV) in monocytes/macrophages and T lymphocytes.[8]

Absence of herpesviral infection or viral reactivation may clarify why some individuals carry periodontopathic bacteria while still maintaining periodontal health or minimal disease. Herpesvirus reactivation may occur spontaneously or as a result of various types of impairment of the host immune defense, including HIV infection, pregnancy, hormonal changes, and psychosocial and physical stress. However, bacterial enzymes or other inflammation-inducing products have probably also the potential to activate periodontal herpesviruses. Factors that activate herpesviruses are also recognized risk indicators of periodontitis.[11]

An active herpesvirus infection initiates periodontal tissue breakdown and that host immune response against the herpesvirus infection are an important component of the etiopathogeny of the disease.[6] This infection would further diminish the resistance of periodontal tissues, thereby allowing subgingival upgrowth of periodontal pathogenic bacteria.[11]

SYNTROPHIC RELATIONSHIP

Like bacteria, Archaea are widely distributed and have been isolated from the human oral cavity, root-canal space, human gut, colon, and vagina.[9,10,11] They resemble bacteria in their shapes and various cell structures, but they differ immensely in the chemical composition of their structures.[9] Thus physically resemble bacteria but have different nucleotide sequences in their 16S rRNA genes and are therefore not in the Bacteria domain. Archaea is a member of microbial life (among Eukarya, Bacteria and Archaea domain). Archaea is the only group in which pathogens have not yet been demonstrated.[12]

Methanobrevibacter oralis is the most commonly found archaean associated with periodontal disease.[2] Members of genus Methanobrevibacter are strict anaerobes.[3] This methane-producing microorganism is the dominant oral methanogens.[12] Although a cause-and-effect relationship has not been shown, Archaea have never been found in the subgingival microbiota of periodontally healthy individuals or at healthy sites in patients with periodontitis. Although M. oralis has been cultured in the laboratory, it is difficult to grow and is not isolated during routine microbiological assessments. It has been shown that M. oralis antigens induce the production of specific IgG antibody by periodontitis patients who harbor the microorganism as part of their subgingival microbiota.[2]

There is some evidence that subgingival methanogens ‘outcompete’ sulfate-reducing bacteria (SRB) and acetogenic bacteria for available H2 in the local environment. These three groups of hydrogenotrophic microorganisms play an important role in the overall subgingival ecology by regulating the levels of H2 and thereby affecting the levels of secondary fermenting periodontal pathogens.[12] Such knowledge could provide basic information on the role of H2 consumption in the regulation of the periodontal biofilm ecosystem (i.e., interspecies hydrogen transfer as a possible driving force to promote proliferation of fermenting pathogens).[12]

The co-occurrence of two (or all three) groups indicates H2 levels to be sufficiently high in periodontal plaque to allow partitioning of H2 consumption or alternative metabolic strategies of SRB and/or acetogens. This could include the use of electron donors others than H2 or the fermentation of short-chain fatty acids with possible production of H2. In the latter case, a mutual relationship with methanogens rather than competition could be possible. Here antagonistic interactions, and hence competition among H2 utilizers, seems to prevail. The SRB should outcompete methanogens for the substrate H2 if sulfate is not a limiting factor.[12] A recent study provided evidence for the possibility of an opposite order of competitiveness (i.e., methanogens outcompeting SRB) in the human colon.[9,12]

Methanogenic Archaea has no direct pathogenic effects but contribute to the overall pathogenicity of subgingival biofilms by syntropic interactions with other microbes. Such interactions are those that promote or otherwise affect the pathogenicity of neighboring microbes.[2] Archaea have not been established as causes of human disease,[5] because no virulence genes have been identified in Archaea.[9]

As Archaea has been found to be capable of colonizing in the human host as the normal flora, however, no virulence genes have been identified in Archaea till now. Recent studies have led to identification of the possibility of a probable role of Archaea in causing virulence. Horizontal gene transfer (HGT) has been thought of as a mode to acquire novel virulence genes in pathogens. Recent sequencing of bacterial and archaeal genomes has shown that inter-domain transfer is common. Since Archaea are
not directly involved in causing a disease but there are similarities between pathogenic bacterial genome and Archaeal genome, *Archaea* might be linked to virulence as donors of virulence-promoting genes to pathogenic bacteria through the process of lateral gene transfer. There are some evidence that HGT has taken place from *Archaea* to bacterial and may have contributed to virulence in bacteria.\[9\]

**DISCUSSION**

Periodontal disease is a polymicrobial anaerobic infection that, besides possibly leading to loss of the involved teeth (if untreated), is considered to constitute a risk factor contributing to the development of life-threatening systemic diseases, such as endocarditis, atherosclerosis, and stroke, as well as being co-causative for preterm birth. The list of oral microorganisms involved in periodontal disease is large, consisting of several hundred species, of which approximately 50% represent as-yet-uncultivable phyotypes.\[12\]

No single species has been identified so far as the ultimate causative agent. Instead, the disease is likely to be a result of the activities of different and varying microbial complexes.\[12\] The periodontal putative pathogens are not limited to Gram-negative anaerobic bacteria, but also a large number of Gram-positive bacteria. Even non-bacterial microbes from the *Archaea* domain and also human viruses may have an etiological role. For example bacteria in the TM7 division which are Gram-positive and now have yet been cultured in the laboratory. This 1025 clone of TM7 has recently been associated with periodontal disease as well as with active inflammatory bowel disease.\[2\]

Human viruses have been established as part of the micro-ecosystem of the oral cavity of periodontitis patients. Some evidence supports co-infection with herpesviruses results in a local increase in proinflammatory cytokines that subsequently disrupts the homeostatic balance between the resident periodontal microbiota and the host. Members of the subgingival microbiota that thrive under inflammatory conditions (e.g. *Porphyromonas gingivalis*/Pg, *Tannerella forsythia*/Tt. *Treponema spp.*) proliferate and contribute to the development and progression of periodontitis.\[1,11\]

Profound hormonal changes at the onset of puberty may re-activate a periodontal CMV infection, resulting in suppression of antibacterial immune defenses and overgrowth of exogenous-like bacteria, such as *Aggregatibacter actinomycetemcomitans* (Aa).\[6\] This Aa has long been known as periodontal putative pathogen. The Aa and Pg have ability to invade and proliferate in human gingival tissues.

Aggressive types of periodontitis also exhibit a close relationship with hvs. Herpesviruses can multiply in gingival tissue and tend to reach higher copy counts in gingival tissue than in subgingival sites. In localized aggressive periodontitis, CMV and Pg seemed to act synergistically to influence the risk for both the occurrence and the extent of the disease. Antibodies against the hvs were predominantly of the IgA isotype in the gingival crevicular fluid, and of the IgG isotype in serum.\[6\]

The recognition that periodontitis is a multifactorial disease involving hvs, bacteria and host reactions may explain why aggressive periodontitis is relatively uncommon in most populations despite a high prevalence of individuals harboring both herpesviruses and bacterial pathogens. It might be that periodontal tissue breakdown is because of simultaneous occurrence of a number of infections disease events as stated by Kamma and Slots (2003).\[11\] Those events including 1) adequate hv load (gingivitis level) in periodontal sites, 2) activation of hvs in the periodontium, 3) inadequate protective antiviral cytotoxic T-lymphocyte response, 4) presence of specific periodontal pathogenic bacteria, and 5) inadequate protective antibacterial antibody response.

*Archaea* are widely distributed in human body. The ubiquity of hydrogenotrophs in periodontal pockets\[2,9,12\] shows the association of *Archaea* domain with periodontitis is quite striking as they appear in progressively greater numbers in the subgingival microbiota as a function of disease severity (i.e. probing depth and clinical attachment loss).\[2\]

The role of H\(_2\) consumption in the regulation of the periodontal biofilm ecosystem is as interspecies hydrogen transfer for a possible force to promote proliferation of fermenting pathogens.\[12\] As the high level of fermenting pathogens shows the high H\(_2\) production, and methanogenic *Archaea* indirectly promote periodontal disease in some patients by serving as a hydrogen sink.

Lepp *et al.* (2004)\[9\] and Mirza *et al.* (2010)\[9\] reported HGT between *Archaea* and pathogenic bacteria. Gene virulence transfers with direction from *Archaea* to bacteria. It has been shown from studies of deep periodontal pockets with high-virulence pathogenic group compared to deep periodontal pockets with non-virulence pathogenic group. The first group has severe destructive periodontal disease and abundant of methanogenic *Archaea*. Hence methanogenic *Archaea* indirectly promote severe destructive periodontal disease.

*Treponema* species are capable of homoacetogenesis, a hydrogen-consuming process. The relative abundance of treponemal rDNA was significantly lower in sites with archaeal rDNA than in sites without archaeal rDNA. This is suggesting that some *Treponema* species may compete with methanogens. Methanogens and treponemes may serve as alternative syntrophic partners with other members of the subgingival biofilm community.\[3,9,12\] This condition supported by Armitage (2010)\[2\] which was stated that in sites with high levels of methanogenic *Archaea* have low levels of *Treponema spp.*, and vise versa.

Armitage (2010)\[2\] stated that subgingival methanogens outcompete sulfate-reducing bacteria (SRB). The competition is in H\(_2\) consumption. This clearly shows that *Treponema spp* also hydrogen-utilizing organisms.

It is concluded that *Archaea* transfer the gene laterally to pathogens putative periodontal and may have contributed
to virulence in bacteria. The infected inflammatory cells by herpesviruses expressed no clinical signs because these viruses still in inactive state. These infected cells enter the periodontal tissues because an event of gingival inflammation induced by highly virulence pathogenic bacteria. These events trigger the active herpesviruses infection, and would further diminish the resistance of periodontal tissues, thereby allowing subgingival upgrowth of periodontal pathogenic bacteria.

Interaction among H$_2$ consumers and H$_2$ producers in plaque biofilms may be as important as those in other anaerobic environments for overall functioning of this disease-associated microbial ecosystem.

The role of *Archaea* as a reservoir of a variety of metabolic innovations for bacteria.

REFERENCES