Under the Microscope

Long in the tooth: Oral bacterial communities and chronic periodontitis

The oral cavity provides a fertile environment for the growth of microorganisms. It has a high and relatively constant temperature, high moisture level and is rich in nutrients. The range of hard and soft tissue surfaces provides a variety of distinctly different microhabitats. The unique, non-shedding hard surfaces of teeth in particular allow for accretion of the thick, complex, structured polymicrobial biofilms known as dental plaque. The majority of oral bacteria exist as components of these biofilms that confer benefit to the host by helping to prevent colonisation by exogenous, often pathogenic, microbes. Colonisation of the oral cavity by bacteria occurs soon after birth and a diverse commensal microbiota becomes established. Over 700 bacterial taxa inhabit the oral cavity making it one of the most bacterially biodiverse regions of the human body. This biodiversity exists despite the large number and variety of antimicrobial agents produced by the host in saliva, gingival crevice fluid and on epithelial surfaces, and the acquired immune response to particular bacterial species. There is a major division between the ecologies of supragingival and subgingival plaque found on the tooth surface above and below the gingival margin respectively.

One of the imperatives for the study of subgingival plaque bacterial communities is to determine how periodontal diseases, the group of inflammatory diseases that result from the host immune responses to specific bacterial species within dental plaque, are initiated and progress. Chronic periodontitis involves destruction of the supporting tissues of the teeth including the periodontal ligament, bone and soft tissues and is associated with a subgingival infection of a consortium of specific Gram-negative bacteria. It has been proposed that an ecological succession of bacterial communities is associated with the development of disease. In a breakthrough study of 13,261 subgingival plaque samples, the levels of 40 bacterial taxa were determined using checkerboard DNA-DNA hybridisation analysis. Five clusters or complexes of subgingival bacterial species were distinguished and these could be correlated to periodontal health status. Three anaerobic, Gram-negative, proteolytic species—Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola—were found to form a tightly related group called the ‘Red Complex’ that was highly associated with the clinical signs of advanced chronic periodontitis. A second tightly related group of bacterial species termed the ‘Orange Complex’ included Fusobacterium nucleatum and Prevotella intermedia. An association between the red and Orange Complexes was evident as members of the Red Complex were rarely found in the absence of members of the Orange Complex.

In a recent study of 474 plaque samples from 37 periodontitis sufferers using real-time PCR, we have shown that the Red Complex species are found more often together, and in deeper periodontal pockets. It also appears that P. gingivalis is dependent on the presence of T. forsythia and/or T. denticola as it is rarely detected in the absence of

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Figure 1 The difference in microbial population in supragingival and subgingival dental plaque, and the subgingival environment in health (A) and periodontal disease (B). Note the loss of connective tissue attachment and bone resulting in the formation of a periodontal pocket in disease (B).
Figure 2 Combination in which Red Complex species Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia were found in 192 subgingival plaque samples positive for at least one of these species from 37 subjects. □ P. gingivalis, ■ T. denticola, ▲ T. forsythia, ▼ P. gingivalis + T. denticola, ▼ P. gingivalis + T. forsythia, ◼ T. denticola + T. forsythia, ▼ all three species. Pockets in which all three Red Complex species were present were significantly deeper than sites with none of the species present (Mann-Whitney test).

these species. These data suggest an ordered colonisation, and possibly proliferation, of the Red Complex species.

Growth as a heterogeneous polymicrobial biofilm on the surface of the tooth confers many benefits on individual species. Some of the advantages are obvious; localisation at a site without being washed away, improved resistance to the host immune response and to antimicrobial agents. Growth as part of a polymicrobial biofilm also allows bacterial communities to approach multicellularity, with interactions between oral bacteria being integral to the development of plaque. F. nucleatum has been shown to produce a reducing environment that aids proliferation of P. gingivalis, which at least in the absence of excess haem, is sensitive to oxidative stress. T. denticola and P. gingivalis exhibit a mutualistic enhancement of growth in vitro as the production of isobutyric acid by P. gingivalis and succinic acid by T. denticola was demonstrated to enhance the growth of the other bacterium. A positive synergy between F. nucleatum and T. forsythia, F. nucleatum and P. gingivalis and T. denticola and P. gingivalis in biofilm formation have been demonstrated. Most oral Gram-negative bacteria have relatively small genomes allowing only limited flexibility in catalytic pathways when growing in isolation, but growth in the presence of other species allows the possibility of cooperativity. Interspecies gene transfer has been demonstrated to increase in oral bacterial biofilms further allowing genome plasticity.

The colonisation of a site by a species depends not only upon the presence of the required environmental and nutritional conditions but also upon highly specific adhesion/receptor interactions between a bacterial cell and those already present in the biofilms. In particular, the presence of the Orange Complex species F. nucleatum is thought to be important for the establishment of a pathogenic plaque, acting as a bridge between early colonisers and these late colonising Red Complex species. For example, T. denticola co-aggregates with Fusobacterium spp., but not with early colonising Actinomyces spp.

Co-infection of mice with P. gingivalis and F. nucleatum, P. gingivalis and T. forsythia, and P. gingivalis and T. denticola has resulted in a synergistic effect on pathogenicity in an abscess model of disease. We have shown that P. gingivalis causes significant alveolar bone loss similar to that seen in human chronic periodontitis, in a mouse periodontitis model. Further to this we have recently demonstrated in this animal model that co-infection with P. gingivalis-T. denticola causes significant alveolar bone loss whereas mono-infection with either species at a similar inocula size failed to result in bone loss (Orth et al; unpublished).

Tissue damage in chronic periodontitis largely results from a dysregulated host immune response to specific bacteria in the subgingival plaque biofilm on the tooth surface. Members of the Red Complex have been shown to invade oral epithelial cells possibly to escape the host immune response or stress. Following the abatement of the immune response or the relaxation of the stress, these bacteria could then escape from the host cells and act as a source of recrudescence. Using a proteomic approach we have recently shown that when P. gingivalis is grown under the stress of haem-limitation there is an increase in the abundance of internalin related proteins on the cell surface that are involved in host cell invasion.

In the past decade, a greater appreciation of the diversity of species colonising the oral cavity has been gained with the advent of laboratory techniques that do not depend upon the culture of fastidious organisms, such as denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism and cloning and sequencing of the 16S rRNA gene. Using tools such as transcriptomics and proteomics, we strive to improve our understanding of the complex interplay between the oral microbiota and the host in both health and disease at a molecular level.

References

How viruses control microbial ecosystems

Viruses are the most abundant nucleic acid containing biological parcel on earth, being ten times more than bacteria and archaea. Most are phage (infect bacteria), have a genome of DNA or RNA, and are encapsulated in a protein coat at concentrations of 10^10/L in aquatic environments. The planet holds 10^31 viruses in its parcel on earth, being ten times more than bacteria and archaea.

Viruses influence on biogeochemical cycles

Today, research in viral ecology is where we were in the 1980s. The resident bacteriophage can move to a lytic (phage multiples and causes lysis of host) life cycle if the host becomes stressed, for example during increased temperatures that cause physiological stress on the host. In my experience, sampling bacteria from a natural environment into a sterile medium can be enough to metabolically stress the host and cause viral lysis (prophage induction) of the host. These populations do not end up in the culture collection. By default, culturing methods select against lysogenic bacterial populations. Changes to the microenvironment that metabolically stress the host can cause the viruses to move from a lysogenic to a lytic life cycle where the virus sacrifices its host to ensure viral survival – the ‘SOS’ response.

Host-viral isolates from the environment

Have you ever wondered why some prokaryotes are hard to isolate from the environment? One reason cultures may sometimes appear fastidious is that you are dealing with a complex set of viral-host interactions. As cited in the review of Weinbauer, at least half of all the bacteria isolated by Ackermann and DuBow were lysogenic (phage genome within host). Similar percentages (40% to 52%) have been reported for bacterial isolates from marine and estuarine environments and most recently 30% to 44% of the bacteria isolated from soils. Hence, lysogeny in bacterial hosts is prevalent, at least for isolates. Lysogeny can confer an environmental robustness on the host over its uninfected counterpart in a mutually beneficial relationship. However, the resident bacteriophage can move to a lytic (phage multiples and causes lysis of host) life cycle if the host becomes stressed, for example during increased temperatures that cause physiological stress on the host. In my experience, sampling bacteria from a natural environment into a sterile medium can be enough to metabolically stress the host and cause viral lysis (prophage induction) of the host. These populations do not end up in the culture collection. By default, culturing methods select against lysogenic bacterial populations. Changes to the microenvironment that metabolically stress the host can cause the viruses to move from a lysogenic to a lytic life cycle where the virus sacrifices its host to ensure viral survival – the ‘SOS’ response.

Viral influence on biogeochemical cycles

Today, research in viral ecology is where we were in the 1980s with bacteria when Azam et al first described the microbial loop; the beginning of our acceptance of the critical role of bacteria and archaea in biogeochemical cycles. With developments in the direct measurement of viral dynamics in ecological processes we see viruses altering prokaryotic diversity, community function, structure, mass, and energy transfer between trophic groups. Via the uptake of dissolved organic carbon (DOC), heterotrophic bacteria mediate total carbon and nutrient flux of freshwater and marine ecosystems. In the oceans, bacterioplankton production is supported by the flow of organic carbon from predominantly phytoplankton, while terrestrial organic carbon inputs underpin freshwater ecosystems.