The major oral diseases of caries and periodontitis are polymicrobial infections initiated by complex microbial biofilms that develop adjacent to the affected tissues. Bacterial plaque adherent to the tooth surface extends below the gingival margin and is increasingly dominated by facultative and obligate anaerobes. The dense plaque adherent to the tooth surface is in apparent equilibrium with a less structured fluid matrix that is in turn in some form of equilibrium with the biofilm adjacent to the surface of the epithelium that lines the gingival sulcus. The organisms that predominate in the epithelial biofilm are of particular interest with respect to the inflammatory disease of chronic periodontitis. Of these, the Gram negative anaerobic pathogen Porphyromonas gingivalis has been most strongly implicated by assessment against the criteria of Koch’s postulates modified to enable evaluation of mixed anaerobic infections. This asaccharolytic organism is a porphyrin auxotroph. It possesses a highly integrated strategy for obtaining essential porphyrin as haem from haemoglobin and other haem proteins.

The capacity of P. gingivalis to obtain essential porphyrin from blood is based on the function of high molecular weight cysteine proteinases, the lysine(Kgp) and arginine(RgpA)-specific gingipains. These proteinases are characterised by C-terminal haemagglutinin domains with location in the outer membrane and glycocalyx of the organism as demonstrated by immuno-gold labelling (Figure 1). The haemagglutinin region contains the porphyrin receptor HA2 which is common to both gingipains and is also found in multiple copies in a putative adhesin HagA. While the structure of RgpA is essentially conserved, there are four variants of Kgp corresponding to recombination in the haemagglutinin region. Kgp is particularly implicated in haemoglobin capture and lysis.

Strategies for targeting capture of the porphyrin macrocycle by $P. gingivalis$

Although regions of the haemagglutinin domain are immunogenic, structural variation in Kgp and the relatively poor immune recognition of HA2 present challenges to an effective immunisation strategy. A compounding influence is the probable low uptake of a vaccine for a disease characterised as non-life threatening, non-painful and extremely chronic. An alternative approach is provided by the unique mechanism of porphyrin recognition by HA2. This receptor binds the porphyrin macrocycle (Figure 2) without reference to the prosthetic metal group and by primary recognition of at least one propionic acid side chain that is exposed in haemoglobin (Figure 3). Consequently there are four variants of Kgp corresponding to recombination in the haemagglutinin region. Kgp is particularly implicated in haemoglobin capture and lysis.

**Figure 1.** Distribution of the HA2 receptor in $P. gingivalis$. Immunogold labelling of $P. gingivalis$ ATCC 33277 showing distribution of gingipains.

**Figure 2.** Protoporphyrin IX.
opportunities to modify the macrocycle to render it unpalatable to other microbial species while retaining high affinity binding for HA2. The mechanism of recognition of the macrocycle by HA2 also permits a variety of derivative adducts to be synthesised without altering the affinity of binding [1,4]. Two modes of targeting are envisaged; location of antimicrobial adducts to the surface of P. gingivalis and uptake of porphyrin analogues that confound metabolic processing by the organism.

A major aetiological role for P. gingivalis in destructive periodontitis has been inferred from the high incidence and relative levels of the organism in disease sites and by its virulence in mono-infected animals. Successful inhibition by a selective agent will provide access to a critical tool, the capacity to specifically suppress or eliminate the organism from disease sites. This extension of Koch’s postulates for complex mixed anaerobic infections is essential to place the role of P. gingivalis in context. Future testing in relevant animal models will provide the basis for prophylactic and supplementary therapy protocols in the management of human disease.

References