Can the Colonisation Resistance of the Oral Microflora be Enhanced?

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Colonisation resistance arises from the integration and synergy of a microbial flora, which thereby excludes extraneous potential colonisers. The factors influencing colonisation resistance in the oral cavity are considered and specific examples of actual or potential clinical applications are discussed. These are based on pre-emptive colonisation or competitive displacement.

INTRODUCTION

The oral cavity offers a large number of shedding and non-shedding surfaces for colonisation. Initial colonisers tend to be aerobes or facultative anaerobes, relatively quick-growing, and able to adhere or be retained at a particular surface. Although many specific interactions are known between microorganisms and oral surfaces, there is an element of chance involved in which organisms initially colonise any particular site and thus fill the related niches in the ecosystem. These initial random events thereby determine the sequence of subsequent ecological succession.[2] The oral cavity, in addition to its spatial heterogeneity, is subjected to temporal fluctuations,[27] e.g. in the rate of salivary flow, and in the host diet which varies in composition and frequency of intake. These characteristics of the oral cavity result in a large number of ecological niches, which may be physical, metabolic and temporal. Therefore, an extremely rich and complex commensal flora develops, primarily in surface-associated microbial films. Once the oral flora has become established, inter-relationships build up and the flora becomes synergistically integrated.[10] Thus, homeostasis tends to restore the initial community when alien microorganisms are introduced. This is observed as the phenomenon known as colonisation resistance.

FACTORS AFFECTING COLONISATION RESISTANCE

Microbial factors

Surface effects   Potential colonising microorganisms have to attach to a surface because the short residence time of saliva is insufficient to support a microbial population. However, although many studies have shown that synergistic interactions can occur enabling organisms to co-aggregate, or to colonise in combination with other species, it appears that even non-adherent bacteria, such as lactobacilli, can colonise if there are sufficient stagnation sites. Attachment inhibition has been proposed as one of the explanations for the observation that human oral streptococci suppressed colonisation of *Candida albicans* in mice.[19] However, metabolic exclusion appears to be far more important than surface interactions in colonisation resistance, shown by the overgrowth of extraneous organisms in the absence of any commensal flora.

Stability and complexity of the flora   Because it takes time for the commensal flora to accumulate, and for microbial metabolic interactions to develop and become integrated, it is unsurprising that colonisation resistance increases with the age of the community. Neonates are especially vulnerable and the phenomenon of overgrowth in sites such as the pharynx has been well-reported.[24]

In caries research, the ability of dental plaque to resist colonisation by *Streptococcus mutans* has been studied. Specific pathogen-free rats were used to show that the longer the resident microflora was allowed to equilibrate before *S. mutans* challenge, the lower was the chance of its establishment.[15] This observation has also been made in the model mouth, where the ability of a three-species mixture of *Actinomyces viscosus*, '*S. mitior*' and *Veillonella dispar*[7] to exclude *S. mutans* developed within 24 h of incubation.[20]

Microbial antagonists   Oral streptococci have been recognised for many years for their potential role in excluding pathogens from the pharynx by hydrogen peroxide production.[22] Microbially-produced hydrogen peroxide has also been shown to inhibit *S. mutans* in mixed culture in the model

mouth.[6] Hydrogen peroxide-producing strepto-cocci appear to play an important role in inhibiting the growth of periodontopathic bacteria in sub-gingival plaque, shown by human epidemiological studies,[11] as well as by *in vitro* and animal exper-iments.[13] Other metabolic end-products such as acids can act as inhibitors, either by a direct effect[5] or by changing the environmental pH.

Many oral commensals produce bacteriocins or similar substances. Their activity and role *in vivo* has been questioned, but studies of mutans streptococci in gnotobiotic rats showed that bacteriocin-producing strains were more competitive and able to prevent the establishment of *A. viscosus*,[16,21] or of non-bacteriocinogenic *S. mutans*,[18] in plaque.

Host factors

The immune system   Antibodies enter the oral cavity via saliva and crevicular fluid. Components of the cellular immune system are also found in crevicular fluid. Immunological control of the oral commensal flora is therefore likely, by inhibiting or promoting microbial adherence to surfaces, and possibly by changing microbial metabolism. Immunocompromised patients are more prone to overgrowth by minor components of the oral flora or by extraneous organisms.

Saliva   Saliva is the main component of the pelli-cle that coats oral surfaces and acts as a source of microbial nutrients, especially in the absence of diet-ary carbohydrates. Many dental plaque bacteria can utilise glycoproteins as a carbon source and saliva also provides minerals, vitamins, and a buf-fering system. However, saliva contains many non-specific antimicrobial substances, such as lysozyme, lactoferrin, and peroxidase, to which the normal commensal flora is relatively resistant. Salivary peroxidase converts salivary thiocyanate and mi-crobially-produced hydrogen peroxide to hypothio-cyanite ($OSCN^-$), the inhibitor of the 'salivary peroxidase system'. Extraneous organisms, such as coliforms, can be killed by the salivary peroxidase system, whereas plaque streptococci and actinomy-cetes are only inhibited temporarily.[1] The peroxi-dase system can protect organisms from the toxic effects of hydrogen peroxide, and in the model mouth this led to a greater accumulation of *S. mutans* when grown with '*S. mitior*'.[6]

Exogenous factors

Diet   Host diet had little effect on the growth rate of plaque bacteria in rats[3] or monkeys,[4] although in starved rats the microbial accumulation declined sooner. Using the model mouth, the colon-isation resistance of a three-species mixture to *S. mutans* implantation was greatest in the absence of any dietary supplement, with saliva as the only nu-trient source (Donoghue & Perrons, unpublished observations).

Antimicrobial agents   Any disturbance of the commensal flora usually results in decreased colonis-ation resistance. However, an example is given below in which bacterial overgrowth was prevented in patients receiving penicillin.

HOW MAY COLONISATION RESISTANCE BE ENHANCED?

Pre-emptive colonisation

It is unlikely, as mentioned in the Introduction, that any particular ecological niche can be filled by only one specific organism. The principle of pre-emptive colonisation is therefore to fill an ecological niche with a harmless or potentially beneficial organism, before the undesired organism has an opportunity to colonise and become established. The initial coloniser becomes integrated into the ecosystem and the pathogen is thereby excluded.

For example, overgrowth of enteric Gram-negative bacteria in the oropharynx of patients due to receive large doses of penicillin was prevented by pre-dosing with low doses of the antibiotic, to select penicillin-resistant, or penicillin-tolerant strepto-cocci.[23] The streptococci excluded the Gram-negative rods, although the authors were aware of the potential hazard of creating a partially penicillin-resistant flora.

There have been several attempts to use pre-emptive colonisation in caries prevention. Low-virulence mutants of *S. mutans* have been produced, deficient in glucosyltransferases,[14] intracellular polysaccharide synthesis,[25] or lactate dehydro-genase.[17] However, wild-type revertants tend to occur, and the effectiveness of mutant strains depends on the animal host.[28]

Competitive displacement

A more competitive organism should be able to displace a pre-existing microorganism in the same ecological niche. Competitive displacement is thus of potentially greater clinical use, as it is not depen-dent on treatment at or before colonisation with the undesired organism.

Several workers have tried to use this technique in caries prevention, i.e. in excluding virulent *S. mutans* from dental plaque. For example, an unusual strain of *S. salivarius* was shown to displace *S. mutans* from the teeth of specific pathogen-free rats and to inhibit tooth decay.[26] However, this organism was much less effective when attempts were made to implant it into dental plaque of adult humans.[8] An *S. mutans* strain which produced a high level of bacteriocin was able to persistently colonise the teeth of three adult human volunteers[12] and a bacteriocin-producing mutant of *S. mutans*, avirulent because of its deficiency in lactate dehydrogenase, is currently under investigation as a possible effector strain for replacement therapy.[9]

In conclusion, colonisation resistance can be enhanced, and the phenomenon has potential clinical applications. However, it is essential to investigate thoroughly the characteristics and stability of any effector strains, and to understand the ecology of the microbial flora.

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