Facing Facts – Making Sense of Oral Infection

Mogens Kilian

Institute of Medical Microbiology and Immunology, Aarhus University, Aarhus C, Denmark

Summary: The recent application of advanced genetic and bioinformatic methods reveal an overwhelming diversity in dental biofilms. Not only do dental biofilms show a considerable degree of individuality, but host genetics may also be crucial to the response to the microbial challenge. The attempts to incriminate single species in the etiology of caries and periodontal diseases may have been too simplistic. Although the challenge posed by this complexity may seem insurmountable, ongoing studies using new comprehensive approaches to understanding both the microbial challenge and the host response *in vivo* render significant progress in the near future realistic.

Key words: dental plaque, biofilm, biodiversity, etiology

Oral Biosci Med 2005; 2/3: 163-165

INTRODUCTION

Realization of the full degree of complexity of the oral microbiota and the fact that the bacteria that have been successfully grown in pure culture only constitute a minor part of the plaque populations has come relatively slowly to oral microbiology. More surprisingly, this realization has had little impact on current concepts about the etiology of periodontal disease and caries. Although early studies noted the significantly lower proportion of particular groups of bacteria recovered from dental plaque by cultivation compared to their proportions in Gram-stained smears, it is only recently that we have obtained unambiguous information on the approximate number of species that is present. Within the past five years oral microbiologists, particularly at Forsyth Institute in Boston, USA, dedicated intense effort to describing the phylogenetic diversity of microbial populations on tooth surfaces associated with health, caries and various forms of periodontal disease. In these studies fragments of DNA encoding 16S rRNA, which is phylogenetically informative, were amplified from samples of whole plaque by PCR technology, cloned in *Escherichia coli*, and the determined nucleotide sequences were analyzed in the context of the global tree of microorganisms (Kroes et al, 1999; Paster et al, 2001, 2002). Many new lineages were detected based on their molecular signature in 16S rRNA sequences and many hitherto unknown and yet uncultured species showed an association with disease (Kroes et al, 1999; Paster et al, 2001, 2002; Brinig et al, 2003, Tanner and, Izard, 2005). It is notable that these studies demonstrate that, in addition to bacteria, dental plaque contains *Archaea* species that are associated with periodontal disease (Kroes et al, 1999). While sequencing is still ongoing, the number of species of microorganisms observed in oral biofilms is rapidly approaching 1,000. It is important to remember that conclusions on relative proportions of the individual species/phylotypes determined in these studies rely on the not necessarily correct assumption that all bacteria are lysed with the same efficiency and that there is no bias either in the subsequent steps of the experimental procedure.

INTRASPECIES DIVERSITY

DNA-based typing of cultured bacteria show that even within individual hosts the microbial populations of oral biofilms contain substantial microheterogeneity. Many species are represented by multiple clones that may change over time in relative proportions or in their actual presence or absence. Although some species appear to be stably present over years as a single cultivable

clone the pattern observed with most oral bacteria that have been examined is a constantly changing mixture of up to 15 clones detectable by culture (Hohwy et al, 2001). Application of more sensitive methods may well increase this number significantly.

This microheterogeneity is not only of academic interest but has important implications. Detailed comparisons of different clones belonging to the same species by comparative genome sequencing or comprehensive hybridization using genome-based microarrays combined with functional or other analyses reveal astonishing differences. Up to 25% of the genome may be variably present in members of the same bacterial species. As the genes that are variably present usually comprise virulence-associated properties, this microheterogeneity may be of substantial functional consequence to the host-parasite relationship. Also the allelic variations in genes that are present in all members of a species may have crucial functional impact as suggested by the *Actinobacillus actinomycetemcomitans* JP2 clone observations (Haubek et al, 2001). Furthermore, the potential of oral bacteria for differential expression of key surface components under different environmental conditions is an important contributor to the individuality and versatility of dental biofilms. Interesting examples of the latter are presented by Darveau and by Cvitkovitch and their colleagues in this issue (Darveau et al, 2005; Korithoski et al, 2005).

DENTAL PLAQUE BACTERIA AS INTEGRATED COMMUNITIES RATHER THAN INDIVIDUALS

Following the tradition of medical microbiology, oral microbiologists have long been searching for specific etiologic agents of periodontal diseases and caries and considerable efforts have been invested in studying disease-associations, putative virulence factors, and immune reactions of and against a very limited number of dental plaque bacteria. New developments strongly suggest that this is a too simplistic approach to the problems. Thus, studies of non-oral and oral biofilms increasingly emphasize that in order to understand their biology and functional implications it is necessary to view them as integrated communities rather than to study what individual members may or may not do under laboratory conditions (Costerton et al, 1995; Kohlenbrander and Palmer, 2004; Marsh, 2004). As discussed by Molin (2005) biofilms often develop into structures in which individual members are present in characteristic spatial relationships that allow direct interspecies communication relevant for gene regulation, collaborative and antagonistic metabolic activities, and horizontal transfer of gene sequences as already anticipated by Guggenheim (1968) more than 35 years ago. It has long been known that bacterial extracellular polysaccharides are important constituents of the biofilms forming on tooth surfaces (Guggenheim and Schroeder, 1967). Recent studies using the Zürich biofilm model suggests that these polysaccharides may facilitate diffusion of macromolecules into the deeper layers of oral biofilms (Thurnheer et al, 2003).

By applying multi-colour fluorescently labelled nucleic acid probes specific for individual species or selected metabolic markers (FISH technique) combined with confocal microscopy it is now possible to study some of these interactions in natural biofilms of oral bacteria formed *in vitro* or *in vivo* (Palmer et al, 2001; Egland et al, 2004; Thurnheer et al. 2004).

The possibility that the microbial factors responsible for periodontal disease and caries are not produced by single species but contributed by a consortium of microorganisms in the dental biofilms and only under certain environmental conditions constitutes an formidable challenge to research in this field.

SPECIFIC HOST-PARASITE INTERACTIONS

The complexity and individuality of the host-parasite interactions is even more striking with the recent recognition that microorganisms have adapted to hosts of particular genetic lineages through million of years of co-evolution and that generalization, as a consequence, is impossible. The racially restricted occurrence and association with aggressive periodontal disease of the JP2 clone of *Actinobacillus actinomycetemcomitans* is one striking example from the oral field (Haubek et al, 2001). The medical field is rich in parallel examples. For example, the significance of the genetic background of the host at the individual level is clearly demonstrated by the crucial effect of tissue type on the outcome of an encounter with superantigens of *Streptococcus pyogenes* (Kotb et al, 2002). Comprehensive monitoration of gene expression in local tissues in response to oral microorganisms as recently made possible by the human genome project and microarrays that represent crucial host genes may further elucidate subject-related differences of host-parasite interactions (Ebersole et al. 2005, Jenner and Young, 2005).

THE POTENTIAL OF METAGENOMIC ANALYSIS OF DENTAL BIOFILMS

Metagenomic analysis (synonyms: community genomics, population genomics) involves isolating DNA from a complex biological sample and circumvents culturing members of the microbial population. After physical shearing of the extracted DNA, fragments are cloned into *E. coli*, and individual clones containing random genome fragments are then sequenced. This strategy was recently used in a monumental study of the micro-

bial flora in the Sargasso Sea, in which Craig Venter and Hamilton Smith and their coworkers sequenced 1.4 billion base pairs and discovered a reported 1.2 million new genes (Venter et al, 2004). Scientists at The Institute for Genomic Research (Rockville, MD), are now, with financial support from the National Institute of Dental and Craniofacial Research in Bethesda, USA, employing their impressive sequencing potential and expertise in bioinformatics in a similar metagenomic study of dental plaque. The data set that will be created during this study will be a gold mine for oral microbiologists. It will make it possible to take a non-biased and comprehensive look at the types of genes that are present and turned on during disease activities. A full appreciation of the functional significance of these findings will require development of new methods. However, by combining this approach with some of the techniques discussed above and in the accompanying papers in this issue our wish to understanding the biology of dental plaque and the etiology of oral infections may indeed become realistic.

REFERENCES

Brinig MM, Lepp PW, Ouverney CC, Armitage GC, Relman DA. Prevalence of bacteria of division TM7 in human subgingival plaque and their association with disease. Appl Envir Microbiol 2003,69:1687-1694.

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Ann Rev Microbiol 1995;49:711-745.

Darveau RP, Reife RA, Al-Qutub MA, Bainbridge BW, Coats SR, Braham PA. *Porphyromonas gingivalis* LPS lipid A heterogeneity significantly effects endothelial cell innate host responses. (this issue).

Duncan M. Comparative microbial genomics. (this issue).

Ebersole JL, Meka A, Stromberg A, Saunders C, Kesavalu L. Host gene expression in local tissues in response to periodontal pathogens. (this issue).

Egland PG, Palmer RJ Jr, Kolenbrander PE. Interspecies communication in *Streptococcus gordonii-Veillonella atypica* biofilms: signaling in flow conditions requires juxtaposition. Proc Natl Acad Sci USA 2004;101:16917-16922.

Guggenheim B. Streptococci of dental plaques. Caries Res 1968;2:147-163.

Guggenheim B, Schroeder HE. Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. Helv Odont Acta 1967;11:131-152.

Haubek D, Ennibi O-K, Poulsen K, Poulsen S, Benzarti N, Kilian M. Early-onset periodontitis in Morocco is associated with the highly leukotoxic clone of *Actinobacillus actinomycetemcomitans*. J Dent Res 2001;80:1580-1583.

Hohwy J, Reinholdt J, Kilian M. Population dynamics of *Streptococcus mitis* in its natural habitat. Infection and Immunity 2001;69:6055-6063.

Jenner RG, Young RA. Insight into host responses against pathogens from transcriptional profiling. Nat Rev Microbiol 2005;3:281-294.

Kohlenbrander PE, Palmer Jr. RJ. Human oral bacterial biofilms. In: Ghannoum M, O'Toole GA (eds): Microbial Biofilms. Washington DC: ASM Press 2004;85-117.

Kotb M, Norrby-Teglund A, McGeer A, El-Sherbini H, Dorak MT, Khurshid A, et al. An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. Nat Med 2002:8:1398-1404.

Korithoski B, Guggenheim B, Cvitkovitch DG. Environmental virulence regulation in *Streptococcus mutans*. (this issue).

Kroes I, Lepp PW, Relman DA. Bacterial diversity within the human subgingival crevice. Proc Natl Acad Sci USA 1999;96:14547-14552.

Marsh PD. Dental plaque as a microbial biofilm. Caries Res 2004:38:204-211.

Molin S. Microbial biofilm development – a discussion of two cases. (this issue).

Palmer Jr. RJ, Kazmerzak K, Hansen MC, Kohlenbrander PE. Mutualism versus independence: strategies of mixed-species oral biofilms in vitro using saliva as the sole nutrient source. Infect Immun 2001;69:5794-5804.

Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, et al. Bacterial diversity in human subgingival plaque. J Bacteriol 2001;183:3770-3783.

Paster BJ, Falkler Jr WA, Enwonwu CO, Idigbe EO, Savage KO, Levanos VA, et al. Prevalent bacterial species and novel phylotypes in advanced noma lesions. J Clin Microbiol 2002;40:2187-2191.

Tanner ACR, Izard J. Etiology of oral disease in view of microbial complexity (this issue).

Thurnheer T, Gmür, R, Guggenheim B. Multiplex FISH analysis of a six-species bacterial biofilm. J Microb Methods 2004;56:37-47.

Thurnheer T, Gmür R, Shapiro S, Guggenheim B. Mass transport of macromolecules within an in vitro model of supragingival plaque. Appl Envir Microbiol 2003;69:1702-1709.

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, et al. Environmental genome shutgun sequencing of the Sargasso Sea. Science 2004;303:66-74.

Reprint requests:
Mogens Kilian, D.D.S., D.Sc.
Professor of Medical Bacteriology
Institute of Medical Microbiology and Immunology
Aarhus University
The Bartholin Building
DK-8000 Aarhus C
Denmark
E-mail: kilian@microbiology.au.dk