J Clin Periodontol 2005; 32 (Suppl. 6): 54–56

Journal of Clinical Periodontology

Consensus Report

Advances in the aetiology of periodontitis

Group A Consensus report of the 5th European Workshop in Periodontology

M. Sanz[1], M. Quirynen[2] on behalf of the European Workshop in Periodontology group A*

[1]Department of Periodontology, University Complutense, Madrid, Spain; [2]Department of Periodontology, Catholic University Leuven, Leuven, Belgium

Sanz M, Quirynen M on behalf of the European Workshop in Periodontology group A. Advances in the aetiology of periodontitis consensus report of the 5th European workshop in periodontology. J Clin Periodontol 2005; 32 (Suppl. 6): 54–56. © Blackwell Munksgaard, 2005.

Key words: dental plaque; oral biofilm; periodontitis; etropathogenesis; periodontal pathogens; vaccination

Accepted for publication 1 April 2005

Dental Plaque – Biological Significance of a Biofilm and Community Lifestyle (Marsh 2005)

Does plaque function as a ''typical'' biofilm and microbial community?

The vast majority of microorganisms in nature are found attached to surfaces, where they grow to form biofilms. Typical biofilms have been defined as matrix embedded microbial populations, adherent to each other and/or to surfaces or interfaces. They are highly structured with channels traversing its depth and creating primitive circulatory systems. The component species are not randomly distributed but are spatially and functionally organized. When cells form a biofilm, their gene expression can alter markedly, resulting in many organisms having a radically different phenotype following attachment to a surface. Within biofilms, sophisticated systems of cell-to-cell communication are used by some bacteria to coordinate gene expression. Many biofilms are comprised of mixtures of interacting microorganisms, the properties of which are more than the sum of the component species; these consortia are termed microbial communities.

Over recent years, the application of novel microscopic and molecular techniques has demonstrated that plaque has properties consistent with those displayed by typical biofilms and microbial communities (Marsh 1994). For example, plaque can have an open architecture, but with gradients that lead to environmental heterogeneity enabling fastidious organisms to grow (a large number of microorganisms in plaque are currently uncultivable). Gene expression by oral bacteria has been shown to alter on attachment and during biofilm maturation; cell to cell signalling occurs via peptides (streptococci) and autoinducer-2 (gram negative bacteria) (Kolenbrander et al. 2002). There is also evidence for gene transfer of antimicrobial resistant traits.

It needs to be highlighted that the information available on the structure and function of dental plaque as a biofilm is derived mostly from in vitro studies on supragingival plaque bacteria (Marsh 2004). Further studies are needed on subgingival bacteria, focusing on their interaction with the gingival crevicular fluid and host cells.

What triggers the change from a ''health-associated biofilm'' to a ''disease-associated biofilm''?

Plaque is natural and exists in harmony with the host in health. Maintenance of health depends on the balance of the homeostatic relationship between the bacterial challenge and the host response. Disease is the consequence of this balanced relationship breaking down, provoked by either changes to the magnitude or nature of the microbial challenge *or* the scale and appropriateness of the host response (Socransky et al. 1998). However, the temporal sequence of events is currently uncertain. The resultant change in the environmental conditions influences biofilm organization, composition and gene expression, all of which may contribute to a modified microbial challenge. Other factors which may affect the homeostasis include: behavioural changes, the onset of other diseases, or changes in the host immune response.

What are the consequences in relation to periodontitis of plaque behaving as a biofilm?

Dental plaque as a biofilm may pose additional challenges to the host. The biofilm by being a three-dimensional exo-polymer matrix structure firmly adhered to the tooth surface creates a physical protection to the organisms from the host response and from some antimicrobial treatments. Plaque by developing a community-life style can display a pathogenic synergism where the bacteria express a plethora of virulence determinants challenging the host and where the virulence of the consortia is higher than of the sum of the individual bacteria.

In vitro studies have shown that plaque is less susceptible to antimicrobial

*Group participants: Sirkka Asikainen, Mike Curtis, Gunnar Dahlén, Ian Douglas, David Herrera, Mogens Kilian, Phil Marsh, Andrea Mombelli, G. Rutger Persson and Arie-jan van Winkelhoff.

agents when behaving as a biofilm, since the agent may be neutralized by or bound to other bacteria and this may result in reduced penetration (depending on the properties of the antimicrobial agent), the bacteria may express a novel phenotype and the bacteria may grow slowly. If these facts apply also to the in vivo situation, strategies other than the mere antimicrobial one should be considered, such as: disruption of the biofilm, interference with signalling, augmentation of the agent dosage or improvement of their pharmacodynamics (such as slow release systems) (Socransky & Haffajee 2002).

Transmission of Periodontal Bacteria and Models of Infection (van Winkelhoff & Boutaga 2005)

Are all periodontal bacteria indigenous (commensal/resident) to the oral microflora?

Most bacterial species currently implicated in periodontitis can be found in periodontally healthy subjects in low numbers. In some geographical regions, some species or clones are infrequently detected in periodontal health, and therefore could be considered as not belonging to the resident microflora in these populations (Van Winkelhoff et al. 2002).

The emergence of highly sensitive PCR techniques has increased the detection of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in healthy subjects, and when present the numbers of these species were low (Slots & Ting 1999).

Is an *A. actinomycetemcomitans* or *P. gingivalis*-associated periodontitis a transmittable disease?

With the improvement of typing techniques it has been shown that there is often only one detectable clonal type of *A. actinomycetemcomitans* and *P. gingivalis* per subject, although many clonal types of these species have been identified in the population (Haubek et al. 2002). These observations have facilitated studies, which demonstrate that *A. actinomycetemcomitans* and *P. gingivalis* are transmittable, especially within families (Okada et al. 2004). However, it still remains to be proven whether transmission increases the risk for disease.

If transmission increases the risk, control of transmission could be a means for preventing the disease. The actual value of such strategies has still to be established.

What are essential factors for transmission of pathogens?

The essential factors making transmission of a pathogen possible are: the source, the environment and the recipient. The species has to be expelled from a host (probably via the saliva) in sufficiently high numbers to survive in the environment and successfully colonize a new host (Asikainen & Chen 1999). The factors that determine the outcome of a transfer event (disease, colonization without disease, transient) are currently unknown.

Critical Pathways in Bacterial Virulence (Curtis et al. 2005)

Have we identified the critical microorganisms causing periodontitis?

A number of species have been associated with the disease based on: their detection by culture and molecular methods, their proportions in plaque, the immunological responses and the biochemical properties consistent with pathogenicity. However, it is becoming clear that there are differences between ethnic groups as well as genetic diversity within species. These organisms may not act singly, but as part of a synergistic consortium. Since a large number of the bacteria in the subgingival plaque have not yet been cultured, and hence are not biochemically characterized, it is clear that further aetiological agents may reside in this fraction of the microflora.

What are the potential pathways in bacterial virulence in regards to pathogenesis?

Current evidence indicates that most destruction of the periodontium is host mediated, driven by a bacterial challenge. A number of microbial factors have been demonstrated that allow them to survive in the presence of an elevated host response (Fives-Taylor et al. 1999). Specific traits recognized so far include:

- protective coats (e.g. polysaccharides),
- full frontal assault (e.g. leukotoxin, proteases),
- invasion of host cells as means of evasion of extracellular immunity,
- manipulation of the host response either by enhancement or suppression.

The molecular characterization of these traits is revealing a sophisticated interplay between the host and parasite in this disease (Darveau et al. 2004). However, the pathways, which are critical for virulence are still not confirmed by in vivo studies. The differential expression of these traits depends on several factors such as the environment, clonal variation, biofilm formation and presence of other species. Similarly, the host response to these traits varies between and within subjects over time.

In the future, comparative analysis of the genome of selected periodontal organisms may allow insight about these critical pathways.

Is there a valid experimental model for the assessment of virulence factors of periodontal bacteria?

There is at the moment no model, which accurately reflects both the poly-microbial and chronic nature of the disease and the specificity of these organisms for the human host.

The development of suitable models is a scientific priority. Candidate models include natural disease in non-humans (e.g. sheep) and genetically modified animals that allow studies of specific aspects of the host-parasite interaction. In addition improved in vitro models (e.g. 3-D epithelial constructs) and more appropriate cell lines will help to elucidate specific pathways.

Immune Responses and Vaccination against Periodontal Infections (Persson 2005)

Is active or passive vaccination a viable strategy in the prevention of periodontitis?

Data derived from vaccination studies *against P. gingivalis* using animal models demonstrate that a significant humoral response is provoked. The effect on periodontal disease progression is inconclusive. Passive immunization using monoclonal antibodies can prevent recolonization of *P. gingivalis* up to 9 months. However, no effect on clinical periodontal variables was found (Persson et al. 1994). Studies in humans aiming at natural active immunization by therapeutic intervention have shown a short rise in antibody titres and function. Both active and passive immuniza-

tion have shown proof of principle, but the impact of antibodies on disease progression is inconclusive. Until we get further knowledge on the infectious nature and the pathogenesis of periodontitis, the vaccination strategy may not be feasible. In light of regulatory (safety) and risk/benefit issues, passive immunization is currently worth exploring.

What is the role of antibody responses against periodontal bacteria (protective, neutral or destructive)?

Although studies in murine and monkey models demonstrate that the rise of antibody titres was not associated with a destructive effect but rather with a protective effect by reducing the rate of bone loss progression, they failed to eliminate the target pathogen (Ross et al. 2004). Data from these animal models cannot easily be extrapolated to the human situation. Studies on humans aimed at natural active immunization have failed to demonstrate a consistent rise in antibody titres, and, therefore, the results are currently inconclusive.

Are there any valid alternatives to vaccination in order to modulate plaque composition?

Current experiments in caries research demonstrate that it is possible to modulate the colonization of caries-related pathogens by inoculation of genetically modified microorganisms (replacement therapy). This concept has not been tested with periodontally related pathogens although the same principle might be valid. Investigation on this field should be pursued (Abiko 2000).

Other strategies could be explored to alter the ecology of the biofilm, for example the use of redox agents and the use of inhibitors of proteases.

References

Abiko, Y. (2000) Passive immunization against dental caries and periodontal disease: development of recombinant and human monoclonal antibodies. *Critical Reviews in Oral Biology & Medicine* **11**, 140–158.

Asikainen, S. & Chen, C. (1999) Oral ecology and person-to-person transmission of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Periodontology 2000* **29**, 65–81.

Curtis, M. A., Slaney, J. M. & Aduse-Opoku, J. (2005) Critical pathways in microbial virulence. *Journal of Clinical Periodontology* **32**, (Suppl. 6), 23–33.

Darveau, R. P., Pham, T. T., Lemley, K., Reife, R. A., Bainbridge, B. W., Coats, S. R., Howald, W. N., Way, S. S. & Hajjar, A. M. (2004) *Porphyromonas gingivalis* lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. *Infection and Immunity* **72**, 5041–5051.

Fives-Taylor, P. M., Meyer, D. H., Mintz, K. P. & Brissette, C. (1999) Virulence factors of *Actinobacillus actinomycetemcomitans*. *Periodontology 2000* **20**, 136–167.

Haubek, D., Ennibi, O. K., Abdellaoui, L., Benzarti, N. & Poulsen, S. (2002) Attachment loss in Moroccan early onset periodontitis patients and infection with the JP2-type of *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Periodontology* **29**, 657–660.

Kolenbrander, P. E., Andersen, R. N., Blehert, D. S., Egland, P. G., Foster, J. S. & Palmer, R. J. (2002) Communication among oral bacteria. *Microbiology and Molecular Biology Reviews* **66**, 486–450.

Marsh, P. D. (1994) Microbial ecology of dental plaque and its significance in health and disease. *Advances in Dental Research* **8**, 263–271.

Marsh, P. D. (2004) Dental plaque as a microbial biofilm. *Caries Research* **38**, 204–211.

Marsh, P. D. (2005) Dental plaque: biological significance of a biofilm and community lifestyle. *Journal of Clinical Periodontology* **32**, (Suppl. 6), 2–10.

Okada, M., Hayashi, F., Soda, Y., Zhong, X., Miura, K. & Kozai, K. (2004) Intra-familial distribution of nine putative periodontopathogens in dental plaque samples analyzed by PCR. *Journal of Oral Science* **46**, 149–156.

Persson, G. R., Engel, D., Whitney., C. W., Weinberg, A., Moncla, B. J., Darveau, R., Houston, L., Braham, P. & Page, R. C. (1994) *Macaca fascicularis* as a model in which to assess the safety and efficacy of a vaccine for periodontitis. *Oral Microbiology & Immunology* **9**, 104–111.

Persson, R. G. (2005) Immune responses and vaccination against periodontal infections. *Journal of Clinical Periodontology* **32**, (Suppl. 6), 39–53.

Ross, C. R., Czajkowski, L., Vandenberg, K. L., Camuglia, S., Woods, J., Agius, C., Paolini, R., Reynolds, E. & Barr, I. G. (2004) Characterization of two outer membrane protein antigens of *Porphyromonas gingivalis* that are protective in a murine lesion model. *Oral Microbiology & Immunology* **19**, 6–15.

Slots, J. & Ting, M. (1999) *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. *Periodontology 2000* **20**, 82–121.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, R. L. Jr. (1998) Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology* **25**, 134–144.5.

Socransky, S. S. & Haffajee, A. D. (2002) Dental biofilms: difficult therapeutic targets. *Periodontology 2000* **28**, 12–55.

Van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A. & van der Velden, U. (2002) *Porphyromonas gingivalis, Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023–1028.

Van Winkelhoff, A. J. & Boutaga, K.. Transmission of periodontal bacteria and models of infection. *Journal of Clinical Periodontology* **32**, (Suppl. 6), 11–22.

Address:
M. Sanz
Facultad de Odontologia
University Complutense de Madrid
Ciudad Universitaria
28040 Madrid
Spain
E-mail: mariano.sanz@odon.ucm.es