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Periodontal microbial complexes associated with specific cell and tissue responses

Moritz Kebschull[1,2] and Panos N. Papapanou[1]

[1]Division of Periodontics, Section of Oral and Diagnostic Sciences, College of Dental Medicine Columbia University, New York, NY, USA; [2]Department of Periodontology, Operative and Preventive Dentistry, University of Bonn, Bonn, Germany

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Abstract

Aims: In this review, we summarize data on the association between specific periodontal bacterial profiles and tissue gene/protein expression, generated from cell culture models and in vivo studies.

Material and Methods: A PubMed search was conducted to identify publications related to the effects of periodontal microbiota on host cells/tissues.

Results and Conclusions: The data indicate the presence of specific host tissue responses to particular microbial complexes, evident by differential regulation of gene or protein expression, ultimately resulting in distinct clinical phenotypes. Transcriptomic analyses showed that periodontal pathogens induce a small, ''common core'' of differentially regulated genes encoding for an inflammatory response, and a larger variable set of genes that may reflect pathogen-specific cellular responses. Limitations of available studies include (i) the unclear role of hundreds of subgingival species not yet investigated, (ii) the fact that in vitro studies utilizing single populations of oral cells challenged with mono-infections of planktonic bacteria may not adequately portray human periodontal diseases and (iii) the cross-sectional nature of most human studies that makes them inherently incapable of allowing temporal or causal inferences. Longitudinal studies in humans hold the potential to be superior to any model, but need to be adequately powered and controlled.

Key words: gene and protein expression; infection; microbial clusters; periodontal

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There is general consensus that periodontal diseases are infections initiated by the bacteria inhabiting the biofilm of dental plaque (Darveau 2010), and that their severity varies significantly in the population largely depending on the quality of the host response to the bacterial challenge (Offenbacher et al. 2008). Among the hundreds of cultivable and yet uncultivable microbiota shown to inhabit the periodontal milieu (Paster et al. 2006), a finite number of bacterial species has been intimately associated with states of periodontal health and disease as well as with different forms of periodontal infection (Borrell & Papapanou 2005). Importantly, synergistic and antagonistic relationships among the individual constituents of the periodontal biofilm have resulted in discernible patterns of co-colonization of the periodontal sulcus or the pathological periodontal pocket by specific microbial clusters (Socransky et al. 1998), the level of which has also been associated with the extent and severity of periodontitis in epidemiologic studies, as well as with progressive loss of periodontal tissue support in clinical trials. Nevertheless, the role of specific bacterial species as causative agents of periodontitis continues to be debatable for a number of reasons, including (i) the poor documentation of a temporal association between colonization by specific bacteria and progressive periodontitis in most of the studies; (ii) the possibility that the allegedly causative bacteria co-vary with the true causative – yet unidentified – pathogens, and are in essence merely markers of disease activity. To address some of the above shortcomings in our current

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inferences, translational research has over the past few years attempted to examine pathobiology-related events that may associate with specific periodontal colonization patterns and defined phenotypes. In this review, we summarize recent data on the association between specific bacterial profiles and gene or protein expression in the periodontal tissue components, generated either from (i) in vitro studies utilizing primary cells of oral tissue origin as experimental models, or (ii) in vivo studies examining transcriptomic and/or proteomic responses in periodontal tissues of experimental animals or humans. In these studies, the investigated bacterial challenge ranged from experimental mono-infections by single bacterial species or co-culture systems to complex subgingival biofilms in clinical cross-sectional studies. This review is limited to publications assessing differential host cell/tissue responses elicited by oral bacteria.

In Vitro Studies

The majority of the in vitro studies of molecular events in periodontal inflammation have relied on experimental infections of primary cells of periodontal origin or of appropriate cell lines by a single periodontal ''model'' organism, primarily the Gram negative, anaerobic pathogen *Porphyromonas gingivalis*. Importantly, these studies have collectively demonstrated that the molecular effects mediated by *P. gingivalis* differ significantly from those elicited by other periodontal species.

Specifically, the activation of the crucial inflammatory nuclear factor kappa B (NF-κB) pathway is less pronounced in a *P. gingivalis*-infected human oral epithelial cell line than in cells challenged by the opportunistic pathogen *Fusobacterium nucleatum* (Milward et al. 2007). Production of a panel of inflammatory interleukins (ILs) in primary human gingival epithelial cells (HGECs) was shown to differ strongly among four common periodontal bacteria. The lowest IL titres were triggered by the commensal *Streptococcus gordonii*, whereas *F. nucleatum* stimulation resulted in the overall highest levels of IL production. *P. gingivalis* stimulation elicited high IL-1β levels, whereas challenge by *Aggregatibacter actinomycetemcomitans* led to high levels of IL-8 (Stathopoulou et al. 2010). Similarly, an assessment of the level of transcripts encoding for the antimicrobial peptides human β-defensin (HBD) 1-3 and LL-37 in a human oral keratinocyte cell line mono-infected by five different periodontal pathogens or three different non-periodontopathogenic oral bacteria resulted in a markedly heterogeneous response (Ji et al. 2007). Thus, the three non-periodontopathogenic species, *Streptococcus sanguinis*, *S. gordonii* and *Veillonella atypica*, elicited a limited up-regulation of a number of anti-microbial peptides, but were unable to trigger a robust inflammatory response in the infected cells, as indicated by the low levels of IL-1α and IL-8. While the ''orange complex'' microbiota *F. nucleatum* and *Prevotella intermedia* were found capable to trigger both anti-microbial peptides and an inflammatory reaction, their effects were not identical, as an *F. nucleatum* infection failed to up-regulate human HBD1, whereas *P. intermedia* stimulation triggered all both β-defensins 1-3 and LL-37. Interestingly, the ''red complex'' pathogens *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola* resulted in mostly unchanged or even down-regulated levels of anti-microbial peptides, pointing to the potential of these pathogens to interfere with the anti-microbial innate immune response. These observations are in line with the earlier documented ability of *P. gingivalis* to suppress IL-8 production and intercellular adhesion molecule 1 in a stratified primary oral epithelium and subsequent inhibition of neutrophil transmigration (Madianos et al. 1997, Darveau et al. 1998). Similarly, *P. gingivalis* was shown to trigger a less pronounced response in human dendritic cells (DCs) than *A. actinomycetemcomitans*, resulting in reduced DC maturation and cytokine production (Vernal et al. 2008).

Importantly, differential cellular responses were not found to be elicited exclusively by whole, live bacteria. A study assessing the effects of *P. gingivalis* and *Escherichia coli* lipopolysaccharide (LPS) on a human monocytic cell line (THP-1), human primary monocytes and DCs demonstrated a striking difference in CXCL5 expression. The chemokine was strongly induced by *E. coli* LPS, and showed only a weak induction by *P. gingivalis* LPS (Barksby et al. 2009).

Another group of studies has examined the effects of individual periodontal species on different host cell populations. Thus, studies evaluating the activation of natural killer (NK) cells with resulting interferon-γ production found that this activation could only be triggered in DCs infected with either *P. gingivalis* or *A. actinomycetemcomitans*, but not in macrophages challenged with the same bacteria (Kikuchi et al. 2004, Kikuchi et al. 2005). Infection of different fibroblast subpopulations from the periodontal tissues revealed vast differences in cytokine production between them, as well as among different tissue donors (Scheres et al. 2010). These differences possibly indicate a variable inherent susceptibility to infection among different individuals, as well as the recognized heterogeneity in responses among different fibroblast cultures.

Additional work has focused on the effects of different serotypes of a single periodontal species on host cells. For example, a comparison of effects of different *A. actinomycetemcomitans* strains on gingival epithelial cells revealed major differences between serotypes, with only a single serotype b species triggering differential cell proliferation and activation (Shimada et al. 2008). These effects may be attributed to dissimilar expression of leukotoxin by the different strains, as demonstrated by the variable IL-1β secretion by human macrophages that appeared to depend on leukotoxin production (Kelk et al. 2008). Likewise, an important determinant of the inflammatory response elicited by individual strains of *P. gingivalis* appears to be its capsule. A study comparing the effects of six encapsulated and one non-encapsulated strain documented stronger inflammatory responses to all strains bearing a capsule, when compared with the non-encapsulated strain (Vernal et al. 2009). However, a recent study that tested the effects of a non-encapsulated mutant of *P. gingivalis* strain W83 showed that the wild-type strain triggered reduced IL-1β, IL-6 and IL-8 transcription in human gingival fibroblasts, suggesting that the capsule is likely a means to evade host defenses (Brunner et al. 2010). These findings are in accordance with earlier reports that partly attributed the pathogenic potential of *P. gingivalis* in the mouse model to its capsule (Laine & van Winkelhoff 1998).

In parallel to studies that focused on the expression of key inflammatory mediators in response to challenge with single, planktonic bacterial species, several studies have investigated the effects of bacterial challenge on whole-genome transcriptomic responses [for review, see Mans et al. (2009)]. Such studies have the distinct advantage of

being free from experimental bias that is inherent in focused studies, as they can detect differences in the expression of transcripts that have not yet been identified to play a role in they inflammatory process. Importantly, these studies can identify functional groups of genes that are differentially expressed in response to infection by specific bacteria, pointing to individual phenotypic responses elicited by these infections.

Handfield et al. (2005) were the first to carry out whole-genome transcriptomic analyses in HGECs, by comparing the effects of two established periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans*, and the opportunistic pathogen *F. nucleatum* and the commensal *S. gordonii* (Hasegawa et al. 2007). In these studies, strong differences in the transcriptomic profiles were evident, most often in line with published data on pathogenic properties of the respective pathogen. For example, a conceivably important property from a pathogenesis point of view is the identified differential ability of *P. gingivalis* and *A. actinomycetemcomitans* to induce apoptosis of infected host cells (Shenker et al. 2001, Nakhjiri et al. 2001). Indeed, the pro-apoptotic phenotype of *A. actinomycetemcomitans* and the anti-apoptotic properties of *P. gingivalis* were reflected by the epithelial transcriptomic profiles elicited by these bacteria. A similar transcriptomic study of a human oral epithelial line challenged with either *P. gingivalis* or *F. nucleatum* revealed that only 20% of all genes differentially expressed in response to the two pathogens were in fact identical and encoded for proteins mediating an inflammatory response (Milward et al. 2007). Thus, at least a part of the remaining 80% of the regulated transcripts likely reflects cellular responses that may be specific to each particular pathogen. This observation points to an important concept generated by analyses of transcriptomic data of host–pathogen interactions, namely the presence of a limited-sized ''common core'' response, consisting of differential regulation of a number of inflammatory genes, as well as of a larger, but variable response, that appears to be pathogen specific (Mans et al. 2006b). However, after challenge with different commensal species, the ''common core'' response was shown to be significantly larger, likely reflecting the better adaption of host cells to these bacteria (Hasegawa et al. 2007).

In parallel to the aforementioned studies that focused on effects of mono-infections, responses triggered by mixed microbial infections or by oral bacterial products have been evaluated as well. For example, the production of IL-8 in human oral keratinocytes challenged with *A. actinomycetemcomitans* was attenuated by co-culture with the potentially ''beneficial'' species *Streptococcus mitis*, *Streptococcus salivarius*, *S. sanguinis* or their cell culture supernatants, while co-culture with the commensal *S. gordonii* did not have any effect. Challenge with *A. actinomycetemcomitans* in combination with *F. nucleatum* showed a synergistic effect, resulting in higher IL-8 levels (Sliepen et al. 2009). Similarily, *Streptococcus cristatus* was shown to attenuate *F. nucleatum* induced IL-8 production in human oral epithelial cells (Zhang et al. 2008).

Bacterial products, such as the *P. gingivalis* gingipain proteases, were also shown to be capable of modifying the host response elicited by bacterial pathogens. Specifically, it was shown that gingipains are capable of degrading secondary cytokines, such as IL-6 or IL-8, produced in response to bacterial challenge by periodontal pathogens (Stathopoulou et al. 2009). Thus, stimulation of oral epithelial cells with *A. acinomycetemcomitans* or *F. nucleatum* resulted in enhanced production of primary and secondary cytokines on both the transcriptional and the protein level, whereas *P. gingivalis*-challenged cells show similar up-regulation on the transcript level only. The produced secondary cytokines were rapidly degraded after secretion, resulting in a seemingly uncommon response limited to primary cytokines only, such as IL-1β.

However, all inferences drawn from the aforementioned in vitro studies are limited due to their reliance on a single cell population as a model representing periodontal tissues. This shortcoming was recently underscored by a transcriptomic study of donor-matched monocytes, macrophages and DCs challenged with *P. gingivalis* (Nares et al. 2009). Interestingly, both the transcriptomic and the proteomic profiles from these distinct, yet closely related, cell populations demonstrated substantial differences. Therefore, it appears that in order to fully appreciate the potential of the various periodontal bacteria to elicit transcriptomic and proteomic responses in the host tissues, use of more sophisticated in vitro models encompassing organ cultures, or experimental in vivo models, rather than single cell cultures, are required.

Likewise, the studies reviewed above have the additional limitation of utilizing microorganism in a planktonic state, and thus may not be adequately portray the bacterial challenge conferred by a polymicrobial, biofilm-mediated disease such as periodontitis (Zijnge et al. 2010). Thus, a critical step towards a more realistic modelling of the host challenge posed by specific microbial complexes is the creation of artificial biofilms of given composition that can be used in vitro [for review, see Kolenbrander et al. (2010) and Kuboniwa & Lamont (2010)].

To date, a single proof-of-principle study exposing HGECs to an artificial biofilm comprising of nine periodontal bacterial species has been published (Guggenheim et al. 2009) and evaluated the effects of the biofilm-mediated challenge on HGEC apoptosis, cytokine production and degradation. Because only a single microbial composition was tested in this work, no inferences can be made that are directly relevant to the present review. However, the study established the feasibility of using artificial biofilms in the study of host–parasite interactions in oral infections, and the described system can be certainly used in the future to characterize biofilm-mediated transcriptomic and proteomic responses in the periodontal tissues or in their cellular components.

The key findings of the in vitro studies reviewed are summarized in Table 1.

In Vivo Studies

Animal studies

Experimental animal studies are frequently utilized in the study of the pathobiology of complex diseases primarily due to (i) the high standardization of experimental procedures and the control of confounding, (ii) the easy access to tissue specimens and (iii) the possibility to use gene knock-in or knock-out models. The main findings of the studies reviewed below are summarized in Table 2.

Despite the well-recognized limitations of rodent models of experimental periodontitis, infection of mice or rats by gavage has been commonly used (Graves et al. 2008). A study in rats infected with the red complex bacteria *P. gingivalis*, *T. forsythia* and *T. denticola* with or without *F. nucleatum* demonstrated an apparent synergism between these pathogens, resulting in

Table 1. In vitro studies assessing differential expression in response to periodontal bacteria

References	Bacteria	Challenge	Outcome	Results	Comments
Ji et al. (2007)	Non-periodontopathogenic: *Streptococcus sanguinis*, *Streptococcus gordonii*, *Veillonella atypica* Periodontopathogenic: *P.g.*, *T.f.*, *T.d.*, *P.i.*, *F.n.*	Infection of human oral keratinocyte line HOK-16B with whole bacteria	Levels of anti-microbial peptides (AMP): human β-defensin 1, 2, 3 and LL37 mRNA Interleukin (IL)-1α, IL-8 production	Non-periodontopathogens No inflammatory response, limited AMP induction Orange complex: inflammation, induction of AMPs Red complex: inflammation, no induction of AMPs	Red complex bacteria potentially interfere with innate immune responses
Kikuchi et al. (2005)	*P.g.*	Infection of human dendritic cells or macrophages with whole bacteria	Production of interferon-γ by co-cultivated natural killer cells	Induction of IFN-γ production only via *P.g.*-stimulated dendritic cells, but not macrophages	Similar study utilizing *A.a.* infection: Kikuchi et al. (2004)
Milward et al. (2007)	*P.g.*, *F.n.*	Infection of an oral epithelial cell line (H400) with whole bacteria	NF-κB activation	Stronger NF-κB activation in *F.n.* than in *P.g.*-infected cells	Same study assessed transcriptomes
Vernal et al. (2008)	*P.g.*, *A.a.*	Infection of human dendritic cells with whole bacteria	DC maturation (CD80, CD86 expression), production of 10 cytokines (mRNA)	Both pathogens induce DC maturation and Th1 response Stronger differentiation and activation after *A.a.* challenge	Similar study assessing the effects of different *P.g.* serotypes (Vernal et al. 2009)
Barksby et al. (2009)	*P.g.*, *Escherichia coli*	Infection of human monocytic cell line, human primary monocytes and dendritic cells	CXCL5 mRNA and protein expression	Stronger CXCL5 induction by *E. coli* LPS	Similar expression pattern for other chemokines/cytokines (in THP-1 cells only)
Stathopoulou et al. (2010)	*A.a.*, *P.g.*, *F.n.*, *S.g.*	Infection of HGECs with whole bacteria	Production of IL-1β, 6, 8, 10	Strongest overall induction: *F.n.* Weakest induction: *S.g.* *P.g.* triggered strong IL-1β release, *A.a.* resulted in highest IL-8 levels	Compare to Stathopoulou et al. (2009) Degradation of secondary cytokines (i.e. IL-8) by *P.g.* gingipains
Scheres et al. (2010)	*P.g.*	Infection of human gingival fibroblasts or periodontal ligament fibroblasts with whole bacteria	Cytokine/chemokine mRNA/protein expression	Strong inter-individual differences in cytokine responses	Compare to Meka et al. (2010) (Table 2)
Kelk et al. (2008)	*A.a.* with no, low or high leukotoxin	Infection of human primary macrophages with whole bacteria	Production of IL-1β	IL-1β secretion mainly due to leukotoxin	Leukotoxin-free strain-induced production, but not secretion of IL-1β
Shimada et al. (2008)	Five different *A.a.* strains (serotypes a, b, c)	Infection of human gingival epithelial cells with bacterial extracts	Cell proliferation, production of IL-8 mRNA, ICAM-1 expression	Growth inhibition and cell activation only in response to extracts from one strain (ATCC 29522, serotype b) Only minimal effects of other strains	Heat-inactivated bacterial extracts were used
Vernal et al. (2009)	Six encapsulated, 1 non-encapsulated *P.g.* strains	Infection of primary human dendritic cells with whole bacteria	Production of IL-1β, 2, 5, 6, 10, 12, 13, interferon-γ, tumour necrosis factor-α and -β mRNA	Differential potential of serotypes to induce dendritic cell responses, strongest cytokine mRNA production in *P.g.* W83 and HG184, weakest in non-encapsulated strain	Assessment of cytokine production on mRNA level only
Brunner et al. (2010)	*P.g.* W83 & epsC (capsule gene) knockout	Infection of human gingival fibroblasts with whole bacteria	Production of IL-1β, 6, 8 mRNA	More inflammatory cytokine production in epsC (non-capsulated) mutant	Compare to Vernal et al. (2009) Host response also triggered by other factors??????

Handfield et al. (2005)	*P.g. versus A.a.*	Infection of HGECs with whole bacteria	Gene expression, effects of challenge with different bacteria	Limited ''common core'' of expression, differences reflect pro-apoptotic properties of *A.a.* and anti-apoptotic properties of *P.g.*	''Common core'' is by far smaller after challenge with pathogenic than commensal species, see Hasegawa et al. (2007)
Mans et al. 2006a ,b	*A.a.*	Infection of HeLa cells or immortalized human oral keratinocytes with live bacteria	Gene expression, effects of *A.a.* stimulation in different epithelial cell lines	Extensive differences in stress response towards pathogens, oral cells $\gg$ HeLa tumour cells	Reported differences are not unexpected, due to the cervical tumour origin of HeLa cells that is supposed to differ strongly from cells of oral origin
Hasegawa et al. (2007)	*F.n. versus S.g.*	Infection of HGECs with whole bacteria	Gene expression, effects of challenge with different bacteria	''Common core'' of $>80\%$, differential ability to trigger pro-inflammatory response *F.n.* $>$ *S.g.*, likely a reason for higher pathogenicity of *F.n.*	Large ''common core'' of gene expression reflects adaption of host to commensal bacteria
Milward et al. (2007)	*P.g. versus F.n*	Infection of H400 oral epithelial cell line with whole bacteria	Gene expression, effects of challenge with different bacteria	*F.n.* challenge resulted in more regulated genes than *P.g.*, ''common core'' of 15–25%	
Nares et al. (2009)	*P.g.*	Donor-matched monocytes, macrophages, DCs	Gene expression	Massive differences of expression profiles between the different cell populations	Underlines the necessity for appropriate cell culture models, or studies in the relevant tissues
Zhang et al. (2008)	*F.n.* with/without *Streptococcus cristatus*	Infection of HGECs with whole bacteria	IL-8 production	Co-culture attenuated IL-8 production triggered by *F.n.*	
Sliepen et al. (2009)	*A.a.* co-culture with *Streptococcus mitis*, *S. sanguinis*, *Streptococcus salivarius*, *S.g.* or *F.n.*	Infection of human oral keratinocytes with whole *A.a.*, co-culture with whole bacteria or supernatants	IL-8 production	Co-culture with *S. mitis/S. sanguinis/S. salivarius*: attenuation of IL-8 production With *S.g.*: no effect With *F.n.*: synergistic effect	
Stathopoulou et al. (2009)	*A.a.*, *F.n.*, *P.g.*	Infection of HGECs with whole bacteria	Production of primary (IL-1β) and secondary (IL-6, IL-8) cytokines	*P.g.* gingipains degrade secondary cytokines (IL-6 and IL-8) *P.g.* infection results in primary cytokine (IL-1β) production only	Corroborates Stathopoulou et al. (2009)
Guggenheim et al. (2009)	Artificial biofilm comprising of nine periodontal bacteria	Infection of HGECs with artificial biofilms	Apoptosis, cytokine production and degradation	Artificial biofilm triggers inflammation with cytokine production and degradation, and induces apoptosis	Proof-of-concept study, because only one distinct biofilm was tested

White panel: studies of single effector molecules in response to challenge by a single species. Light grey panel: studies of single effector molecules in response to challenge by different serotypes of the same species. Medium grey panel: whole-genome transcriptomic studies of cells stimulated with single species. Dark grey panel: effects of co-cultures of bacterial combinations.
A.a., *Aggregatibacter actinomycetemcomitans*; *F.n.*, *Fusobacterium nucleatum*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *S.g.*, *Streptococcus gordonii*; *T.d.*, *Treponema denticola*; *T.f.*, *Tannerella forsythia.*

Table 2. Responses to different microbial challenges in animal tissues

References	Bacteria assessed	Study type; challenge	Primary outcome	Results	Comments
Kesavalu et al. (2007)	*P.g.*, *T.f.*, *T.d.* with/without *F.n.*	Oral infection of rats	Bone loss (radiographic)	Combined infection of *P.g./T.f./T.d.* resulted in most bone loss	Demonstrates apparent synergism between ''red complex'' pathogens
Polak et al. (2009)	*P.g.*, *F.n.*	Oral infection of Balb/c mice	Bone loss (micro-CT)	Combination resulted in more bone loss than mono-infections	Possibly influenced by immune state of Balb/c mice
Verma et al. (2010)	*P.g.*, *F.n.*	Oral/anal infection of rats	Bone loss (morphometry, radiographic)	No synergistic effect of *P.g.* and *F.n.* detected	Assessment of bone loss likely less accurate than micro-CT
Kesavalu et al. (2002)	*P.g.*, *A.a.*, *S.g.*	Subcutaneous injection over calvarian bone in Balb/c mice	Cytokine expression in soft tissue	Stronger cytokine induction by *P.g.* than *A.a.* Only weak reaction to *S.g.*	
Gemmell et al. (2006)	*P.g.*	Intra-peritoneal immunization of Balb/c mice	Gene expression of splenic CD4 and CD8 T cells	Strong down-regulatory effect of *P.g.*, only limited ''common core'' of genes down-regulated in both cell types	Possible contamination of splenic T-cell preparations with B cells
Meka et al. (2010)	*P.g.*	Subcutaneous injection over calvarian bone in Balb/c mice	Gene expression in soft tissue and calvarian bone	Pro-inflammatory reaction, differs in hard and soft tissue	
Bakthavatchalu et al. (2010)	*T.d.*	Subcutaneous injection over calvarian bone in Balb/c mice	Gene expression in soft tissue and calvarian bone	Pro-inflammatory reaction, differs in hard and soft tissue	No comparison with data by Meka et al. (2010) attempted

White panel: oral infection models assessing the potential of different bacterial complexes to initiate periodontal bone loss. Light grey panel: whole-tissue expression following microbial challenge.

A.a., *Aggregatibacter actinomycetemcomitans*; *F.n.*, *Fusobacterium nucleatum*; *P.g.*, *Porphyromonas gingivalis*; *S.g.*, *Streptococcus gordonii*; *T.d.*, *Treponema denticola*; *T.f.*, *Tannerella forsythia*.

accelerated bone loss, as compared with the mono-infected animals (Kesavalu et al. 2007). A similar synergism was demonstrated in Balb/c mice infected with *P. gingivalis* and/or *F. nucleatum* (Polak et al. 2009). However, this effect was not reproducible in the rat model of periodontitis (Verma et al. 2010), possibly due to the immuno-compromised state of Balb/c mice. Further research is therefore warranted to unequivocally demonstrate these potential synergistic effects, preferably utilizing additional animal models, such as rabbits (Hasturk et al. 2007). Thus far, no animal studies of experimental periodontitis have reported on an association between the quality of the infectious challenge and transcriptomic or proteomic expression patterns in the periodontal tissues. This is understandable, given the dimensions of the periodontal niche in small rodents, particularly mice, that renders the harvesting of both periodontal microbial samples and gingival tissue specimens problematic. In an attempt to circumvent these difficulties, a model using injection of bacterial suspensions into the subcutaneous soft tissue over the calvaria of Balb/c mice and subsequent analysis of protein secretion in the soft tissues was established, and demonstrated higher levels of inflammatory cytokine expression after *P. gingivalis* than *A. actinomycetemcomitans* injection, and only minimal effects after *S. gordonii* injection (Kesavalu et al. 2002). In a more recent study, responses to *P. gingivalis* injection on both soft tissue and calvarian bone gene expression were analysed 3 days post-infection (Meka et al. 2010). The findings demonstrated that *P. gingivalis* infection resulted in differential expression of multiple gene ontology groups suggesting an overall pro-inflammatory response. Importantly, the observed transcriptional profiles differed between soft and hard tissue, underscoring the need to study separately the individual tissue components in order to dissect the molecular mechanisms involved in the host response to the bacterial challenge. A recent study of *T. denticola* subcutaneous injections by the same group showed similar results (Bakthavatchalu et al. 2010). Unfortunately, no comparisons of the tissue-specific responses following infection by each of the two pathogens were reported. A predominantly down-regulatory effect of *P. gingivalis* was, however, found in splenic CD4 and CD8 T cells isolated from Balb/c mice after intra-peritoneal immunization. The obtained expression profiles indicated a suppression of T cell function, as well as a shift away from Th1 responses. Importantly, only a subset of approximately 20% of all down-regulated genes was common to both T cell types, indicating a host-cell-specific response to the bacterial challenge (Gemmell et al. 2006).

Human studies

A limited number of human studies have explored the association between distinct subgingival microbial complexes and gingival tissue, and their main findings are summarized in Table 3. Observations stemming from animal studies suggesting synergistic effects among bacterial species (Kesavalu et al. 2007, Polak et al. 2009) and clinical findings demonstrating that colonization by red complex species resulted in site-specific increased severity of periodontal destruction (Mineoka et al. 2008) appear to corroborate the hypothesis that distinct periodontal microbial colonization patterns may result in individual gene or protein expression profiles, ultimately translating into distinct clinical phenotypes. Clearly, human studies are free from a number of shortcomings inherent in in vitro or experimental animal studies, as the assessment of the bacterial challenge involves pathogens inha-

Table 3. Studies of microbial complexes associated with specific tissue responses in humans

References	Study type	Study population	Assessment of bacteria infection	Relevant outcome	Results	Comments
Jin et al. (2002)	Longitudinal	16 subjects with untreated PD	DNA probes for *P.g.*, *P.i.*, *T.f.*, *T.d.*, *A.a.* at baseline and 4 weeks post SRP	Interleukin (IL)-8 levels in GCF at baseline and 4 weeks post SRP	Reduction of IL-8 levels by treatment dependent on eradication of simultaneous presence of *P.g./P.i./T.f./T.d.*	
Airila-Mansson et al. (2006)	Cross-sectional	82 PD, 31 healthy	PCR for *P.g.*, *P.i.*, *T.f.*, *P.n.*, *A.a.*	Prostaglandin E2 and granulocyte elastase levels in GCF	Higher levels of PGE2 and granulocyte elastase in sites with *T.f./P.n.* or *T.f./P.g.*	These sites also show higher disease activity
Söder et al. (2006)	Cross-sectional	33 PD, 31 controls	PCR for *P.g.*, *P.i.*, *T.f.*, *T.d.*, *P.n.*, *A.a.*	MMP9 levels in GCF	*T.f.* is main predictor of MMP9 levels in GCF	
Offenbacher et al. (2007)	Cross-sectional	6768 subjects	Eight species by checkerboard	*Biofilm-gingival interface* (BGI) classification	BGI phenotypes differ significantly in bacterial profiles, different profiles partially determined by change in specific complexes	
Sakellari et al. (2008)	Cross-sectional	35 CP, 39 healthy	Checkerboard for *P.g.*, *T.d.*, *T.f.*, *A.a.*	sRANKL levels in GCF	Correlation of sRANKL and levels of *P.g.* or *T.d.*	Correlation of *T.d.* and sRANKL levels perhaps due to co-occurrence of *P.g.* and *T.d.*?
Teles et al. (2010)	Cross-sectional	31 AP, 25 healthy	40 species by checkerboard	GM-CSF, IL-1β, IL-2, IL-6, IL-10, IL-13, IFN-γ, TNF-α levels in GCF	Cytokine profiles very similar in different bacterial profiles in health, strong differences in bacterial clusters in AP patients (Fig. 1)	
Papapanou et al. (2009)	Cross-sectional	57 AP, 63 CP	11 species by checkerboard, plaque was sampled from both pockets adjacent to the biopsy site	Gene expression in 311 gingival tissue samples	Significant correlations between pathogens and gene expression, red complex pathogens cluster together	Data corroborate in vitro transcriptomic data (Handfield 2005, Table 1)

White panel: studies assessing expression of cytokines in the gingival crevicular fluid in response to specific bacterial colonization. Light gray panel: specific microbial complexes in the subgingival plaque and whole-genome gene expression of gingival tissues.

A.a., *Aggregatibacter actinomycetemcomitans*; *F.n.*, *Fusobacterium nucleatum*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *P.n.*, *Prevotella nigrescens*; *T.d.*, *Treponema denticola*; *T.f.*, *Tannerella forsythia*.

biting their native environment, i.e., the periodontal biofilm, and the characterization of their effects is directly assessed in the relevant tissues, i.e., the soft and hard tissues of the periodontium, or in the gingival crevicular fluid (GCF). In fact, most studies to date have evaluated the association between subgingival bacterial profiles and presence or levels of specific inflammatory biomarkers in the GCF.

In a series of papers, Söder and coworkers established an association between markers of periodontal disease activity and the presence or absence of a limited number of periodontal pathogens. Specifically, they demonstrated higher levels of prostaglandin E2 and granulocyte elastase in lesions that simultaneously harboured *T. forsythia* and *P. nigrescens* or *P. gingivalis*, coinciding with increased disease severity (Airila-Mansson et al. 2006). Subgingival levels of *T. forsythia* were the main independent predictors of MMP9 levels in the GCF (Söder et al. 2006) while a treatment-induced reduction of IL-8 levels was dependent on eradication of *P. gingivalis*, *P. intermedia*, *T. forsythia* and *T. denticola* in sites that harboured these pathogens at baseline (Jin et al. 2002).

Furthermore, GCF levels of the soluble receptor activator of NF-κB ligand (sRANKL), a marker of bone destruction (Taubman et al. 2007), were found to correlate positively with subgingival levels of *P. gingivalis* and *T. denticola*, but not with levels of *A. actinomycetemcomitans* or *T. forsythia* (Sakellari et al. 2008). These data are in line with observations of *P. gingivalis* as a potent activator of RANKL (Belibasakis et al. 2010), but also point to a recognized problem of cross-sectional association studies, i.e., their inability to support causal inferences. In other words, the observed association between GCF sRANKL levels and *T. denticola*, a pathogen not previously related to RANKL activation, could be due to the well-established co-occurrence of *P. gingivalis* and *T. denticola* in subgingival plaque samples (Socransky et al. 1998).

In a recent study, Teles et al. (2010) examined the association between eight cytokines in the GCF and subgingival profiles comprising 40 investigated bacterial species in aggressive periodontitis subjects, and in periodontally healthy subjects. Interestingly, while the cytokine profiles were very similar for six different bacterial profiles in periodontal

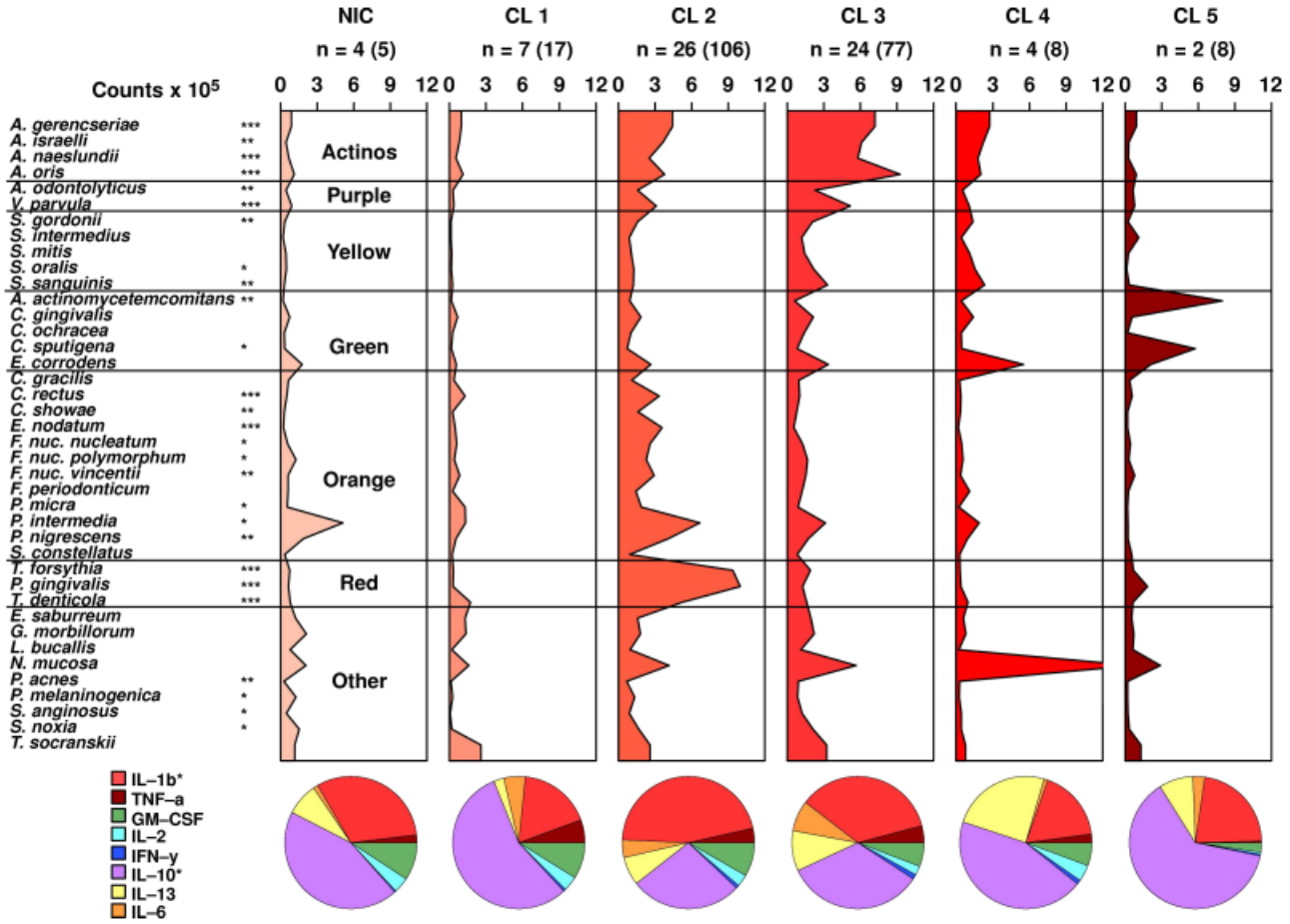

Fig. 1. Microbiological clusters in aggressive periodontitis patients and cytokine profiles in the GCF (gingival crevicular fluid). In 31 patients with aggressive periodontitis, up to 14 sites/subject were assessed for 40 periodontal species. Cluster analyses of the obtained microbiological profiles revealed five distinct clusters. Four subjects had ⩾1 site that did not fit any cluster [''not in cluster'' (NIC) group]. Bacterial counts are displayed as mean counts × 105 subgingival taxa. The numbers above the clusters represent the number of subjects with ⩾1 site fitting the cluster (first number), as well as the total number of sites represented by the cluster (second number). The pie charts display the proportion of GCF cytokine production in each cluster. Statistical testing was performed using Kruskal–Wallis tests. Note the dramatic differences in cytokine production in the different clusters [interleukin (IL)-1β – red, IL-10 – purple]. In contrast, in periodontally healthy subjects, an equal number of microbiological clusters displayed very similar GCF cytokine profiles. These data suggest a direct influence of periodontal pathogen complexes on protein expression in the tissue. Figure reproduced with permission from Teles et al. (2010).

health, they differed drastically among the bacterial clusters identified in aggressive periodontitis patients (Fig. 1). Therefore – and with the inherent limitations of a cross-sectional study design in mind – it appears that the subgingival bacterial profiles influence the production of inflammatory cytokines in the adjacent tissues and thereby contribute to the clinical phenotype.

A similar approach was adopted in an earlier study by Offenbacher et al. (2007), in which GCF levels of 16 inflammatory biomarkers, along with subgingival bacterial levels of eight periodontal bacteria and serum IgG titres to 17 periodontal bacteria were to characterize the gingival inflammation at the biofilm–gingival interface (BGI). These authors proposed five different gingival phenotypes termed BGI-healthy (BGI-H); BGI-gingivitis (BGI-G); and BGI-deep lesion (BGI-DL) displaying either low, moderate or severe bleeding-on-probing (LB/MB/SB). Interestingly, these phenotypes were also significantly different in their corresponding subgingival bacterial profiles. Differences in bleeding-on-probing severity between BGI-DL/LB and BGI-DL/MB were determined primarily by the numbers of red complex bacteria that were found to be lower in BGI-DL/LB than in BGI-DL/MB, as well as in BGI-H and BGI-G. Differences between BGI-DL/MB and BGI-DL/SB were determined by an overall increase of all tested bacterial species with no specific complex prevailing.

So far, the most extensive in vivo investigation of pathogen–host interactions in periodontitis has been a transcriptomic study of 311 gingival tissue samples from 120 patients (Papapanou et al. 2009) that analysed the correlation between the colonization patterns by 11 periodontal bacterial species, assessed in a total of 611 subgingival plaque samples from the periodontal pockets adjacent to the biopsied sites, with specific gebe expression profiles. After adjustments for clinical periodontal status, the red complex bacteria *T. forsythia* and *P. gingivalis* were shown to be associated with 9392 and 8537 differentially expressed probe sets, respectively, while the health-associated *Actinomyces naeslundii* was merely associated with eight probe sets. Clustering of the differentially regulated genes into gene ontology groups identified strong differences in tissue responses between individual bacteria.

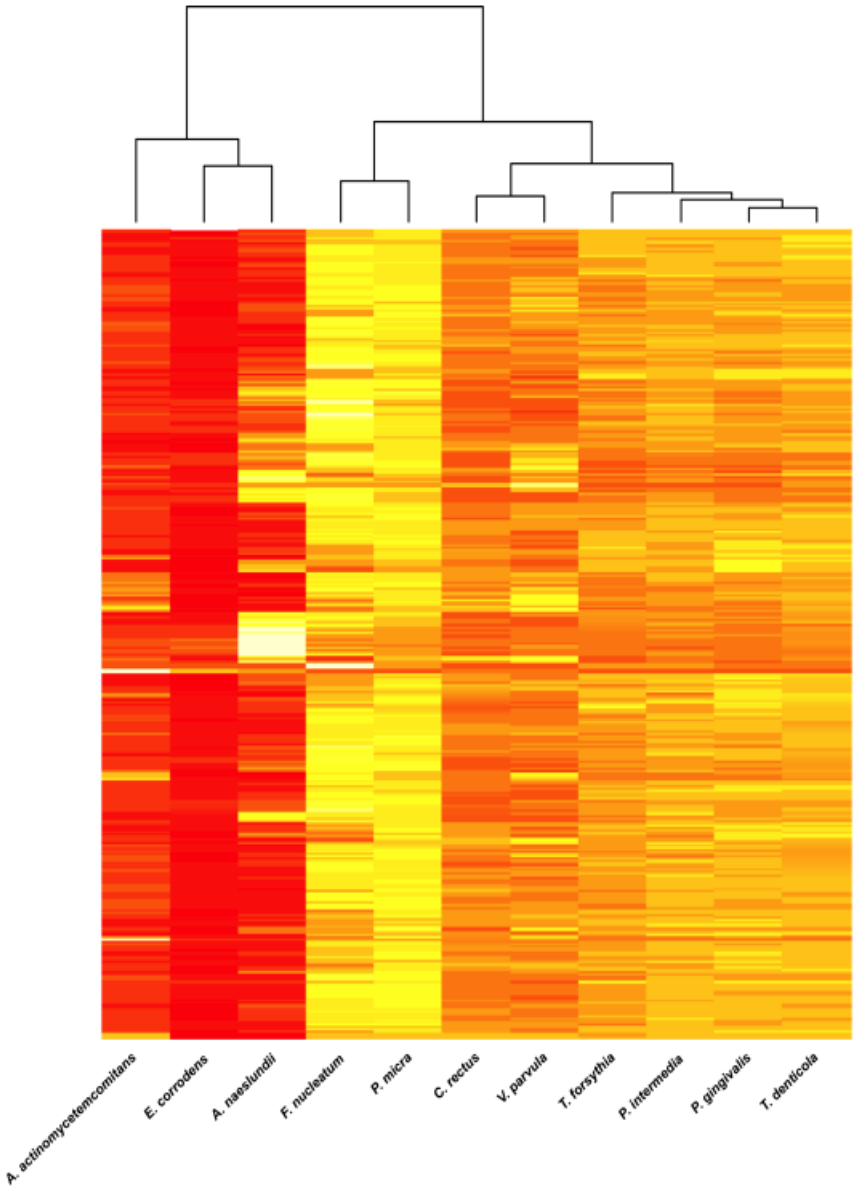

Fig. 2. Cluster analysis of functional gene groupings associated with specific bacteria in the neighbouring periodontal pockets. The analysis identifies bacterial species whose subgingival levels correlate with specific gene expression profiles in the adjacent gingival tissues. Each pixel row displays a functional group of genes (Gene Ontology Group) as a pixel row, and the strength of the differential regulation is indicated by a colour range from red for weak regulation to white/yellow for stronger regulation (heat map). The relative similarity of tissue expression profiles elicited by the different species is displayed on the *x*-axis, i.e., bacterial species clustering together are associated with similar transcriptomic responses in the gingival tissues. Figure reproduced with permission from Papapanou et al. (2009).

For example, when the differentially expressed gene ontology groups were ranked according to the magnitude of their differential regulation, the second top functional group for *A. actinomycetemcomitans*, ''apoptotic mitochondrial changes'' was ranked 96th, 101st and 96th for *P. gingivalis*, *T. forsythia* and *T. denticola*, respectively. These observations are in line with the data described above from in vitro studies comparing apoptosis-related transcriptomic profiles in gingival epithelial cells stimulated with *A. actinomycetemcomitans* and *P. gingivalis* (Handfield et al. 2005). However, high levels of colonization by all red complex pathogens and *A. actinomycetemcomitans* resulted in a common top-regulated functional group in the adjacent tissues, ''antigen presentation and processing'', corroborating data from in vitro transcriptomic studies suggesting a ''common core'' of genes regulated by most pathogens, as well as variable, pathogen-specific expression profiles (Mans et al. 2006a). Furthermore, cluster analyses of the gene ontology groups associated with specific colonization patterns identified the bacterial species eliciting relatively similar responses in the adjacent gingival tissues (Fig. 2). Interestingly, *A. actinomycetemcomitans* did not cluster with the red complex bacteria, that expectedly were associated with relatively similar gene expression patterns, but with its green complex peer *Eikenella corrodens* and the health-associated *A. naeslundii*. Thus, this study demonstrated that the bacterial content of the pocket is an important determinant of gene expression in the adjacent gingival tissues and, conceivably, of the clinical phenotype. However, this study is still not free from the limitations inherent in the cross-sectional design mentioned above. Importantly, the documented transcriptomic responses (i) should not necessarily be ascribed to the individual bacterial species studied but may partly be owed to the hundreds of non-investigated species that co-varied with the indicator bacteria assessed, and (ii) stemmed from a mixed pool of host cells (including the epithelial lining of the pocket, connective tissue fibroblasts, endothelial cells and infiltrating inflammatory cells). Logical next steps in future studies would be to utilize laser-capture microdissection (Chowdhury et al. 2007) or similar approaches to selectively analyse expression profiles in uniform cell populations of the gingival tissues, as well as to investigate whether the transcriptomic responses indeed result in differential expression of corresponding proteins. However, the lack of a high-throughput proteomics platform makes a comprehensive confirmation of these data on the protein level unfeasible at the present time.

Conclusions

Limited data from in vitro and in vivo studies in animals and humans support the concept of specific host tissue responses to particular microbial complexes that are evident by differential regulation of both gene or protein expression, ultimately resulting in a

distinct clinical phenotype. Whole-genome expression analyses showed that periodontal pathogens induce a small, ''common core'' of differentially regulated genes encoding for an inflammatory response, and a larger subset of genes that may reflect cellular responses specific to each particular pathogen.

Several limitations of the currently available studies are apparent and need to be addressed in future research. First, the role of the hundreds of bacterial species identified in the subgingival plaque but not assessed in any of the available studies is unclear. Second, data from in vitro studies utilizing single populations of oral cells challenged with mono-infections of planktonic bacteria may not adequately portray human periodontal diseases. Studies using organ culture models of periodontal tissues interacting with artificial biofilms may overcome these limitations in the future. Lastly, the majority of the published human studies are cross-sectional in nature, and therefore inherently incapable of allowing temporal or causal inferences. Longitudinal studies in humans hold the potential to be superior to any model, but need to be adequately powered and controlled.

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Address:
Panos N. Papapanou
Division of Periodontics
Section of Oral and Diagnostic Sciences
Columbia University College of
Dental Medicine
630 W 168th Street
PH-7-E-110
New York, NY 10032
USA
E-mail: pp192@columbia.edu

Clinical relevance

Scientific rationale for the study: To evaluate the evidence supporting the notion of a local host response in the periodontal tissues that is specific to the subgingival bacterial challenge.

Principal findings: Different periodontal pathogens appear to trigger distinct gene and protein expression profiles, ultimately driving the clinical phenotype.

Practical implications: The presence of specific subgingival pathogen complexes may be indicative of a particular disease phenotype with a possible bearing in diagnosis and treatment.