Host Gene Expression in Local Tissues in Response to Periodontal Pathogens

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Purpose: Periodontal tissue destruction is a host mediated immunoinflammatory lesion, triggered by the accumulation of a complex microbial ecology in biofilms juxtaposed to the tissues. Current evidence is providing substantive support that characteristics of the portfolio of responses of individual hosts, e.g. controlled by gene expression variation, may be more significant than the commensal microbial biofilm dissimilarities in explaining disparities in the expression and severity of disease across the population. We report our molecular approaches, including microarray technology, to describe the responses in local host tissues to infection by oral microorganisms.

Materials and Methods: We describe a murine calvarial model (MCM) and a rat oral infection model (ROIM). The MCM focused on gene expression patterns of soft tissue and bone following monoinfection with *Porphyromonas gingivalis (P. gingivalis)*, *Treponema denticola (T. denticola)*, or *Tannerella forsythia (T. forsythia)*, or a polybacterial challenge with this consortium. The ROIM evaluated gene expression in gingival tissues following oral infection with each of the microorganisms.

Results: The MCM identified gene families and genes that demonstrated a significantly altered expression after local infection with the oral bacteria. Importantly, gene expression profiles were substantially different between soft tissue and calvarial bone, and the gene profiles with the polybacterial infection showed extensive differences from the monoinfections. The ROIM demonstrated for the first time the ability to successfully orally infect rats with members of this human pathogenic consortium. We noted significantly increased bone loss with the consortium, and variations in gene expression profiles related to the type of infection.

Conclusions: The results from both models supported a dramatically different pattern of gene expression than what might have been expected based upon previous 'reductionist' approaches. Microarray technology should better describe the range of host responses that occur in local tissue challenged with oral bacteria, and may provide a very different gene expression footprint of homeostasis versus critical molecular mechanisms that enable disease progression.

Key words: microarray, polybacterial infection, animal models, gene expression.

Oral Biosci Med 2005; 2/3: 175-184

INTRODUCTION

The predominant polybacterial infection of mankind is expressed clinically as periodontal disease, which afflicts nearly half of the population by the age of 50 and is related to development of a microbial biofilm colonizing the subgingival sulcus. Variations in onset, severity and clinical parameters have resulted in the recognition of different types of periodontitis, which have been suggested to exhibit different microbial characteristics. However, the concept that periodontitis is a chronic disease, expressed by exacerbations and remissions, strongly suggests that deviations in regulation of host responses should occur and may provide a more robust mechanistic description for these variations in disease expression (Kornman, 1999).

The trigger for the initiation of disease is the presence of complex microbial biofilms (Socransky and Haffajee, 2002) that colonize the sulcular regions of the teeth. The characteristics of microbiological progression from periodontal health to gingivitis (e.g. chronic inflammation of the gingival tissues without tissue destruction), and eventually to periodontal disease is complicated, with an estimate of nearly 700 bacterial

taxa, phylotypes and species can colonize the oral cavity of humans showing some structural organization in the biofilms, although it remains unclear how this multitude of bacteria compete, co-exist, and/or synergize to initiate this chronic disease process (Paster et al, 2001). Studies over the past three decades have attempted to focus on the microbial ecology comprising this pathogenic biofilm and to link specific bacteria or bacterial consortia with progressing disease (Haffajee and Socransky, 1994; Paster et al, 2001). Major complexes have been observed in subgingival plaque (Socransky et al, 1998), with the 'red complex' consisting of *P. gingivalis*, *T. forsythia*, and *T. denticola* proposed as a portion of the climax community in the biofilms at sites expressing progressing periodontitis (Haffajee et al, 1998; Kigure et al, 1995). While these observations have routinely noted the presence of the consortium, and *in vitro* studies have suggested that they may actually exist in a supportive ecology leading to virulence, minimal direct evidence of synergistic pathogenicity is available to buttress this hypothesis.

Various aspects of human periodontal disease may be assessed in animal models (Maddon and Caton, 1994) that enable the examination of microbiological, immunological, and clinical features of periodontal disease and its prevention and treatment (Weinberg and Bral, 1999). Animal models of periodontal bone loss include extensive studies in nonhuman primates (Holt et al, 1988; Shou et al, 1993; Persson et al, 1993; Assuma et al, 1998; Ebersole et al, 1999, 2000), and in mice and rats that provide unique opportunities to evaluate microbial and host response aspects of this disease (Taubman et al, 1983; Yamashita et al, 1991; Klausen, 1999; Baker et al, 1994, 1999, 2000; Wang and Stashenko, 1993). Our studies and those of others using these models showed that infections with various oral bacteria including *P. gingivalis*, *Campylobacter rectus (C. rectus)*, *Fusobacterium nucleatum (F. nucleatum)* and *Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans)* induced bone resorption (Zubery et al, 1998; Chiang et al, 1999; Kesavalu et al, 2002; Li et al, 2002) related to prostaglandins, IL-1, TNFα and other signaling molecules.

Substantial evidence has established the crucial contribution of host derived inflammatory cytokines and mediators in periodontal inflammation and disease. Current theory emphasizes that gingival tissue destruction around affected teeth results from a local immunoinflammatory response secondary to colonization by the complex biofilms (Page, 1991; Stashenko et al, 1989, 1991; Rossomando and White, 1993; Manolagas, 1995; Darveau et al, 1997; Salvi et al, 1998) and drives the destructive processes. While a number of mediators, e.g. IL-1, TNFα, IL-6 etc., have substantial support as target molecules for destruction of alveolar bone, it is clear that a much more complex host response takes place during infection with a single microorganism. This becomes even more complicated during a polybacterial infection, in which complementary and potentially competing signals are provided to the host tissues.

The completion of the human genome project and sequencing numerous model species suggests that changes in individual genes within the tissue milieu of a chronic inflammatory site are likely to not adequately address the complexity of molecular interactions that occur, nor satisfactorily describe the host responses that are genetically regulated to fully understand variations in disease. The use of DNA microarrays to survey transcriptional host responses after exposure to microbial pathogens is becoming a powerful approach to understanding host-polybacterial interactions. Thus, host response profiling may also identify transcripts uniquely affected by individual pathogens (Cummings and Relman, 2000; Kato-Maeda et al, 2001). Various applications of gene expression profiling of host responses in microbial pathogenesis have been reported (Eckmann et al, 2000; Rosenberger et al, 2000; Belcher et al, 2000; Cohen et al, 2000; Saban et al, 2000; Salama et al, 2000; Wells et al, 2001). Despite their power and application to a variety of diseases, pathogens, and animal models, few studies have been published on virulence studies of oral pathogens.

This report provides a brief overview of exploratory approaches that we have been using to implement array technology to elucidate mouse and rat genes in tissues that are differentially regulated in mono- and polybacterial infections with periodontal pathogens. These studies will provide an initial profiling of the host-bacterial interactions and will generate additional hypothesis-driven experimental designs to explore host-multi-pathogen interactions occurring in oral infections.

MATERIALS AND METHODS

Murine Calvarial Model

The murine calvarial bone resorption model of Boyce et al (1989) has been adapted the to examine the impact of oral bacteria traversing into the connective tissue, eliciting local inflammation and altering bone metabolism (Zubery et al, 1998; Chiang et al, 1999; Kesavalu et al, 2002; Li et al, 2002). In this study, mice were injected s.c. over the calvaria with 1.5×10^9 (high) and 5×10^8 (low) doses of live *P. gingivalis*, *T. forsythia*, or *T. denticola*, a consortium of *P. gingivalis*/*T. forsythia*/*T. denticola* (5×10^8) of each species, or a control group injected with reduced transport fluid (RTF) once daily for three days. Mice were euthanized eight hours after the last injection. Six mice from each group were used to prepare the tissues over the calvaria, as well as the calvarial bone, and snap frozen for gene expression studies.

Rat Oral Infection Model

P. gingivalis strain 381 (Kesavalu et al, 1992, 2003), *T. forsythia* ATCC 43037 (Sharma et al, 1998; Yoneda et al, 2001; Sakamoto et al, 2002), and *T. denticola* ATCC 35404 (Kesavalu et al, 1998, 1999) used in this study were cultivated using standard conditions in broth or on blood agar plates. The bacteria were harvested, suspended in reduced transport fluid at 2 x 10^{10}/ml, and purity, viability and motility assessed (Kesavalu et al, 1998, 1999). For oral monobacterial infection or Pg/Tf/Td polybacterial infection, the bacteria were mixed with equal amounts of 2% carboxymethylcellulose (CMC) and 1ml of the bacterial suspension administered orally to isoflurane inhalation-anaesthetized rats within 15 minutes. Prior to oral infection, the rats were administered 4 consecutive days of antibiotic treatment delivered through drinking water (Baker et al, 1994, 1997, 2000) and the oral cavities were swabbed with a 0.12% chlorhexidine gluconate rinse (Fine et al, 2001). The animals were rested for three days before the oral infection regimen. Oral microbial samples were collected using sterile cotton veterinary swabs at 1, 3, 5, 7, 9, and 11-week intervals to document infection. DNA was isolated from the oral samples (Wizard® Genomic DNA purification kit; Promega, Madison, WI). PCR oligonucleotide species-specific primers with defined product size were used and the products analyzed by 0.8% agarose gel electrophoresis. Animals were euthanized, blood collected and serum IgG antibody to *P. gingivalis*, *T. forsythia*, and *T. denticola* were assessed using an ELISA (Kesavalu et al, 1992, 1998, 1999). The head was detached, autoclaved, mandible and maxilla separated from the skull, the bulk of flesh removed, and hemisected for radiographic analysis of alveolar bone resorption. The crestal alveolar bone loss for each surface was defined as the distance along the tooth surface from the cemento-enamel junction (CEJ) to the alveolar crest using a technique outlined by Reed and Polson (1984). The summation of bone loss, in mm, was tabulated and analyzed for intra and inter-group analysis.

RNA Isolation

Total RNA was isolated from murine calvarial soft tissue and calvarial bone, and rat gingiva (both mandibular and maxillary jaws) using Trizol reagent (Invitrogen, CA) and RNA cleanup with a Qiagen RNeasy mini kit (Qiagen, Valencia, CA). The Murine GeneChip® MOE 430A array and the Rat GeneChip® 230 2.0 (Affymetrix, Santa Clara, CA) were used to profile the MCM and ROIM transcriptome changes. Reverse transcription was performed on 10µg of total RNA, SuperScript polymerase and an oligo-(dT)24 primer with a T7 RNA polymerase promotor (Invitrogen, CA), according to Affymetrix Corp. Technical manual. The fragmented complimentary RNA (cRNA) was hybridized to the GeneChips, arrays stained with streptavidin-conjugated phycoerythrin, and probe arrays were scanned.

For any particular experiment, Affymetrix microarray chips were normalized and standardized using Affymetrix Version 5 software and then exported to Excel, resulting in gene intensities for each gene-chip combination. The following steps were then performed: 1) All positive control genes and genes that are labeled absent for all chips were removed from the analysis. 2) All expressed sequence tags (ESTs) were removed from the analysis. 3) An overall F test for each ANOVA model was used to rank the genes from most significant to least. 4) The False Discovery Rate (Benjamini and Hochberg, 1995) method (FDR) was used to truncate the list in 3). Multiple comparison techniques such as Bonferroni are far too conservative for microarray experiments. 5) The list from 4) was divided into categories, e.g. significantly down regulated compared to control rat tissues for further analysis. Based on our preliminary data, we initiated the studies limiting our focus to differences of >2 fold change (up and down-regulated), with a $p<0.01$ level of significance (Hughes et al, 2000; Smoot et al, 2001; Boyce et al, 2002). We have used this approach in combination with web-based genomics/proteomics databases to identify characteristics of major tissue gene changes resulting from infection with these oral pathogens. We also investigated genes with high statistical significance, but less than two-fold change. These studies should help identify specific host genes that may play an important role in the host protective or pathologic response to oral mono- and polybacterial infections. Furthermore, the ability to access the broad array of gene expression changes in this model system will also enable additional hypothesis generating capacity by exploring broader gene expression patterns, and identification of unique genes that have not been evaluated or predicted to contribute to the periodontal tissue destructive processes.

RESULTS

Murine Calvarial Model

Our principal focus in using this MCM was to test the hypothesis that a purported pathogenic consortium from humans had the capacity to synergistically elicit a destructive inflammatory response and bone resorptive processes. Heretofore, this consortium has only been identified as an 'associated cluster' that appears with an elevated incidence in sites of periodontal disease. However, negligible evidence is available to support that they actually 'conspire/consort' to enhance tissue destructive processes. We have initially examined this potential by exploring the profiles of gene expression in infected soft tissues and underlying calvarial bone following monobacterial infections versus the polybacerial consortium. Fig 1 schemati-

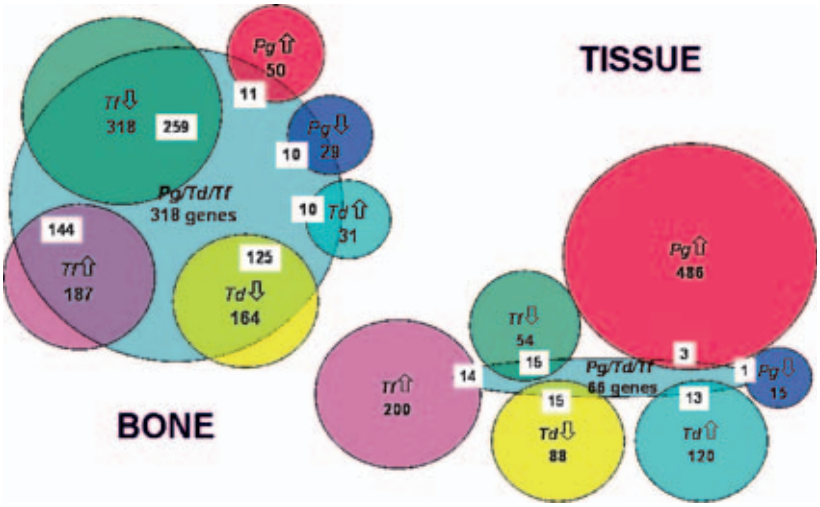

Fig 1 Number of genes that were upregulated (p<0.01 & >2-fold) in tissues from mice infected with high (⇧) or low (⇩) dose monoinfection, or with the *Pg/Td/Tf* consortium. Numbers in white box denote gene that overlapped in upregulated expression between mono- and polybacterial infection.

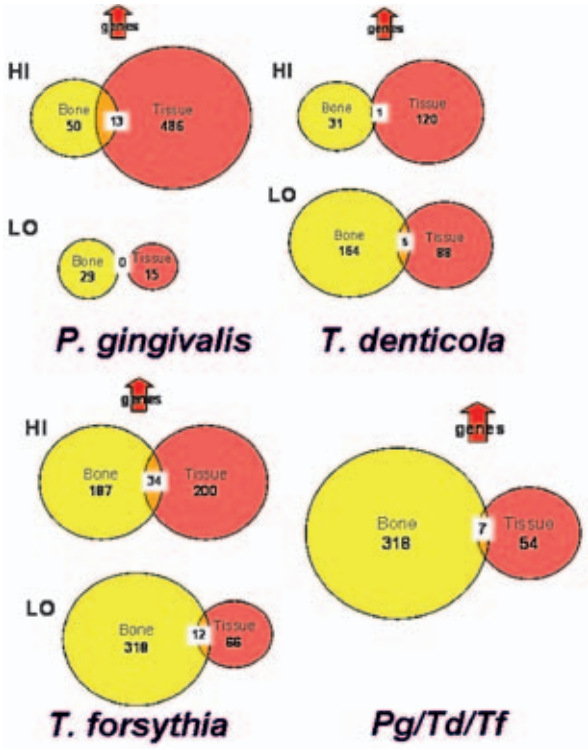

Fig 2 Number of genes that were upregulated (p<0.01 & >2-fold) in tissues from mice infected with HI or LO dose monoinfection, or with the *Pg/Td/Tf* consortium. Numbers in white box denote gene that overlapped in upregulated expression between bone and soft tissue in the calvaria.

cally depicts the characteristics of gene expression regulation in bone and soft tissue of the mice. The number of genes upregulated by the individual monoinfections, vary substantially across the species, within and between tissues, and related to the challenge dose. *P. gingivalis* appeared to alter expression of considerably more genes in the soft tissues than in bone, while *T. forsythia* showed much greater activity in the bone. The consortium was particularly effective in altering gene expression in bone, although it appeared to modulate the number of genes that were upregulated in soft tissues, compared to the various monoinfections. Finally, there was extensive overlap in gene expression between the mono- and polybacterial infections in the bone, while generally unique gene profiles were observed in the soft tissue responses. Fig 2 provides a schematic comparison of the upregulated gene expression profiles comparing bone and soft tissue with mono- or polybacterial infections. The results

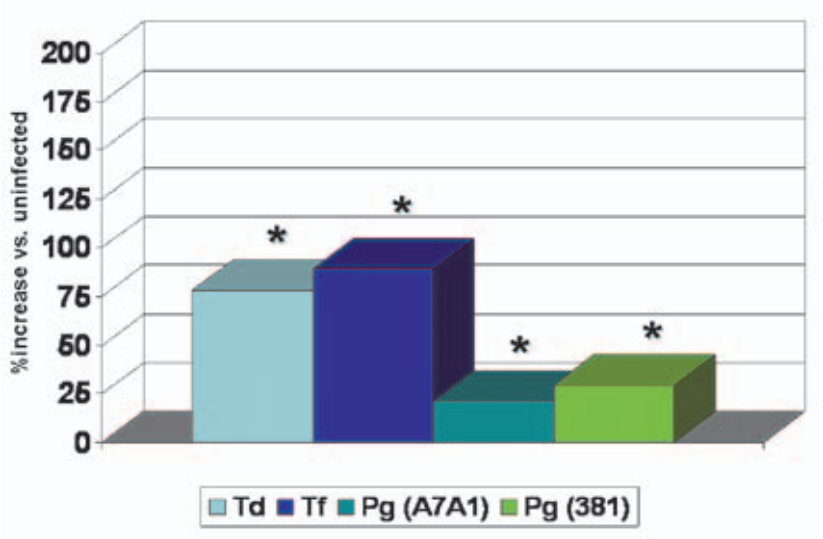

Fig 3 Total alveolar bone loss in rats orally infected with single or multiple bacteria. The bars denote mean bone loss of group of animals (5-20) infected with individual or consortia of bacteria compared to control, uninfected rats. * denotes significantly different at p<0.0001 vs. controls.

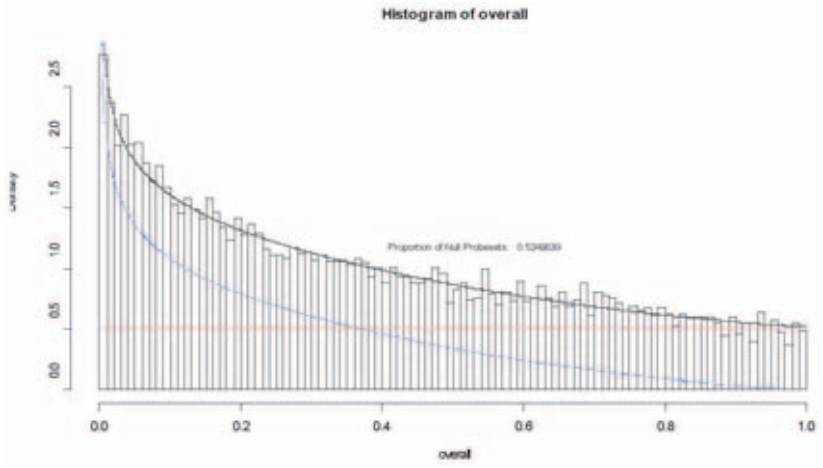

Fig 4 Histogram of the observed p-values for the p-values for the overall test of difference among treatment groups. The evaluation was applied to the *P. gingivalis, T. denticola, T. forsythia* induced gingival tissue gene expression microarray data. The horizontal axis is the p-values for the entire dataset of gene expression and the vertical axis is the corresponding probability density of genes within each p-value category. The blue line corresponds to the estimated weighted beta distribution, the red line corresponds to the estimated weighted uniform distribution, the black line corresponds to the estimated mixture of beta and uniform distributions.

demonstrated little overlap in the genes that were altered in the tissues, irrespective of the microbial challenge.

Rat Oral Infection Model

PCR analysis using species-specific primers was used to document infection of the rats with the human oral bacteria and serum antibody analyses confirmed the oral infection by the bacteria. Using this ROIM, we demonstrated significantly increased alveolar bone loss in the rats following the monobacterial infections. Interestingly, while previous studies may have suggested *P. gingivalis* as a prototype species for enhancing bone loss, we noted that both *T. denticola* and particularly *T. forsythia* were robust in eliciting significant alveolar bone loss (Fig 3).

Gingival tissue analysis examined an array of nearly 16,000 genes in the controls and in tissues from animals with the monobacterial infections. Using a one-way analysis of variance the genes that had a significant difference, at p<0.01, were grouped together based on if there was or was not a significant difference when they were compared with the control group in the corresponding post hoc test. The gene lists were further refined by comparing the magnitude of the mean expression levels to the control group mean expression levels. We have done a false discovery analysis of the data by fitting a mixture of uniform and beta distribution to the empirical cumulative distribution of the p-values using non-linear least squares (Fig 4). Under the null hypothesis, the p-value distribution is approximated

by the uniform distribution. If the null hypothesis does not hold, the distribution of the p-values is approximated by a beta distribution. We estimate that 47.5% of the probe-sets have a detectable difference among the groups, for any sample size.

We noted that 327 genes were significantly different ($p<0.01$) between control and infected gingival tissues. Table 1 depicts a strategy to examine gene expression alterations across the groups infected with the individual bacteria. This table organizes the gene expression patterns into common genes, up- or down-regulated, among the tissues infected with multiple bacteria. These comprised 47% of the genes that were significantly changed. The second grouping of 41% of the genes represents those that changed uniquely with a particular bacterium. Interestingly, only 2% of the 327 genes exhibited changes that disagreed across the infections (i.e. up with one infection, down with another), supporting some consistency for the characteristics of gingival responses when challenged with these exogenous human pathogens.

We have used the EASE (Expression Analysis Systematic Explorer) analytical approach to provide an exploration of biological functions, represented by groups of gene changes, occurring in the gingival tissues of infected rats (Table 2). EASE performs three basic functions with the 327 genes selected by a $p<0.01$ level of change: 1) over-representation analysis of functional gene categories, 2) customizable linking to online tools (DAVID EASE), and 3) creation of descriptive annotation tables. The statistical measure of over representation is a one-tailed Fisher exact p-value or 'EASE score'. The results demonstrate a number of biological processes, cellular components, and molecular functions that are altered in representation of gene changes in the gingival tissues of rats orally infected with *P. gingivalis*, *T. denticola* or *T. forsythia*. These changes in response to oral infection provide a somewhat different perspective than previous studies, which limited their focus to changes in one or a few inflammatory genes in the gingiva. Drilling deeper into the gene families representing these functions should enable a more rational approach to not only the mechanisms of disease progession, but also provide potential biomarkers for risk and/or progressing disease.

Finally, we queried the dataset to examine the characteristics of gene expression in infected gingival tissues from rats, for genes/products that have been previously suggested to be altered in gingival tissues/fluids from patients with periodontitis. The results in Table 3 demonstrate that a number of the biomolecules that have been suggested to be altered in humans, are also well represented as changes in gingival tissues of infected rats. Moreover, about 2/3 of the markers reported to be associated with human disease were detected in control and infected rat gingiva; however, many of these host responses were not the predominant gene changes that occurred in the rat following infection and correlated with alveolar bone loss.

DISCUSSION

The interaction between oral biofilms associated with marginal and subgingival areas of the oral cavity, clearly represents a dynamic and complex process. The host inflammatory/immune armamentarium is directed towards 'managing' this commensal microbial ecology, and must successfully respond to alterations in the characteristics of the biofilms reacting to fluctuations in the local environment. The ultimate goal of this interaction is not to eliminate the biofilms, or even individual members of the ecology, but to develop a 'mutual non-aggression pact' to maintain homeostasis of the tissues. These concepts would imply at least two major considerations in examining the mechanisms of periodontal tissue destructive processes: 1) while certain specific bacteria provide a fingerprint of biofilms at sites of disease, the transition of the biofilm components from health to disease likely reflects a microbial response to environmental changes (ie. inflammatory response with new/increased nutrients coupled with pH and oxygen tension alterations) rather than a driving force of pathogenicity; and 2) the hopes of identifying a 'single analyte' that reflects these complex interactions and accounts for the variation in disease presentation across the population is unlikely. This report attempts to address these issues using various animal models to explore the effects of polybacterial challenge of host tissues and using genomic microarray technology to elucidate the complexity of the local host response. We believe that these initial findings will enable new hypothesis testing of patterns of host molecules that may describe the tissue destructive processes leading to disease and describing variations in disease progression in the population.

Our initial results of a polybacterial infection with *P. gingivalis*, *T. denticola*, and *T. forsythia*, resulted in some unexpected findings. First, we noted major differences in gene expression profiles with the individual bacteria and the consortium. This included both absolute numbers of genes that were altered, as well as the profiles of specific genes. This was particularly noticeable in demonstrating the unique gene sets that changed in bone versus the inflamed soft tissue overlying the calvaria. We observed that while *P. gingivalis* was quite adept at affecting gene changes in soft tissue, it appeared much less active in bone, compared to both *T. denticola* and particularly *T. forsythia*. Moreover, while altered gene profiles in bone demonstrated substantial similarity between the mono- and polybacterial infection, the patterns in the soft tissue were essentially

Table 1 *P. gingivalis*, *T. denticola*, and *T. forsythia* gene expression patterns. Oral monoinfection with *P. gingivalis*, *T. denticola*, or *T. forsythia* induced changes in mean expression levels of 327 genes (p≤0.01) in gingival tissues from infected vs. control rats. The gene expression changes are designated: up-regulated (Up), down-regulated (Dn), and no observed difference (Nd)

Pattern Pg-Td-Tf	# of Genes	Pattern Pg-Td-Tf	# of Genes
Common gene patterns			
Dn-Dn-Dn	32	Up-Up-Up	12
Dn-Dn-Nd	19	Up-Up-Nd	28
Dn-Nd-Dn	14	Up-Nd-Up	14
Nd-Dn-Dn	20	Nd-Up-Up	14
TOTAL	**85**		**68**
Unique gene patterns			
Dn-Nd-Nd	26	Up-Nd-Nd	24
Nd-Dn-Nd	20	Nd-Up-Nd	34
Nd-Nd-Dn	13	Nd- Nd-Up	27
TOTAL	**59**		**75**
No Change		**Non-agreeing patterns**	
Nd-Nd-Nd	23	Disagree	7

Table 2 EASE analysis of altered gene expression in rat gingival tissues infected by oral pathogens

System	Category	List Hits	List Total	Pop Hits	Pop Total	EASE Score	Fisher Exact
Biological process	Protein biosynthesis	9	76	92	2535	0.00506	0.00141
"	Circadian rhythm	4	76	13	2535	0.00575	0.00044
"	Rhythmic behavior	4	76	15	2535	0.00876	0.00079
"	Behavior	4	76	29	2535	0.0524	0.01
"	Nuclear division	3	76	17	2535	0.0882	0.013
"	M phase	3	76	18	2535	0.0974	0.0153
Cellular component	Ribosome	7	67	47	2154	0.00263	0.00048
"	Ribonucleoprotein complex	7	67	54	2154	0.00535	0.00114
"	Small ribosomal subunit	3	67	15	2154	0.0751	0.01
"	Cytosolic ribosome	3	67	26	2154	0.188	0.0449
Molecular function	Structural constituent of ribosome	7	78	47	2761	0.00167	0.00028
"	Structural molecule activity	10	78	121	2761	0.00574	0.0018
"	Metal ion binding	15	78	276	2761	0.0182	0.00883
"	Binding	50	78	1484	2761	0.0488	0.0397
"	Monovalent inorganic cation transporter activity	4	78	32	2761	0.058	0.0116
"	Cation transporter activity	5	78	58	2761	0.0764	0.0225
"	Transition metal ion binding	6	78	91	2761	0.108	0.0416
"	RNA binding	5	78	68	2761	0.12	0.0412
"	Hydrogen ion transporter activity	3	78	28	2761	0.183	0.0428

unique. This was also quite striking with the consortium infection and response in the soft tissues. In contrast to an additive/synergistic effect of the polybacterial infection eliciting gene expression changes, the three bacteria together appeared to substantially modify the host response by modulating the number of genes expressed, consistent with a microbial strategy to escape antimicrobial host responses. These findings will continue to be pursued to develop a clearer understanding of how these commensal microbes function in a consortium at sites of infection.

Table 3 Homologies in gene expression in rat gingival tissue following *P. gingivalis (Pg)*, *T. denticola (Td)*, or *T. forsythia (Tf)* infections compared to selected gene products associated with human periodontitis. Numbers in red are values up- or down-regulated by at least two-fold with more than one bacterial challenge and bold depicts values that are significantly changed at p<0.01

HUMAN periodontal disease genes	Rat gene	Ratio (Infected/Control)		
		Pg	Td	Tf
Matrix metalloproteinase-2 (MMP-2) (gelatinase)	*Mmp2*	0.81	1.25	0.96
Matrix metalloproteinase-3 (MMP-3) (stromelysin)	*Mmp3*	1.56	**4.33**	2.09
Matrix metalloproteinase-12 (MMP-12) (collagenase)	*Mmp12*	1.52	1.17	1.63
Matrix metalloproteinase-12 (MMP-13)	*Mmp13*	0.59	1.41	0.97
Matrix metalloproteinase-14 (MMP-14)	*Mmp14*	**0.52**	**0.58**	**0.55**
Matrix metalloproteinase-24 (MMP-24)	*Mmp24*	**2.28**	**2.0**	1.4
Tissue inhibitor of metalloproteinase –1	*Timp1*	0.9	1.82	0.77
Tissue inhibitor of metalloproteinase –2	*Timp2*	0.82	1.02	**0.22**
Prostaglandin E synthase	*Ptges*	1.34	1.4	1.1
Cathepsin D	*Ctsd*	0.87	0.76	0.57
Interleukin-1α	*Il1α*	2.17	1.31	1.16
Interleukin-1β	*Il1β*	1.66	1.81	1.43
Interleukin-7	*Il7*	**2.34**	**2.15**	**2.3**
Chemokine (C-C motif) ligand 5	*Ccl5*	1.29	2.36	0.48
Chemokine (C-C motif) ligand 22	*Ccl22*	1.37	2.63	1.01
Tumor necrosis factor α	*Tnfα*	1.11	1.52	1.14
S100 calcium binding protein (MRP14)	*S100a9*	**31.16**	1.80	2.94
S100 calcium binding protein (MRP8)	*S100a8*	**21.93**	2.62	3.17
β–glucuronidase	*Gusb*	0.96	0.88	1.16
Aspartate aminotransferase	*Aat*	2.07	1.73	2.08
Fc-Á receptors-FcÁRII (CD32) and FcÁRIII (CD16)	*Fcgr3, Fcgr3a*	**0.55**	0.89	**0.75**
1,25(OH)$_2$D$_3$ (vitamin D$_3$) receptor	*Vdr*	0.46	1.49	1.05
Prostaglandin E synthase	*Ptges*	1.34	1.4	1.1
Defensin beta 3	*Defb3*	**4.57**	**4.67**	1.86
Defensin beta 1	*Defb1*	2.32	2.74	1.5
Fibrinogen, B beta polypeptide	*Fgb*	**2.73**	**2.4**	**2.98**
Fc receptor, IgE, high affinity I, alpha polypeptide	*Fcer1α*	1.98	2.3	2.28
Ceruloplasmin	*Cp*	2.1	1.57	1.21
Collagen, type 1, alpha 1	*Cpl1a1*	**0.43**	0.69	0.62
Collagen, type V, alpha 3	*Col5a3*	0.26	0.59	0.56
β-N-acetyl-hexosaminidase	*Hexa*	0.73	0.77	0.82

The following list of genes associated with human periodontal disease are not expressed in rat gingival tissues under the conditions tested. MMP1, MMP8, MMP9, MMP10, MMP11, MMP16, TIMP3, IL1R1α, IL-2Rβ, IL-4, IL-6, IL-8, IL-10, IL-11R1α, IL-12, IL-13R1α, TNFβ, IFNγR1, IFNγR2, MPO, FMLPR, and PTH (parathyroid hormone).

We were able to document oral infection of the rats with each of the consortium species. This infection resulted in a systemic antibody response and oral bone loss. Molecular studies of the host responses demonstrated a substantial number of significant changes in gene expression in the rat gingiva. Interestingly, the profile of 327 genes with significant changes demonstrated that 88% showed common patterns among the three microorganisms, or unique patterns dependent upon the individual bacterium, and only 7/327 lacked agreement in the gene expression levels observed across the infections. We have also begun to use this oral infection model system to examine specific ontologies of gene functions and individual genes, using multiple bioinformatics tools for data exploration. These preliminary findings demonstrate a group of processes and functions in the gingiva that are responsive to the oral infection, and should help elucidate both biomarkers of the infectious process and targeting molecular mechanisms of tissue destruction. Finally, we provided a snapshot of genes/gene products that have been measured in human periodontitis tissues or gingival crevicular fluid, and implicated as 'risk factors/indicators/markers' for periodontitis. Interestingly, while there was some overlap in altered expression of this array of genes in rat gingiva, many of the gene alterations that would be predicted to occur in human gingiva were not particularly evident in the rat. Moreover, a number of prominent responses in rat gingiva, have not been evaluated or reported in human disease. Since many of the identified genes have homologies in mice, rats and humans, extension of these studies will enable more definitive comparative molecular assessment of common features of mammalian tissue responses to infection by oral bacteria.

ACKNOWLEDGEMENTS

This work was support by UKRF pilot grant from the University of Kentucky, and USPHS grants DE-15720 and RR-20145.

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