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Differential gene induction in macrophage-like human cells by two types of *Porphyromonas gingivalis*: a microarray study

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Abstract: Several studies have provided clinical evidence that FimA clonal variation may contribute to the periodontopathogenicity of *Porphyromonas gingivalis (P.g.)*. We studied the gene expression profiling of the macrophage-like human cell line U937 after infection of two types of *P.g.* (*fimA* type I; Pg-I and *fimA* type II; Pg-II) using microarray. Of 1088 genes examined, 394 genes were detectable. Bioinformatics algorithms were used to analyze the detectable genes. Hierarchical clustering analysis showed that gene expression patterns of Pg-II and the control (no infection) were grouped together. *K*-means clustering grouped 79 genes into Pg-II dominance and 88 genes into Pg-I dominance. A large number of genes related to cell signaling, extracellular communication proteins, cell receptors (by ligands), protein turnover and cell adhesion receptors/proteins were grouped into clusters of Pg-I dominance. Our results indicate that compared with Pg-I, Pg-II induces a low host response as measured by its weak induction of gene expression. (J. Oral Sci. 46, 9-14, 2004)

Key words: microarray; gene expression; FimA; *Porphyromonas gingivalis*; human macrophage-like cell.

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Introduction

Porphyromonas gingivalis (P.g.) is considered to be the most important pathogen of adult periodontitis in humans (1-4). *P.g.* produces many cell components and macromolecules that have been proposed to function as virulence factors. These factors include lipopolysaccharide, the outer membrane, fimbriae, and numerous end products of metabolism (5-8). Fimbriae of *P.g.* are filamentous components on the bacterial cell surface and are thought to play an important role in pathogenesis. Fimbrillin (FimA), a subunit protein of fimbriae, is classified into five types on the basis of their nucleotide sequences (9, 10). Type II *P.g.* (Pg-II) is predominant in periodontitis patients. In contrast, most healthy subjects carry type I *P.g.* (Pg-I) (11, 12). Pg-II, which causes periodontitis, has been investigated in a large number of studies that focused on the virulence factors, fimbrillin proteins, Arg-specific protease and immunological properties (13-19). However, definitive causes have yet to be identified. We recently demonstrated that compared with Pg-I, Pg-II prolongs IL-1β, IL-8, IL-12 and TNF-α induction (20). In this study, we used microarray to analyze the gene expression patterns of the macrophage-like human cell line U937 after infection with two types of *P.g.*.

Materials and Methods

Bacterial strains

P.g. 381 (*fimA* type I; Pg-I) and *P.g.* ATCC 49417 (A7A2-10, *fimA* type II; Pg-II) were grown in GAM broth (Nissui, Japan) anaerobically (80% N_2, 10% H_2, 10% CO_2) at 37°C. *Actinomyces naeslundii* (ATCC 12104;

An) which also has fimbriae was cultured in BHI broth (Becton and Dickinson Microbiology Systems, USA).

Cell cultures

The U937 cell line was maintained in RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (0.1 μg/ml), and L-glutamine (2 mM) in 75 cm^2 tissue culture flasks at 37°C in 5% CO_2 with high air humidity. For differentiation into macrophage-like cells, logarithmic phase cultures at 1×10^6 cells/ml were preincubated with 10 nM phorbol myristate acetate (PMA, Sigma, St Louis, MO, USA) for 48 h, after which they were made quiescent for 24 h by incubation in PMA-free fresh medium before the experiments.

Bacterial infection

Concentrations of bacteria were estimated photometrically. Bacteria were grown in log-phase cultures and added to U937 cells at approximately 10 : 1 multiplicity of infection in RPMI1640 complete medium without antibiotics.

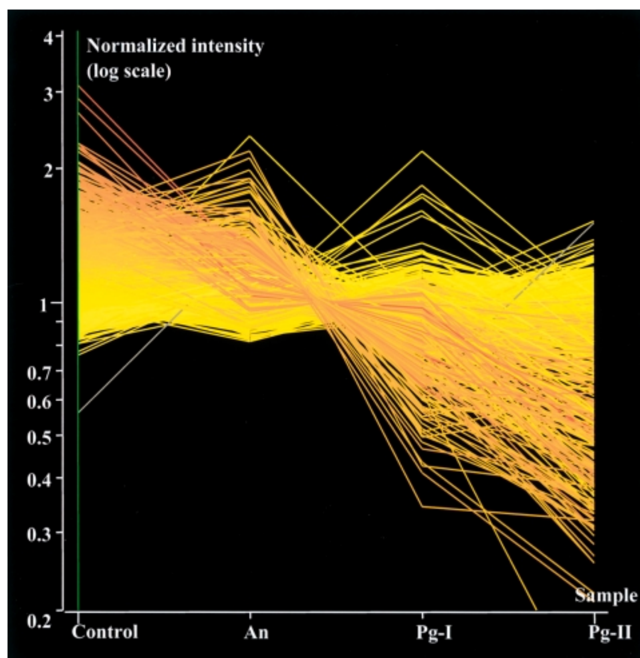

Fig. 1 Analysis of detectable genes from microarray data. Total RNA was extracted from macrophage-like U937 cells 24 h after addition of three bacteria (*P.g. fimA* type I; Pg-I, *P.g. fimA* type II; Pg-II and An). Each RNA sample was used as a template for synthesis of cDNA probes, which incorporated Cy3. The probes were hybridized to the Atlas Glass Human 1.0. The slides were scanned in a confocal microarray scanner. Signal intensities greater than two-fold above the average lambda DNA intensities (negative control) in at least one condition were considered as detectable genes. Data for the detectable genes were normalized with GeneSpring software. The data presented are the average of three separate experiments.

Analysis of gene expression using cDNA arrays

Analysis of gene expression was performed with the Atlas Glass Human 1.0 (Clontech Laboratories, Palo Alto, USA) according to the manufacturer's instructions. Fluorescence (Cy3) labeled cDNA probes were prepared from 6 μg total RNA using an Atlas PowerScript Fluorescence Labeling Kit (Clontech). Fluorescent DNA bound to the microarray was detected with a ScanArray Lite array scanner (Packard BioScience, Billerica, MA, USA), using QuantArray software to locate individual spots, quantitate the Cy3 fluorescence intensity at each spot, and determine background signal intensities. Data from spots that were determined to be the result of hybridization anomalies or microarray errors were excluded from further analysis. Fluorescence intensity values were determined by subtraction of the local background from the foreground. Only those signal intensities greater than two-fold above the average negative control intensity (lambda DNA) in at least one condition were considered as detectable genes for further analysis to avoid errors as a result of low signal intensity. Data analyses were performed with GeneSpring software (Silicon Genetics, Redwood City, USA)

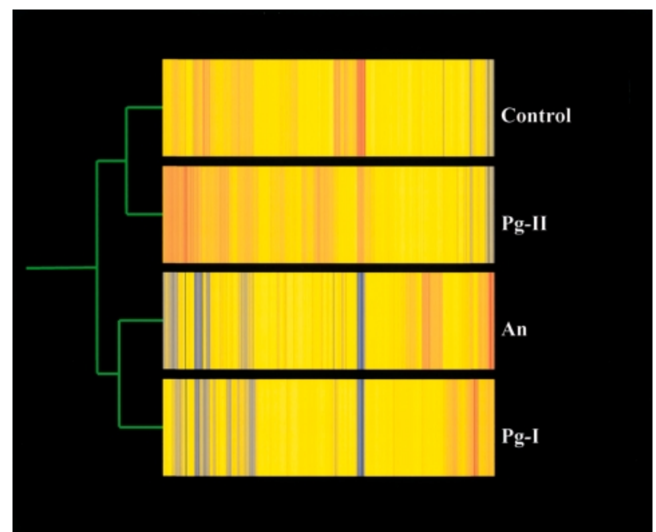

Fig. 2 Hierarchical clustering analysis of detectable genes of infected cells. Experiment tree clustering similarity by standard correlation was applied to normalized microarray data (*P.g. fimA* type I; Pg-I, *P.g. fimA* type II; Pg-II and An). Red, yellow and blue lines indicate high, moderate and low signal intensities, respectively. Green lines indicate a potential inter-relationship between experimental conditions.

bioinformatics algorithms. Per-chip and per-gene normalization was performed to control chip-wide variations in intensity and detection efficiency between spots. The data presented are the average of three separate experiments.

Results

We examined the gene expression profile using microarray at 24 h post infection. Of 1088 genes examined, 394 genes were detectable. Figure 1 shows the gene expression patterns. The vertical axis indicates normalized intensity. Bioinformatics algorithms were used to analyze the detectable genes.

First, hierarchical clustering analysis showed that gene expression in Pg-II infection and the control (PMA treated cells) were grouped together, and Pg-I infection and An infection were grouped together (Fig. 2).

Second, GeneSpring software was used to organize the genes into different expression patterns. The genes were divided into five clusters using *K*-means clustering, representing specific patterns of regulation (Fig. 3A). Clusters of Pg-II dominance (79 genes), Pg-I dominance (88 genes) and An dominance (25 genes) included genes highly expressed in Pg-II, Pg-I and An infection, respectively. The Cluster Control (36 genes) includes genes repressed in bacterial infection and the Cluster No change (166 genes) represents genes whose expression is not affected by bacterial infection (Fig. 3B).

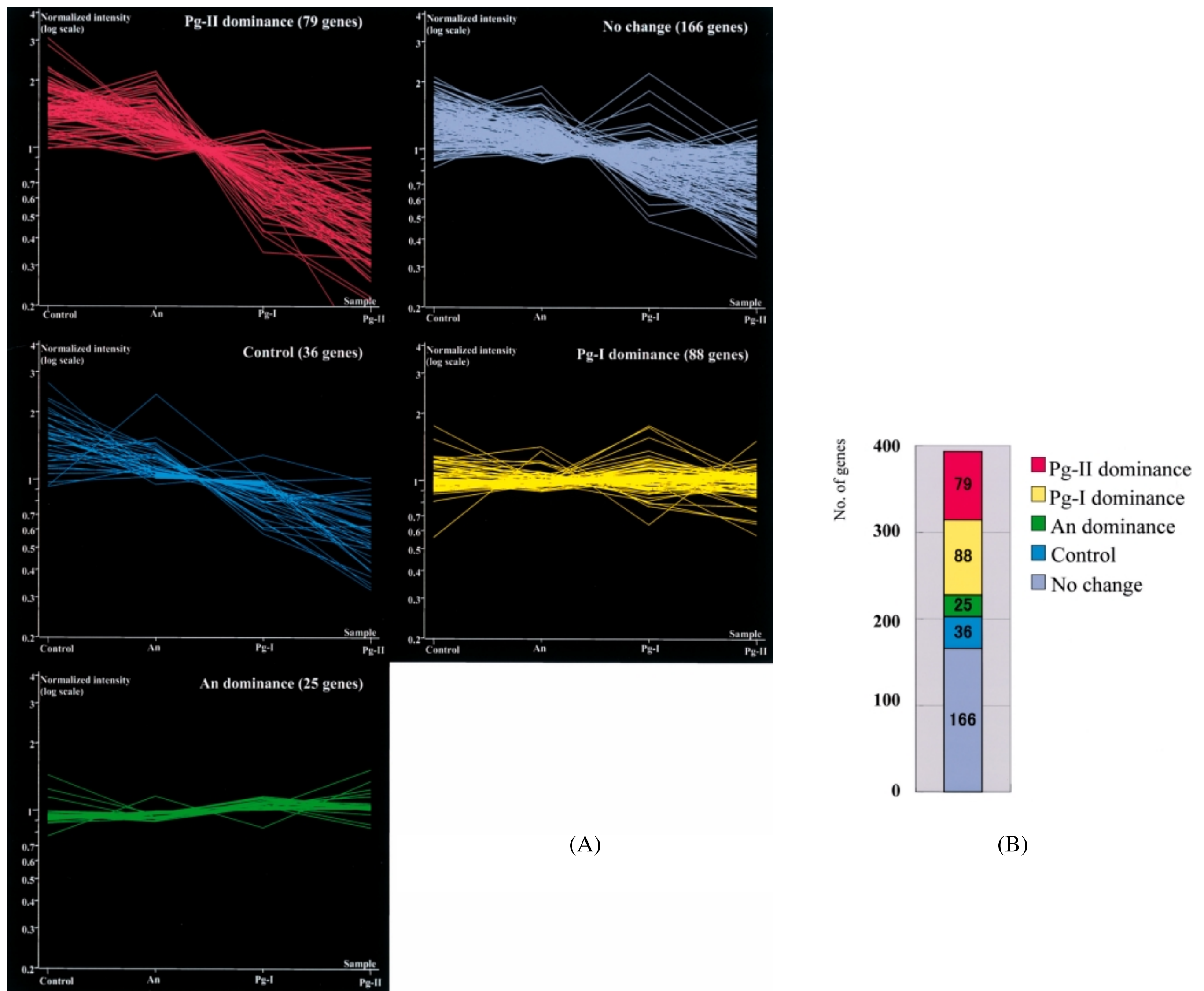

Fig. 3 *K*-means classification of detectable genes. (A) *K*-means clustering measuring similarity by standard correlation was applied to group genes that share similar expression profiles. (B) Number of genes identified in each cluster.

Table 1 List of genes grouped into clusters of Pg-I dominance and Pg-II dominance.

Pg-I		
Gene name and function	GeneBank	Ratio (PgI/PgII)
cell signaling, extracellular communication proteins		
delta-like homolog	U15979	2.6
prostate differentiation factor	AF019770	2.4
glucagon	J04040	2.4
hepatocyte growth factor	M77227	2.3
S100 calcium-binding protein A9	X06233	2.1
hepatocyte growth factor	M60718	2.0
ribonuclease/angiogenin inhibitor	M36717	2.0
cysteine-rich, angiogenic inducer, 61	M36717	2.0
fibroblast growth factor 3	X1445	1.9
brain-derived neurotrophic factor	M61176	1.6
KIT ligand	M59964	1.5
S100 calcium-binding protein A8	X06234	1.5
transforming growth factor, beta 1	X02812	1.4
thrombopoietin	L36052	1.4
vascular endothelial growth factor C	U43142	1.4
transforming growth factor, beta 2	M19154	1.3
fibroblast growth factor 7	M60828	1.3
colony stimulating factor 3	X03438	1.2
endothelin 3	J05081	1.2
pro-platelet basic protein	M54995	1.2
interferon, gamma	X01992	1.2
interleukin 15	U14407	1.2
interleukin 9	X17543	1.2
5-hydroxytryptamine receptor 1D	M89955	1.1
small inducible cytokine B subfamily, member 13	X02530	1.1
small inducible cytokine subfamily E, member 1	U10117	1.1
small inducible cytokine subfamily B, member 10	X02530	1.1
fibroblast growth factor 6	U46010	1.1
interleukin 5	X04688	1.1
macrophage migration inhibitory factor	M25639	1.1
manic fringe homolog	U94352	1.1
wingless-type MMTV integration site family 2	X07876	1.1
quiescin Q6	L42379	1.0
vascular endothelial growth factor B	U48801	1.0
inhibin, beta A	J03634	1.0
intracellular transducers/effectors/modulators		
leukemia inhibitory factor	X13967	1.5
RAP1, GTP-GDP dissociation stimulator 1	X63465	1.4
intersectin 2	U61167	1.3
ems1 sequence	M98343	1.2
G protein-coupled receptor kinase 5	L15388	1.1
ephrin-A1	M57730	1.1
ephrin-A5	U26403	1.1
ephrin-B2	L38734	1.1
mitogen-activated protein kinase kinase kinase 5	D84476	1.1
cell receptors (by ligands)		
EphB1	L40636	3.0
TRK-fused gene	X85960	2.3
interferon receptor 2	X77722	2.2
interleukin 8 receptor, alpha	M68932	2.0
Epstein-Barr virus induced gene 3	L08187	1.6
tumor necrosis factor receptor superfamily	X60592	1.6
interleukin 2 receptor	D11086	1.6
protein tyrosine phosphatase, receptor type, H	D15049	1.6
transforming growth factor, beta receptor II	D50683	1.5
endothelin receptor type B	L06623	1.5
interleukin 1 receptor, type II	X59770	1.5
EphA5	X95424	1.5
chemokine (C-C motif) receptor 2	U03882	1.4
interleukin 3 receptor, alpha	M74782	1.2
interferon gamma receptor 1	L03143	1.2
ciliary neurotrophic factor receptor	M73238	1.1
interleukin 7 receptor	M29696	1.1
insulin receptor	M10051	1.0
protein turnover		
inter-alpha (globulin) inhibitor	X07173	1.9
putative ovarian carcinoma marker	X63187	1.7
inter-alpha (globulin) inhibitor H4	D38595	1.5
matrix metalloproteinase 3	X05232	1.2
serine protease inhibitor, Kazal type, 2	M91438	1.2
serine (or cysteine) proteinase inhibitor, clade I	Z81326	1.2
serine (or cysteine) proteinase inhibitor, clade A	X02920	1.1
KIAA0231 protein	D86984	1.1
carboxypeptidase E	X51405	1.0
proteasome subunit, alpha type, 1	D00759	1.0
proteasome subunit, alpha type, 4	D00763	1.0
transcription		
v-rel oncogene homolog A (p65)	L19067	1.3
signal transducer and activator of transcription 2	U18671	1.3
oncogenes and tumor suppressors		
platelet-derived growth factor beta polypeptide	X02818	1.1
cell adhesion receptors/proteins		
desmocollin 1	X72925	1.3
cadherin 6, type 2, K-cadherin	D31784	1.1
cadherin 13, H-cadherin	L34058	1.1
desmocollin 3	X83929	1.1
desmocollin 2	X56807	1.1
ninjurin 1	U72661	1.0
apoptosis associated proteins		
BCL2-interacting killer	X89986	1.4
cell cycle		
none		

Pg-II		
Gene name and function	GeneBank	Ratio (PgII/PgI)
cell signaling, extracellular communication proteins		
bone morphogenetic protein 1	M22488	1.7
interleukin 8	Y00787	1.7
HGF activator	D14012	1.4
endothelin 2	M65199	1.4
interleukin 6	X04602	1.4
placental growth factor	X54936	1.3
interleukin 1, beta	K02770	1.3
wingless-type MMTV integration site family	Z71621	1.3
interleukin 11	M57765	1.1
intracellular transducers/effectors/modulators		
protein kinase, cAMP-dependent	U95299	1.7
transferrin receptor (p90, CD71)	X01060	1.6
Rho guanine nucleotide exchange factor (GEF) 5	U02082	1.6
CD86 antigen	L25259	1.5
PCTAIRE protein kinase 3	X66362	1.5
MARCKS-like protein	X70326	1.5
phosphorylase kinase, gamma 2	M31606	1.4
Janus kinase 3	U09607	1.4
guanine nucleotide binding protein	M36429	1.4
phosphatidylinositol 4-kinase	L36151	1.4
linker for activation of T cells	AF036905	1.4
transforming growth factor, beta receptor I	L11695	1.2
zeta-chain (TCR) associated protein kinase	L05148	1.2
NCK adaptor protein 1	X17576	1.2
mitogen-activated protein kinase kinase 3	L36719	1.2
neurogranin	Y09689	1.1
cell receptors (by ligands)		
chemokine (C-C motif) receptor 2	U03905	1.5
platelet-derived growth factor receptor	M21616	1.5
complement component 5 receptor 1	M62505	1.4
Duffy blood group	U01839	1.4
patched homolog	U43148	1.3
protein turnover		
matrix metalloproteinase 12	L23808	1.7
basigin	L20471	1.3
matrix metalloproteinase 8	J05556	1.1
transcription		
CCAAT/enhancer binding protein	U34070	1.6
nucleobindin 1	M96824	1.5
chromodomain helicase DNA binding protein 3	AF006515	1.4
transcription factor-like 1	D43642	1.4
B-cell CLL/lymphoma 3	M31732	1.4
hepatic leukemia factor	M95585	1.3
butyrate response factor 1	L36719	1.3
cut (Drosophila)-like 1	L12579	1.3
immediate early protein	M62831	1.2
transcription factor AP-2 alpha	M36711	1.2
oncogenes and tumor suppressors		
Notch homolog 4	U95299	1.7
v-mos viral oncogene homolog	J00119	1.7
proline oxidase homolog	AF010310	1.6
ets variant gene 6 (TEL oncogene)	U11732	1.4
mouse double minute 2	M92424	1.4
melanoma adhesion molecule	M28882	1.3
v-myb viral oncogene homolog-like 2	X13293	1.3
Cas-Br-M retroviral transforming sequence	X57110	1.3
ras-related C3 botulinum toxin substrate 2	M64595	1.1
cell adhesion receptors/proteins		
trophinin associated protein	U040810	1.7
apoptosis associated proteins		
lymphotoxin alpha	D12614	1.7
death-associated protein 6	AF015956	1.2
BCL2-related protein A1	U29680	1.2
fms-related tyrosine kinase 3 ligand	U04806	1.2
CASP8 and FADD-like apoptosis regulator	AF010127	1.2
myeloid cell leukemia sequence 1	L08246	1.1
cell cycle		
cyclin E1	M73812	1.7
mitogen-activated protein kinase 7	U25278	1.7
growth arrest-specific 1	L13698	1.5
cyclin D2	M90813	1.3
ubiquitin carrier protein E2-C	U73379	1.3
prothymosin, alpha (gene sequence 28)	M26708	1.2

Third, the different behaviors revealed by clustering analysis reflect the complexity of the different bacterial infections. To this end, the clustered genes were classified according to biological function. We established a list of 10 different categories to sort the 394 genes according to their biological roles as defined by the Clontech database (Fig. 4). When genes grouped into Pg-I dominance and Pg-II dominance were analyzed, a large number of genes related to cell signaling, extracellular communication proteins, cell receptors (by ligands), protein turnover and cell adhesion receptors/proteins were grouped into the cluster of Pg-I dominance (Fig. 4, Table 1).

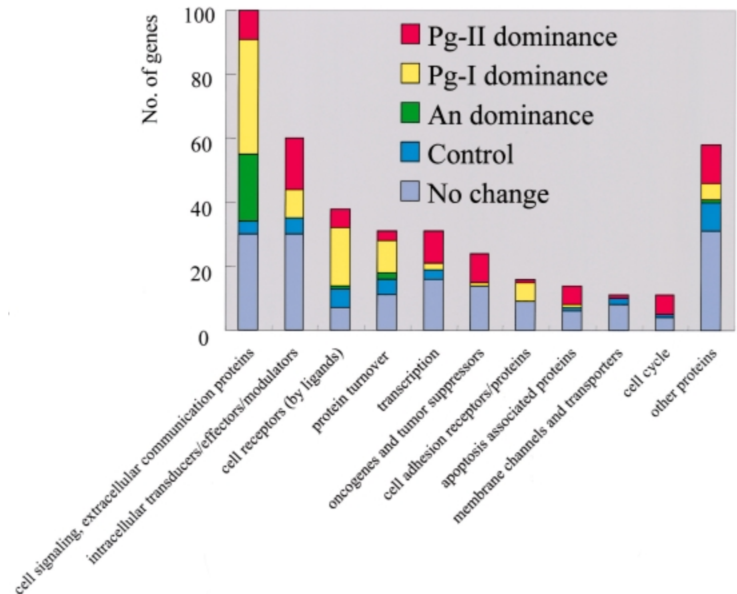

Fig. 4 General functional classification of clustered genes. Clustered genes were classified according to their known general function.

Discussion

In the present study, we used microarray technology to study host-microbe interactions. Microarray technology is a powerful tool that can be used to expand our current understanding of this relationship for a number of reasons (21-23).

Firstly, this technology permits the study of simultaneous changes in the expression of a large number of genes under uniform experimental conditions. Secondly, microarray permits comparison of expression profiles obtained from multiple conditions. Thirdly, microarray measures changes in individual genes in the context of how the expression of other members of the gene family, their receptors, ligands, or transcriptional activators are altered. This allows a more comprehensive understanding of host responses to bacterial infection by identifying patterns of gene expression that would be evident from studying each gene in isolation. Our hierarchical analysis of microarray data showed that the expression profiles of Pg-II and the control, Pg-I and An appear very similar. In contrast to Pg-I, the biological classification indicated that Pg-II is not a potent inducer of genes related to cell signaling, extracellular communication proteins, cell receptors, and cell adhesion proteins. These results demonstrate that compared with Pg-I, Pg-II induces a low host response as measured by its weak induction of gene expression. This may allow Pg-II to evade innate host response, resulting in colonization and development of periodontitis. Microarray analysis of infected cells has helped to reveal the mechanisms by which the host responds to periodontopathic bacteria and some of the mechanisms by which this pathogen attempts to subvert host responses. A more comprehensive examination of the transcriptional responses of *P.g.* and their host will help to define conserved and specific strategies used by both.

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