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# Induction of Signal Transduction Pathway Genes in Dendritic Cells by Lipopolysaccharides from *Porphyromonas gingivalis* and *Escherichia coli*

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***Porphyromonas (P.) gingivalis* lipopolysaccharide (Pg LPS) is the major pathogenic component of periodontal disease. In this study, we have attempted to determine the expression profiles of the signal transduction pathway genes induced by Pg LPS in comparison with *Escherichia (E.) coli* LPS (Ec LPS). DC2.4 cells were treated for two hours with 1 μg/ml of Pg LPS or 0.5 μg/ml of Ec LPS. The total RNA from these cells was then isolated and reverse-transcribed. Gene expression profiles were then analyzed with a signal transduction pathway finder GEArray Q series kit and significant changes in expression were confirmed by real-time PCR. The microarray results indicated that several genes, including *Tnfrsf10b, Vcam1, Scyb9, Trim25, Klk6,* and *Stra6* were upregulated in the DC2.4 cells in response to Pg LPS treatment, but were downregulated or unaffected by Ec LPS. Real-time PCR revealed that the expression of *Trim25, Scyb9* and *Tnfrsf10b* was increased over the untreated control. Notably, *Trim25* and *Tnfrsf10b* were more strongly induced by Pg LPS than by Ec LPS. These results provide greater insight into the signal transduction pathways that are altered by *P. gingivalis* LPS.**

**Key words: signal transduction, *Porphyromonas gingivalis*, *Escherichia coli*, lipopolysaccharide, dendritic cell**

## Introduction

Dendritic cells (DCs) are known to be the most efficient antigen presenting cells (APCs), and are capable of stimulating both primary and secondary immune responses (Fearon *et al*., 1996; Hart *et al*., 1997). DCs are distributed widely throughout both the lymphoid and nonlymphoid organs (Hart *et al*., 1997, Banchereau *et al*., 1998). Recent studies have also reported the detection of DCs in oral tissues, including dental pulp (Sakurai *et al*., 1999), the periodontal membranes, (Kan *et al*., 2001) and the gingiva (Cutler *et al*., 1999), and the results of these studies suggest that DCs function as sentinels in host defense. DCs are also efficient antigen-capturing cells while in their immature state, but as they mature they undergo phenotypic changes which facilitate their migration toward the lymphoid organs, and potentiate their unique ability to prime T cells (Hart *et al*., 1997; Banchereau *et al*., 1998). The initiation of protective immune responses is generally thought to require DC activation.

Specific pathogens associated with plaque biofilm have been implicated in the induction of strong systemic and mucosal immune responses within the oral cavity (Ogawa *et al*., 1993). Among these pathogens, *Porphyromonas gingivalis* (*P. gingivalis*), a gram-negative, black-pigmented anaerobic rod, has been suggested as one of the principal periodontopathic organisms associated with chronic inflammatory periodontal disease (Van Winkelhoff *et al*., 1988) occurring via the stimulation of the host's immune responses.

During the gram-negative bacterial infection, outer membrane LPS is one of the primary molecules recognized by the innate immune system (Fearon *et al*., 1996; Hart *et al*., 1997; Sallusto *et al*., 1995). The injection of LPS, as well as TNF and IL-1, has been determined to induce the migration of murine DCs. The chemical and biological properties of *P. gingivalis* LPS (Pg LPS) are different from those of classical enterobacterial LPS variants, and the endotoxic ability of Pg LPS is significantly less pronounced than has been reported with enterobacterial preparations (Nair *et al*., 1983; Ogawa *et al*., 1994). This may be attributable

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to differences between the structures of Pg LPS and *Escherichia coli* LPS (Ec LPS). Structurally, Pg LPS lacks an ester-linked phosphate at the 4' position of the glucosamine disaccharide, as well as the presence of long acyl chain-bearing fatty acids (Kinane *et al*., 1999). Recently, lipid A from *E. coli* and its related species has been shown to induce mammalian cell activation via the innate immunity receptor, Toll-like receptor (TLR) 4 (Nagai *et al*., 2002). However, LPS and lipid A from *P. gingivalis* have been suggested to induce cell activation independently of TLR4, instead operating via TLR2-mediated signal transduction (Tanamoto *et al*., 1997; Kirikae *et al*., 1999; Hirschfeld *et al*., 2001; Lorenz *et al*., 2002). However, little information is currently available regarding the differences between Pg LPS- or Ec LPS-induced signal transduction pathways in DCs.

DNA microarray technology is a new and powerful tool, which enables rapid and efficient simultaneous analyses of a large number of nucleic acid hybridizations. Recently, microarray analysis of immunoregulatory genes induced by Pg LPS and Ec LPS was reported (Barksby *et al*, 2009).

In this study, we have employed a defined cDNA array in order to target and characterize the expression profiles of signal transduction pathway finder genes in Pg LPS and Ec LPS-treated DC2.4 cells, and to highlight the signal transduction pathway or genes specifically induced by Pg LPS.

## Material and Methods

### Reagents

LPS derived from *Escherichia coli* (O111:B4, L3024) was obtained from the Sigma Chemical Co. (St. Louis, MO).

### Bacterial culture

*P. gingivalis* was cultured in a brain-heart infusion broth, containing 5 mg/ml of hemin and 0.5 mg/ml of vitamin K, at 37°C in an anaerobic chamber, in an atmosphere of 80% $N_2$, 10% $H_2$, and 10% $CO_2$.

### Purification of LPS

*P. gingivalis* was grown under anaerobic conditions, and harvested at the end of the logarithmic growth phase. LPS extraction was conducted via the hot-phenol-water method (Westphal *et al*., 1965). In brief, the bacterial cell pellets were suspended in pyrogen-free water, after which an equal volume of 90% phenol at 60°C was added dropwise for 20 minutes, with constant stirring. The aqueous phase was separated via 15 minutes of centrifugation at 7000 rpm and 4°C, and then collected. This process was repeated, and the aqueous phase was pooled and dialyzed against deionized water for 3 days at 4°C. The dialyzed LPS preparations were then centrifuged for 90 minutes at 40,000 rpm at 4°C in a Beckman (Palo Alto, CA) Ultracentrifuge. The precipitates were suspended in 30 ml of pyrogen-free water, dialyzed for 3 days against distilled water, lyophilized, and maintained at 4°C. The LPS samples were separated via SDS-PAGE and stained for protein with Coomassie's Brilliant Blue in order to verify the purity of the LPS moieties.

### Cell culture

DC2.4 cells were maintained in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Inc., Paisley, Scotland), 100 U/ml penicillin, and 100 g/ml of streptomycin, and were then incubated at 37°C in a humidified atmosphere of 5% $CO_2$.

### RNA preparation

The DC2.4 cells were treated for 2 hours with either 1 μg/ml of Pg LPS or 0.5 μg/ml of Ec LPS. The total RNA from these cells was isolated using TRIzol (Invitrogen, Carlsbad, CA), in accordance with the manufacturer's instructions.

### Microarray

Gene expression array analysis was conducted using the nonradioactive signal transduction pathway finder GEArray Q series kit (SuperArray Inc., Bethesda, MD), in accordance with the manufacturer's instructions. This array membrane is comprised of 96 signal transduction pathway genes, a plasmid pUC18 negative control, and four housekeeping genes, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cyclophilin A, ribosomal protein L13a, and β-actin, each of which was printed with the Tetra Spots format. The biotin-16-dUTP-labeled cDNA probes were synthesized from 5 μg of total RNA. After 2 hours of pre-hybridization at 60°C, with GEAhyb Hybridization Solution (SuperArray) containing 100 μg of DNA/ml of denatured salmon sperm DNA (Invitrogen), the array membranes were hybridized overnight with denatured cDNA probes at 60°C. The membranes were then washed twice with 2x SSC, 1% SDS, and twice with 0.1x SSC, 0.5% SDS, for 15 minutes each at 60°C, and the membrane was blocked for 40 minutes with GEAblocking Solution Q (SuperArray), then incubated with alkaline phosphatase-conjugated streptavidin for 10 minutes at room temperature. Chemiluminescent detection was conducted using CDP-Star chemiluminescent substrate, and images of the arrays were acquired on X-ray film. The images were then scanned with a TouchToss SIS-3800 scanner (Samsung, Seoul, Korea). The resultant scanned images were converted to raw data files using Scanalyse software. GEArray Analyzer software (SuperArray Inc.) was used for data analysis. The relative expression levels of the different genes were estimated via comparisons of their respective signal intensities with that of the internal β-actin control.

### Real-time PCR

Real-time PCR was performed in the system of Light cycler (Roche Applied Science. Indianapolis, IN, USA) for the quantification of the mRNA expression levels. In a LightCycler, 1 μl of first strand cDNA template was added

into a 25ul PCR mix, containing 12.5ul 2X RT$^2$ Real-time$^{TM}$ SYBR Green PCR master mix, 1ul RT$^2$ PCR primer set (Superarray Bioscience Corp, Frederick, MD. USA). The mixture was incubated for an initial denaturation at 95°C for 15min, followed by 40 PCR cycles. Each cycle consisted of 95°C for 30s, 55°C for 30s, and 72°C 30s. A standard curve was generated for the housekeeping gene, GAPDH to enable normalization of each gene to a constant amount of GAPDH.

## Results

**Differential mRNA expression as measured by microarray**

In order to characterize the signal transduction pathways that might be differently modulated by Pg LPS or Ec LPS at the gene expression level, we conducted a series of microarrays. The GEArray Signal Transduction Pathway Finder Gene Array membrane contains 96 marker genes, which are associated with 18 different signal transduction pathways (Mitogenic Pathway, Wnt Pathway, Hedgehog Pathway, TGF-β Pathway, Survival Pathway, p53 Pathway, Stress Pathway, NF-κB Pathway, NFAT Pathway, CREB Pathway, Jak-Stat Pathway, Estrogen Pathway, Androgen Pathway, Calcium and Protein Kinase C Pathway, Phospholipase C Pathway, Insulin Pathway, LDL Pathway, and Retinoic Acid Pathway) and four housekeeping genes, including GAPDH, cyclophilin A, ribosomal protein L13a, and β-actin. A change of at least 1.5-fold in gene expression, as compared to the untreated control, was considered to be significant. Expression profiles of the signal transduction pathway finder genes are shown in Table 1. Pg LPS treatment resulted in an increase in the mRNA levels of the Wnt pathway gene (Myc), Survival pathway gene (Myc), p53 pathway gene (Tnfrsf10b), Stress pathway gene (Myc), NFkB pathway genes (Lta, Vcam1), Jak-Stat pathway gene (Scyb9), Calcium and Protein Kinase C pathway genes (Csf2, Myc), Phospholipase C pathway gene (Vcam1), LDL pathway genes (Csf2, Vcam1), and the Retinoic acid pathway gene (Stra6). Ec LPS treatment was shown to modulate the expression of a significantly wider variety of genes than was seen in the case of Pg LPS treatment.

In order to allow for discrimination between the signal

**Table 1.** Genes increased by DC2.4 cells in response to Pg LPS or Ec LPS treatment

| | Genebank | Symbol | Description | Pg LPS | Ec LPS |
|---|---|---|---|---|---|
| Mitogenic Pathways | M20157 | Egr1 | Mouse Egr-1 (Early growth response 1) | 1.41 | 1.79 |
| | V00727 | Fos | Mouse c-fos oncogene (FBJ osteosarcoma oncogene) | 1.33 | 1.70 |
| Wnt Pathway | X01023 | Myc | normal c-myc gene and translocated homologue from J558 plasmocytoma cells | 1.52 | 1.56 |
| TGF-β Pathway | U22399 | Cdkn1c | Cyclin-dependent kinase inhibitor p57Kip2 | 1.31 | 2.30 |
| | L76150 | Cdkn2a | Mouse CDK4 and CDK6 inhibitor p16ink4a | 1.43 | 1.91 |
| | U19597 | Cdkn2d | Cyclin-dependent kinase inhibitor p19 | 1.31 | 1.94 |
| Survival Pathway | NM_009741 | Bcl2 | B-cell leukemia/lymphoma 2 | 1.02 | 2.94 |
| PI3 Kinase/AKT Pathway | M18194 | Fn1 | Mouse fibronectin (FN) mRNA | 1.27 | 1.75 |
| | X01023 | Myc | normal c-myc gene and translocated homologue from J558 plasmocytoma cells | 1.52 | 1.56 |
| Jak/Src Pathway | NM_009741 | Bcl2 | B-cell leukemia/lymphoma 2 | 1.02 | 2.94 |
| | L35049 | Bcl2l | Bcl2-like | 0.63 | 1.22 |
| NF-kB Pathway | NM_007536 | Bcl2a1d | B-cell leukemia/lymphoma 2 related protein A1d | 0.64 | 1.95 |
| | NM_008670 | Birc1a | Baculoviral IAP repeat-containing 1a | 0.67 | 2.51 |
| | NM_010872 | Birc1b | Baculoviral IAP repeat-containing 1b | 0.54 | 1.83 |
| | U88908 | Birc2 | Mus musculus inhibitor of apoptosis protein 1 | 1.24 | 2.03 |
| | U88909 | Birc3 | Mus musculus inhibitor of apoptosis protein 2 | 1.41 | 2.23 |
| p53 Pathway | L22472 | Bax | Bcl2-associated X protein | 1.01 | 1.57 |
| | AF176833 | Tnfrsf10b | Tumor necrosis factor receptor superfamily, member 10b | 1.52 | 0.97 |
| Stress Pathway | U46026 | Atf2 | Activating transcription factor 2 (CRE-BP1 transcription factor) | 1.17 | 1.60 |
| | V00727 | Fos | Mouse c-fos oncogene (FBJ osteosarcoma oncogene) | 1.33 | 1.70 |
| | X01023 | Myc | normal c-myc gene and translocated homologue from J558 plasmocytoma cells | 1.52 | 1.56 |
| | K01700 | Trp53 | Transformation related protein 53, Tumor antigene | 1.32 | 0.62 |

**Table 1.** Continued

| | Genebank | Symbol | Description | Pg LPS | Ec LPS |
|---|---|---|---|---|---|
| NFkB Pathway | M31585 | Icam1 | Mouse (clone lambda-c5e) intercellular adhesion molecule 1 (ICAM-1) | 1.09 | 1.54 |
| | M16819 | Lta | Mouse tumor necrosis factor-beta mRNA | 1.62 | 2.00 |
| | M84487 | Vcam1 | Vascular cell adhesion molecule 1 | 1.62 | 1.26 |
| CREB Pathway | M20157 | Egr1 | Mouse Egr-1 (Early growth response 1) | 1.41 | 1.79 |
| | V00727 | Fos | Mouse c-fos oncogene (FBJ osteosarcoma oncogene) | 1.33 | 1.70 |
| Jak-Stat Pathway | NM_007376 | A2m | Mus musculus alpha-2-macroglobulin (A2m), | 1.28 | 1.68 |
| | NM_008599 | Scyb9 | Mouse monokine induced by gamma interferon (MIG) | 1.75 | 1.43 |
| Estrogen Pathway | NM_009741 | Bcl2 | B-cell leukemia/lymphoma 2 | 1.02 | 2.94 |
| | D63902 | Trim25 | Mouse mRNA for estrogen-responsive finger protein | 1.36 | 0.23 |
| Androgen Pathway | NM_010639 | Klk6 | Mus musculus kallikrein renal/pancreas/salivary (Klk1), | 1.49 | 0.63 |
| Calcium and | X03020 | Csf2 | Colony stimulating factor, granulocyte macrophage | 1.56 | 1.52 |
| Protein Kinase C Pathway | V00727 | Fos | Mouse c-fos oncogene (FBJ osteosarcoma oncogene) | 1.33 | 1.70 |
| | X01023 | Myc | normal c-myc gene and translocated homologue from J558 plasmocytoma cells | 1.52 | 1.56 |
| Phospholipase C Pathway | NM_009741 | Bcl2 | B-cell leukemia/lymphoma 2 | 1.02 | 2.94 |
| | M20157 | Egr1 | Mouse Egr-1 (Early growth response 1) | 1.41 | 1.79 |
| | V00727 | Fos | Mouse c-fos oncogene (FBJ osteosarcoma oncogene) | 1.33 | 1.70 |
| | M31585 | Icam1 | Mouse (clone lambda-c5e) intercellular adhesion molecule 1 (ICAM-1) | 1.09 | 1.54 |
| | M84487 | Vcam1 | Vascular cell adhesion molecule 1 | 1.62 | 1.26 |
| LDL Pathway | X03020 | Csf2 | Colony stimulating factor, granulocyte macrophage | 1.56 | 1.52 |
| | M84487 | Vcam1 | Vascular cell adhesion molecule 1 | 1.62 | 1.26 |
| Retinoic Acid Pathway | NM_009291 | Stra6 | stimulated by retinoic acid gene 6 | 2.58 | 0.78 |

transduction pathways differently induced by Pg LPS and Ec LPS, the number of genes increased by Pg LPS or Ec LPS per pathway was counted, and reported as a % expression (number of genes increased by treatment of LPS/ number of genes included in each gene family × 100). 67% of mitogenic pathway genes, 43% of TGF-β pathway genes, 50% of Kak/Src pathway (survival pathway) genes, 100% of NF-kB pathway (survival pathway) genes, 100% of CREB pathway genes, and 17% of estrogen pathway genes were ultimately determined to have been up-regulated as the result of Ec LPS treatment, but Pg LPS treatment resulted in no up-regulations of the genes associated with those pathways. Moreover, we did not detect any pathways that were specifically induced by Pg LPS treatment in the array membranes assessed in this study (Table 2). These findings indicate that Ec LPS may be a significantly more potent stimulator than is Pg LPS, and may be capable of inducing a wide range of signal transduction pathways in DC2.4 cells.

In order to identify the genes that were specifically increased as the result of Pg LPS treatment during this experiment, those genes that were increased by Pg LPS by more than 1.3-fold as compared to the LPS-untreated control, but which were not increased as the result of Ec LPS treatment, are summarized in Table 3. Several genes, including Tnfrsf10b, Vcam1, Scyb9, Trim25, Klk6, and Stra6, were found to have been up-regulated in the DC2.4 cells in response to Pg LPS treatment, but were down-regulated or unmodulated via Ec LPS treatment. These data suggest that those genes specifically increased as the result of Pg LPS exposure may perform important functions in the specific immune responses induced by Pg LPS.

### Differential mRNA expression as measured by real-time PCR

In order to confirm the results of microarray, real-time PCR was carried out using SYBR Green I as a fluorescent indicator of double stranded DNA production. After relative quantification and normalization, the expression levels of six genes were calculated. Similar to the results of microarray experiments, both LPSs enhanced the mRNA expression of Trim25, Scyb9 and Tnfrsf10b compared with the control (Fig. 2). Especially, the expression of Trim25 and Tnfrsf10b mRNA was increased only by Pg LPS treatment not by Ec LPS.

## Discussion

With regard to the role of local etiologic factors in periodontal disease, it is known that dental plaque and its associated bacteria (which populate the periodontal pockets) effect the release of LPS into the sulcus. LPS then affects immune cells, including monocytes/macrophages, neutrophils,

**Table 2.** Fraction of gene family increased by Pg LPS or Ec LPS (%)

| | Increased gene fraction (%) | |
|---|---|---|
| Pathways | Pg LPS | Ec LPS |
| Mitogenic Pathway | 0 | 67 |
| Wnt Pathways | 20 | 20 |
| Hedgehog Pathway | 0 | 0 |
| TGF-b Pathway | 0 | 43 |
| Survival Pathway | | |
| PI3 Kinase/AKT Pathway | 14 | 29 |
| Jak/Src Pathway | 0 | 50 |
| NF-kB Pathway | 0 | 100 |
| p53 Pathway | 13 | 13 |
| Stress Pathway | 14 | 43 |
| NF-kB Pathway | 25 | 25 |
| NFAT Pathway | 0 | 0 |
| CREB Pathway | 0 | 100 |
| Jak-Stat Pathway | 13 | 13 |
| Estrogen Pathway | 0 | 17 |
| Androgen Pathway | 0 | 0 |
| Calcium and Protein Kinase C Pathway | 20 | 30 |
| Phospholipase C Pathway | 11 | 44 |
| Insulin Pathway | 0 | 0 |
| LDL Pathway | 40 | 20 |
| Retinoic Acid Pathway | 11 | 0 |

% = number of genes increased by LPS/number of genes included each gene family × 100

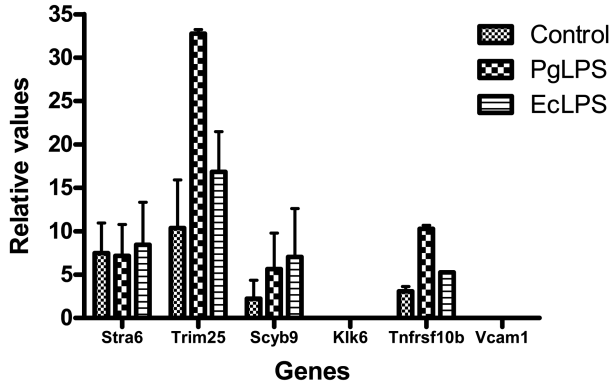


**Fig. 1.** Normalized relative quantification of mRNA expression by real-time PCR for Tnfrsf10b, Vcam1, Scyb9, Trim25, Klk6, and Stra6. Values are expressed as mean ± the SD obtained from three independent experiments.

and dendritic cells within the gingival and periodontal tissues. LPS has been suggested to induce the generation of local cytokines in immune cells. Several signaling pathways have been implicated downstream of the binding of LPS to their receptors on immune cells. In fact, more than one signaling pathway appears to be required for the regulation of the expression of many immunomodulatory genes. Pg LPS has been identified as a key factor in the development of periodontitis. The unique endotoxic activities of Pg LPS are attributed to its distinctly different structure from that of enterobacterial LPS. DCs are the most efficient antigen-presenting cells, and are able to stimulate both primary and secondary immune responses (Fearon *et al*., 1996; Hart *et al*., 1997). Also, the initiation of protective immune responses is known to generally require DC activation. Thus, in this present study, in order to highlight the signal transduction pathways or genes specifically induced by Pg LPS, gene expression profiles were evaluated in Pg LPS- or Ec LPS-treated DC2.4 cells, by virtue of the described cDNA microarray technique.

DNA microarray technology is a new and powerful tool which allows for the rapid and efficient simultaneous analyses of a large number of nucleic acid hybridizations (Lipshutz *et al*., 1999). This technology has proven extraordinarily useful in terms of our knowledge regarding the interrelationships of gene expression involved in the functioning of immune systems. The Signal Transduction Pathway Finder GEArray monitors the activation of 18 distinct signal transduction pathways. The microarray represents several genes that encode downstream effectors, the expression of which responds at the transcriptional level to the activation or deactivation of these signal transduction pathways. The determination of which genes change their relative signal intensity on the microarray can thus reveal the relevant pathway or pathways.

In the present study, which involved cDNA expression array analyses using the Signal Transduction PathwayFinder GEArray system, the expressions of Myc, Lta, VcamI, Scyb9, Csf2, and Stra6 were found to be up-regulated at the transcriptional level by Pg LPS in DC2.4 cells. Among those

**Table 3.** Genes expressed specifically by Pg LPS more than by Ec LPS

| Genebank | Symbol | Description | Pg LPS | Ec LPS |
|---|---|---|---|---|
| AF176833 | Tnfrsf10b | Tumor necrosis factor receptor superfamily, member 10b | 1.52 | 0.97 |
| M84487 | Vcam1 | Vascular cell adhesion molecule 1 | 1.62 | 1.26 |
| NM_008599 | Scyb9 | Mouse monokine induced by gamma interferon (MIG) | 1.75 | 1.43 |
| D63902 | Trim25 | Mouse mRNA for estrogen-responsive finger protein | 1.36 | 0.23 |
| NM_010639 | Klk6 | Mus musculus kallikrein renal/pancreas/salivary (Klk1), | 1.49 | 0.63 |
| NM_009291 | Stra6 | stimulated by retinoic acid gene 6 | 2.58 | 0.78 |

genes up-regulated by Pg LPS, Myc, Lta, and Csf2 were also up-regulated in response to Ec LPS treatment. Ec LPS modulated the expression of a significantly larger variety of genes than did Pg LPS. Genes up-regulated in the DC2.4 cells in response to Pg LPS, but not by Ec LPS, included Tnfrsf10b (tumor necrosis factor receptor superfamily, member 10b), VcamI (vascular cell adhesion molecule 1), Scyb9 (mouse monokine induced by gamma interferon, MIG), Trim25 (mouse mRNA for estrogen-responsive finger protein), Klk6 (mouse musculus kallikrein renal/pancreas/salivary), and Stra6 (stimulated by retinoic acid gene 6).

Tnfrsf10b is a gene of TRAIILR2 (DR5), a receptor harboring a death domain, which is known to be capable of activating TRAIL-dependent apoptosis (MacFarlane *et al*., 1997). Monokine induced by interferon-γ (MIG, Scyb9), which is induced in macrophages by IFN-γ almost exclusively, has been shown to be involved in chronic inflammation and viral and protozoan infections, as well as in T-cell trafficking, chemotaxis, and activation (Vanguri *et alet al*., 1990; Narumi *et al*., 1997; Luster *et al*., 1985; Liao *et al*., 1995; Amichay *et al*., 1996; Asensio *et al*., 1997; Loetscher *et al*., 1996). MIG may also perform an important function in the regulation of tissue granulation and remodeling via the inhibition of angiogenesis (Strieter *et al*., 1995; Strieter *et al*., 1995). Estrogen-responsive finger protein (Efp, protein product of Trim25) belongs to a member of the RING-finger B-box Coiled-Coil family (Inoue *et al*., 1993), which is considered to be involved in the regulation of a variety of cellular functions, including cell-cycle regulation and transcription (Saurin *et al*., 1996). Klk6 is the recently identified serine protease, kallikerin 6. Kallikerin 6 has been shown to be expressed abundantly by perivascular and infiltrating inflammatory cells in cases of central nervous system inflammatory disease (Sachiko *et al*., 2004). The function of elevated levels of kallikerin 6 in activated immune cells remains to be thoroughly elucidated. As a secreted serine protease, it may be able to hydrolyze extracellular matrix proteins. However, the functions of these genes in immune response or Pg LPS-induced dendritic cell activation also remain to be fully defined.

When real-time PCR was carried out to confirm the expression levels of six genes, similar to the results of microarray experiments, both LPSs enhanced the mRNA expression of Trim25, Scyb9 and Tnfrsf10b compared with the control. The expression of Trim25 and Tnfrsf10b mRNA was more increased only by Pg LPS treatment but not by Ec LPS. However, Kik6 and Vcam1 were not detected by real-time PCR and Stra6 expression was low and similar in Pg LPS and Ec LPS-treated cells.

It should be noted that the results reported herein represent data collected at only one time point (2 h of stimulation), and that the parameters of this study were focused on the expression of only a very limited number of genes. In this study, therefore, we may have likely failed to detect some early- and late-response genes, and possibly other genes that were not included in this array. Although this does not compromise the conclusions of this report, further studies of gene expression profiles in DC2.4 cells, involving the collection of data at more time points, and encompassing a wider variety of genes, will undoubtedly provide us with a more complete understanding of the signal transduction pathways specifically induced by Pg LPS. Insight into these signal transduction pathways or genes, as well as the functions of the genes involved in the activation of DC2.4 cells or in Pg LPS-induced immune responses, may provide improvement in therapeutic modalities or vaccines against periodontal diseases induced by *P. gingivalis.*

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