

Genome-wide transcriptome induced by *Porphyromonas gingivalis* LPS supports the notion of host-derived periodontal destruction and its association with systemic diseases

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Abstract

Chronic periodontitis (CP) is a prevalent pathogen-associated inflammatory disorder characterized by the destruction of tooth-supporting tissues, and linked to several systemic diseases. Both the periodontopathogen *Porphyromonas gingivalis* (Pg), and the genetically determined host immune response, are hypothesized to play a crucial role in this association. To identify new target genes for CP and its associated systemic diseases, we investigated the transcriptome induced by Pg in human monocytes using a genome-wide approach. Monocytes were isolated from healthy male volunteers of European origin and challenged with the Pg virulence factor LPS. Array-based gene expression analysis comprising >47,000 transcripts was performed followed by pathway analyses. Transcriptional data were validated by protein and cell surface markers. LPS Pg challenge led to the significant induction of 902 transcripts. Besides known periodontitis-associated targets, several new candidates were identified (CCL23↑, INDO↑, GBP 1/4↑, CFB↑, ISG20↑, MIR155HG↑, DHRS9↓). Moreover, various transcripts correspond to the host immune response, and have been linked to cancer, atherosclerosis and arthritis, thus highlighting the systemic impact of CP. Protein data of immunological markers validated our results. The present findings expand understanding of Pg elicited immune responses, and indicate new target genes and pathways of relevance to diagnostic and therapeutic strategies.

Keywords

Porphyromonas gingivalis, human monocytes, periodontitis, innate immunity, transcriptome

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Introduction

Chronic periodontitis (CP) is a prevalent inflammatory disease characterized by the irreversible destruction of tooth-supporting tissues.¹ CP has become the focus of increasing interest, owing to its rising prevalence and its association with life-threatening systemic disorders such as atherosclerosis, autoimmune disease and cancer.² DNA-based phylotyping studies have indicated that bacterial diversity has been influenced by the introduction of Neolithic and industrialization diets, resulting in an increase in periodontal pathogens, which are key players in CP.³

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Porphyromonas gingivalis (*Pg*), a Gram-negative anaerobic microbe, is highly related to the severity of CP.⁴ However, there is evidence that a certain genetic predisposition also plays a role in CP, reinforcing the notion of host-derived pathogen-associated periodontal destruction.⁵ In the last decade, research has investigated associations between periodontitis and various single nucleotide polymorphisms (SNPs). Based on the hypothesis that a pro-inflammatory environment and immune cell recruitment are implicated, the primary focus of this research was the investigation of SNPs located in immunological, pro- and anti-inflammatory genes like IL-1 and receptor antagonist (IL1RN), TNF- α or IL-10.⁶ Specific innate immune receptors such as TLRs may also play a role in CP. TLRs are highly conserved pathogen recognition receptors, and are responsible for the detection of PAMPs such as LPS. Toxic LPS, a cell envelope component of Gram-negative bacteria, activates TLR4-MD2 complex. This activation is followed by the nuclear translocation of transcription factors, like NF- κ B, and the secretion of pro-inflammatory cytokines and chemokines. The LPS that is derived from *Pg* is structurally and functionally distinct from the LPS of *Escherichia coli*, an enteric bacterium, and research suggests that LPS *Pg* interacts with TLR2, TLR4 and TLR7.⁷

Monocytes play a crucial role in systemic pathogen signaling as they migrate to the sites of infection via PAMP recognition, and differentiate into macrophages or dendritic cells (DCs). Recent investigations have shown that periodontal pathogens reach the bloodstream (bacteremia), and that the level of bacteremia is related to the periodontal pathogen density and the severity of periodontal destruction.⁸ Hence, the interaction between periodontal pathogen bacteria and the host immune response is crucial. Hypo- and hyper-responsiveness of the host immune response may predispose to, and aggravate, tissue destruction, thus influencing both periodontal and systemic disease.

The aim of the present study was to identify new target genes and pathways of relevance to CP disease and the associated systemic immune response. The investigation focused on the transcriptome induced by LPS *Pg* in human monocytes. Therefore, >47,000 transcripts were analyzed to provide genome-wide transcriptional coverage of well-characterized genes derived from the National Center for Biotechnology Information Reference Sequence. The present findings expand understanding of the *Pg*-elicited immune response, and indicate new target genes and pathways of relevance to diagnostic and therapeutic strategies, and explain the development of the associated systemic disorders.

Materials and methods

Monocyte isolation

The study was approved by the ethics committee of the University of Bonn (Nr. 352/13), and all participants

provided written informed consent prior to inclusion. Ten healthy male volunteers of European origin (18–35 yr) were recruited, and peripheral blood samples were obtained. Females were excluded, as hormonal factors may influence transcriptional expression patterns. The following inclusion criteria were applied: non-smoker; no acute or chronic infection; no vaccination within the 4 wk prior to blood sampling; C-reactive protein levels < 2.5 mg/dl; monocyte purity > 95%; and overnight survival > 85%. On the basis of these criteria, three individuals were excluded. The cells of the remaining seven individuals underwent further processing (Figure 1A–C).

Peripheral blood mononuclear cells from 100 ml whole blood were purified using a Ficoll-Plaque density gradient. CD14⁺ monocytes were then separated using magnetic-activated cell sorting and CD14-microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), in accordance with the manufacturer's instructions. Cell purity was determined by flow cytometry followed by cell resuspension in RPMI 1640 GlutaMAX. The latter was supplemented with 10% heat-inactivated FBS (all from Gibco, Life Technologies, Waltham, MA, USA); 100 U/ml penicillin; and 100 μ g/ml streptomycin (PAA Laboratories/GE Healthcare, Pasching, Austria).

Monocyte stimulation

Monocytes were cultured overnight in 96-well round-bottom wells at a density of 500,000 cells/well in 100 μ l. The next morning (approximately 18–20 h after cell isolation) cell survival was analyzed optically using trypan blue staining, followed by Annexin V (Apoptosis Detection Kit) and propidium iodide detection (eBioscience, San Diego, CA, USA) via flow cytometry. The cells were then stimulated with ultrapure LPS from *Pg* (InvivoGen, Darmstadt, Germany) for 90 min or 6 h. On the basis of the proliferation assay results (XTT assays; PromoKine, Heidelberg, Germany), a 200-ng/ml LPS challenge was selected for cell stimulation. Untreated monocytes served as controls.

After stimulation, the supernatants were collected, cells were lysed in RLT Plus buffer (Qiagen, Venlo, the Netherlands), and stored at -80°C until analysis.

RNA extraction

RNA was extracted from lysed monocytes using the Qiagen AllPrep DNA/RNA Mini Kit in accordance with the manufacturer's instructions. For quality control purposes, RNA concentrations were measured using NanoDrop (PeqLab, Erlangen, Germany), and RNA degradation was measured using Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Transcriptome analysis

RNA was amplified and biotinylated using the Illumina TotalPrep RNA Amplification Kit (Life Technologies).

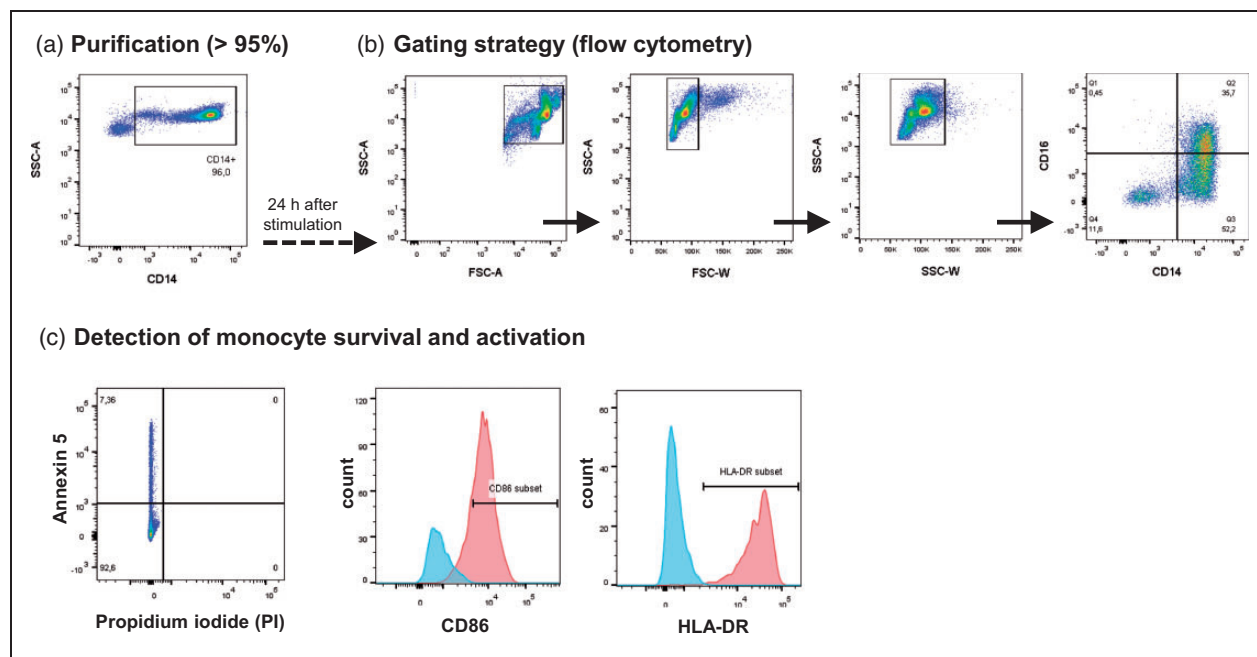


Figure 1. Experimental setting and methods. (a) Blood of healthy volunteers was collected for CD14⁺ monocyte isolation. Purity (>95%) was determined using CD14-microbeads (Miltenyi Biotec GmbH) and flow cytometry. (b) Gating strategy to identify CD14⁺ expressing human primary monocytes. (c) Detection of monocyte survival and activation: Prior to LPS stimulation (24 h after cell isolation), human monocyte survival was determined using the Annexin V-FITC Apoptosis Detection Kit (eBioscience) and flow cytometry. Monocyte activation was evaluated using specific surface markers [CD86 (eBioscience) and HLA-DR (BD Bioscience)]. SSC: side scatter; FSC: forward scatter.

Subsequent array-based gene expression analysis was performed using the Human HT-12 v4 Expression BeadChip (Illumina, San Diego, CA, USA) and the automated protocol, in accordance with the manufacturer's manual. The HT-12 v4 Expression BeadChip comprises 47,231 probes. The arrays were scanned with an iScan microarray scanner (Illumina). Statistical analysis was performed using functions implemented in the statistical software packages R and Bioconductor. All data were subjected to quantile normalization using the limma package.^{9,10} Selection of differentially expressed genes was performed using the following statistical filter criteria: fold change (FC) ≥ 1.7 and $P \leq 0.05$. The FC is the ratio of the group mean values. Transcripts with at least a 1.7-fold change were selected. All adjusted P values were calculated using a Student's t -test, and adjusted for multiple testing using Benjamini–Hochberg correction.

Protein analysis

To determine the impact of specific transcripts, ELISAs or Multiplex Immunoassays were performed in accordance with the manufacturer's instructions (eBioscience or R&D (Minneapolis, MN, USA)). The supernatants of monocytes challenged with LPS *Pg* for 6 h were analyzed for IL-10, TNF- α , TIMP-2, CXCL8 (IL-8), CCL8 (MCP-2), CCL23 (variant Ck β 8-1, MIP-3) and CCL4 (MIP-1) concentrations.

Cell surface markers

To assess monocyte activation, surface markers were determined using flow cytometry. First, monocytes were directly blocked with PBS/1% BSA containing 0.1% Fc block (Sigma-Aldrich, St. Louis, MO, USA). Second, monocytes were stained with the following Abs: CD14 FITC (Clone: 62D3); CD16 APC (Clone: eBioCD16); human leukocytes Ag (HLA)-DR PerCP-Cy5.5 (Clone: LN3) (eBioscience); and CD86 (known as B70/B7-2) PE [Clone: 2331(FUN-1)] (BD Bioscience, San Diego, CA, USA). Flow cytometry was performed using BD FACS Canto followed by analysis with FACS Diva software (BD Biosciences) and FlowJo X software (FLOWJO, Ashland, OR, USA).

IPA analysis

Transcriptional data were evaluated using Ingenuity Pathway Analysis (IPA, Systems Inc., Redwood City, CA, USA).¹¹ Here, each gene is represented in a global molecular network that is designed by information from the Ingenuity Pathways Knowledge Base. 'Networks' were generated algorithmically, based on their connectivity in terms of activation, expression and transcription. Molecular relationships between genes are represented by connecting lines between nodes, as supported by published data stored in the Ingenuity Pathways Knowledge Base and/or PubMed.

Data analysis

Protein and surface marker expression profiles were analyzed using Student's *t*-test and GraphPad Prism Version 5.0 (GraphPad Software, San Diego, CA, USA). Data are shown as the mean with SEM. $P \leq 5\%$ was considered statistically significant.

Results

Transcriptome induced by LPS *Pg* in *CD14*⁺ monocytes

Initial experiments demonstrated that 90 min of LPS *Pg* challenge induced 423 differentially regulated transcripts (260 overexpressed and 167 repressed genes; see

Supplementary Table S1). The ‘immediate’ genes included candidates of inflammasome activation and immunity (e.g. TNF- α ↑, IL-6↑, NLRP3↑, CCL14↑, CCL20↑, ICAM1↑). Comparison of these results with those obtained after 6 h of LPS *Pg* stimulation revealed distinct differences in expression profiles (Figure 2A). In view of the higher outcomes of prolonged stimulation, LPS challenge for 6 h was further investigated.

In these analyses, 902 transcripts were significantly differentially regulated after 6 h of LPS *Pg* challenge compared with control ($n = 7$; FC ≥ 1.7 ; $P < 0.05$; Figure 2B, C; see also Supplementary Table S2). In total, 486 genes were overexpressed and 416 were repressed. The top transcripts (FC > 5) are listed in Table 1. These included genes

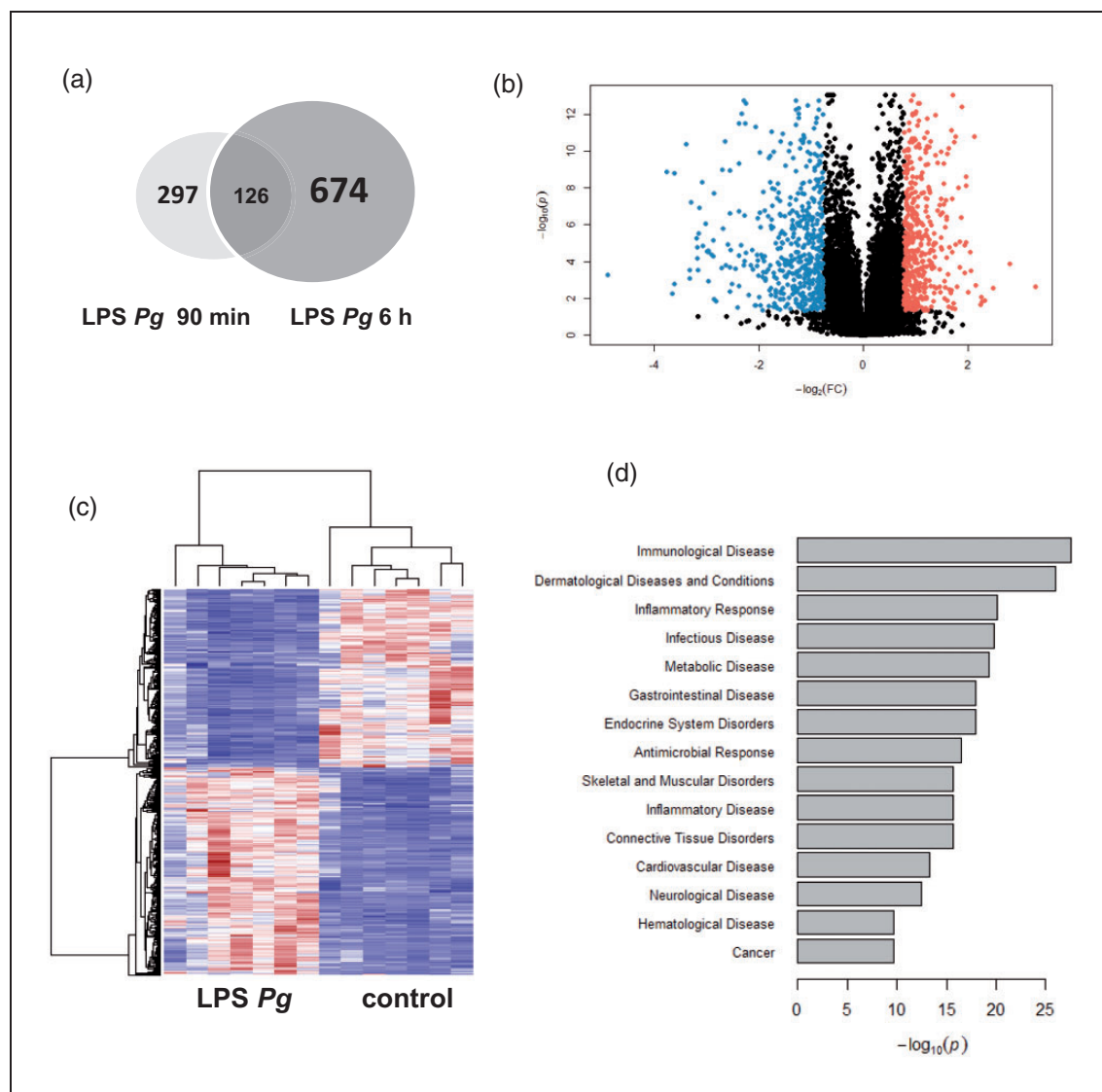


Figure 2. Genome-wide transcriptome induced by LPS *P. gingivalis* challenge in human monocytes. (a) Venn diagram of differentially expressed transcripts (FC ≥ 1.7 and $P < 0.05$) after LPS *Pg* challenge, demonstrating differences and similarities between 90 min and 6 h exposure. (b) Volcano plot analysis of LPS *Pg*-induced genes after 6 h. Red indicates overexpressed and blue indicates repressed genes ($n = 902$). For the complete list of differentially regulated genes and detailed information on the individual probes, see Supplementary Table S2. (c) Hierarchical cluster analysis of differentially expressed genes ($n = 902$) after 6 h of LPS exposure. Data are based on seven individuals and independent experiments. Columns represent subjects and rows represent transcripts. Red indicates high relative expression levels and blue indicates low relative expression levels. A hierarchical cluster analysis was performed using Pearson's correlation as distance measurement and average linkage. (d) Overview of diseases associated with LPS *Pg*-induced genes visualized by bar plot constructed using IPA (www.ingenuity.com).

Table 1. Top transcripts ($FC \geq 5$) significantly induced in human monocytes ($n = 7$ different donors) after 6 h of LPS Pg challenge.

Illumina_ID/ transcript	ILMN_Gene	Definition	FC of Pg	P-Value adjusted
ILMN_1838319	LOC730249	PREDICTED: <i>Homo sapiens</i> similar to Immune-responsive protein 1 (LOC730249), mRNA.	25.4	8.00E-04
ILMN_3239965	IDO1	<i>Homo sapiens</i> indoleamine 2,3-dioxygenase 1 (IDO1), mRNA.	14.1	<1.00E-16
ILMN_2100209	CCL4L1	<i>Homo sapiens</i> chemokine (C-C motif) ligand 4-like 1 (CCL4L1), mRNA.	13.12	2.90E-03
ILMN_1656310	INDO	<i>Homo sapiens</i> indoleamine-pyrrole 2,3 dioxygenase (INDO), mRNA.	12.67	<1.00E-16
ILMN_1716276	CCL4L2	<i>Homo sapiens</i> chemokine (C-C motif) ligand 4-like 2 (CCL4L2), mRNA.	11.42	<1.00E-16
ILMN_3235832	LOC728835	PREDICTED: <i>Homo sapiens</i> similar to cytokine, transcript variant 3 (LOC728835), mRNA.	10.85	<1.00E-16
ILMN_1659913	ISG20	<i>Homo sapiens</i> interferon stimulated exonuclease gene 20 kDa (ISG20), mRNA.	10.31	4.00E-04
ILMN_3193271	IRG1	PREDICTED: <i>Homo sapiens</i> immunoresponsive 1 homolog (mouse) (IRG1), mRNA.	10.2	8.60E-03
ILMN_1686109	CCL23	<i>Homo sapiens</i> chemokine (C-C motif) ligand 23 (CCL23), transcript variant CKbeta8, mRNA.	9.91	<1.00E-16
ILMN_2218856	CCL3L1	<i>Homo sapiens</i> chemokine (C-C motif) ligand 3-like 1 (CCL3L1), mRNA.	9.4	4.00E-04
ILMN_1773352	CCL5	<i>Homo sapiens</i> chemokine (C-C motif) ligand 5 (CCL5), mRNA.	9.39	<1.00E-16
ILMN_2098126	CCL5	<i>Homo sapiens</i> chemokine (C-C motif) ligand 5 (CCL5), mRNA.	9.34	<1.00E-16
ILMN_1802653	EBI3	<i>Homo sapiens</i> Epstein-Barr virus induced 3 (EBI3), mRNA.	8.92	<1.00E-16
ILMN_1691341	IL7R	PREDICTED: <i>Homo sapiens</i> IL-7 receptor (IL7R), mRNA.	8.9	<1.00E-16
ILMN_1774287	CFB	<i>Homo sapiens</i> complement factor B (CFB), mRNA.	8.71	1.20E-03
ILMN_2093343	PLAC8	<i>Homo sapiens</i> placenta-specific 8 (PLAC8), mRNA.	8.55	<1.00E-16
ILMN_2170814	LAMP3	<i>Homo sapiens</i> lysosomal-associated membrane protein 3 (LAMP3), mRNA.	8.3	<1.00E-16
ILMN_1747355	CCL3L1	<i>Homo sapiens</i> chemokine (C-C motif) ligand 3-like 1 (CCL3L1), mRNA.	8.29	<1.00E-16
ILMN_1701114	GBP1	<i>Homo sapiens</i> guanylate binding protein 1, IFN-inducible, 67 kDa (GBP1), mRNA.	8.28	1.00E-04
ILMN_2148785	GBP1	<i>Homo sapiens</i> guanylate binding protein 1, IFN-inducible, 67 kDa (GBP1), mRNA.	8.25	<1.00E-16
ILMN_2054019	ISG15	<i>Homo sapiens</i> ISG15 ubiquitin-like modifier (ISG15), mRNA.	8.14	1.20E-03
ILMN_1671509	CCL3	<i>Homo sapiens</i> chemokine (C-C motif) ligand 3 (CCL3), mRNA.	7.84	<1.00E-16
ILMN_1651832	EHD1	<i>Homo sapiens</i> EH-domain containing 1 (EHD1), mRNA.	7.42	1.00E-04
ILMN_1653026	PLAC8	<i>Homo sapiens</i> placenta-specific 8 (PLAC8), mRNA.	7.33	1.00E-04
ILMN_1772964	CCL8	<i>Homo sapiens</i> chemokine (C-C motif) ligand 8 (CCL8), mRNA.	7.18	1.58E-02
ILMN_1764030	CCL23	<i>Homo sapiens</i> chemokine (C-C motif) ligand 23 (CCL23), transcript variant CKbeta8-1, mRNA.	7.15	1.00E-04
ILMN_1773245	CCL3L1	<i>Homo sapiens</i> chemokine (C-C motif) ligand 3-like 1 (CCL3L1), mRNA.	7.15	1.56E-02
ILMN_1660462	MCOLN2	<i>Homo sapiens</i> mucolipin 2 (MCOLN2), mRNA.	6.95	<1.00E-16

(Continued)

Table 1. Continued.

ILLUMINA_ID/ transcript	ILMN_Gene	Definition	FC of Pg	P-Value adjusted
ILMN_2105573	CCL3L3	<i>Homo sapiens</i> chemokine (C-C motif) ligand 3-like 3 (CCL3L3), mRNA.	6.93	<1.00E-16
ILMN_3248910	MIR155HG	<i>Homo sapiens</i> MIR155 host gene (non-protein coding) (MIR155HG), non-coding RNA.	6.91	<1.00E-16
ILMN_1674811	OASL	<i>Homo sapiens</i> 2'-5'-oligoadenylate synthetase-like (OASL), transcript variant 2, mRNA.	6.87	1.00E-04
ILMN_1695316	SLC39A8	<i>Homo sapiens</i> solute carrier family 39 (zinc transporter), member 8 (SLC39A8), transcript variant 1, mRNA.	6.78	<1.00E-16
ILMN_1785732	TNFAIP6	<i>Homo sapiens</i> TNF- α -induced protein 6 (TNFAIP6), mRNA.	6.63	<1.00E-16
ILMN_1776967	LRRC50	<i>Homo sapiens</i> leucine rich repeat containing 50 (LRRC50), mRNA.	6.52	<1.00E-16
ILMN_2233539	SLC39A8	<i>Homo sapiens</i> solute carrier family 39 (zinc transporter), member 8 (SLC39A8), transcript variant 1, mRNA.	6.34	<1.00E-16
ILMN_2342579	IL7R	<i>Homo sapiens</i> IL7R, mRNA.	6.28	4.00E-04
ILMN_1787897	CXCL1	<i>Homo sapiens</i> chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (CXCL1), mRNA.	5.85	3.00E-04
ILMN_1775501	IL1B	<i>Homo sapiens</i> IL-1 β , mRNA.	5.79	2.20E-03
ILMN_1785202	STAT4	<i>Homo sapiens</i> signal transducer and activator of transcription 4 (STAT4), mRNA.	5.75	<1.00E-16
ILMN_1667966	C1ORF24	<i>Homo sapiens</i> chromosome 1 open reading frame 24 (C1orf24), transcript variant 2, mRNA.	5.73	<1.00E-16
ILMN_1807372	ADORA2A	<i>Homo sapiens</i> adenosine A2a receptor (ADORA2A), mRNA.	5.57	<1.00E-16
ILMN_2233783	CD38	<i>Homo sapiens</i> CD38 molecule (CD38), mRNA.	5.47	3.00E-04
ILMN_1805827	PPA1	<i>Homo sapiens</i> pyrophosphatase (inorganic) 1 (PPA1), mRNA.	5.45	1.00E-04
ILMN_1769388	GJB2	<i>Homo sapiens</i> gap junction protein, beta 2, 26 kDa (GJB2), mRNA.	5.43	<1.00E-16
ILMN_1782389	LAD1	<i>Homo sapiens</i> ladinin 1 (LAD1), mRNA.	5.37	<1.00E-16
ILMN_1776188	MAP1LC3A	<i>Homo sapiens</i> microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), transcript variant 2, mRNA.	5.33	<1.00E-16
ILMN_2406501	SOD2	<i>Homo sapiens</i> superoxide dismutase 2, mitochondrial (SOD2), nuclear gene encoding mitochondrial protein, transcript variant 3, mRNA.	5.31	<1.00E-16
ILMN_1810725	FAM129A	<i>Homo sapiens</i> family with sequence similarity 129, member A (FAM129A), transcript variant 2, mRNA.	5.23	<1.00E-16
ILMN_1771385	GBP4	<i>Homo sapiens</i> guanylate binding protein 4 (GBP4), mRNA.	5.19	<1.00E-16
ILMN_1684585	ACSL1	<i>Homo sapiens</i> acyl-CoA synthetase long-chain family member 1 (ACSL1), mRNA.	5.12	2.00E-04
ILMN_1679268	PEL11	<i>Homo sapiens</i> pellino homolog 1 (Drosophila) (PEL11), mRNA.	5.02	<1.00E-16
ILMN_1733998	DHRS9	<i>Homo sapiens</i> dehydrogenase/reductase (SDR family) member 9 (DHRS9), transcript variant 1, mRNA.	-6.58	2.00E-04
ILMN_1797731	MS4A6A	<i>Homo sapiens</i> membrane-spanning 4-domains, subfamily A, member 6A (MS4A6A), transcript variant 2, mRNA.	-8.53	3.40E-03

A total of 902 genes showed significant differential expression at a false discovery rate (Benjamini-Hochberg adjusted *P*-value). The complete list of differentially regulated genes and detailed information on the individual probes are provided in Supplementary Table S2.

Table 2. Highly induced canonical pathways in human monocytes after 6 h of *Pg* challenge, as determined using the Ingenuity Pathways Knowledge Base (IPA).

Ingenuity canonical pathways	−log (<i>P</i> -value)	Molecules
TREMI signaling	9,04E00	NFKB1, CCL3, TLR5, TLR7, CCL7, IL1B, CASP1, CD40, CXCL8, NFKB2, CD83, TNF, CCL2, CASP5, IL10, CD86, MAPK3
Role of pattern recognition receptors in recognition of bacteria and viruses	8,91E00	EIF2AK2, OAS1, NFKB1, TLR5, OSM, TLR7, CCL5, IL1B, ATM, OAS2, CASP1, CXCL8, NFKB2, IRF7, PIK3R2, TNF, OAS3, IFIH1, IL1A, PRKCB, IL10, MAPK3
Communication between innate and adaptive immune cells	8,53E00	CCR7, CCL3, TLR5, TNFSF13B, TLR7, CCL5, IL1B, CD40, CCL3L3, CXCL8, CD83, IL1RN, TNF, IL1A, CD80, IL10, CD86
TLR signaling	7,84E00	EIF2AK2, NFKB1, TLR5, TLR7, IL1B, NFKBIA, ELK1, NFKB2, LY96, IL1RN, TNFAIP3, TNF, IL1A, MAP2K3, TRAF1, IRAK2
Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis	7,05E00	OSM, TLR5, CALM1 (includes others), SOCS3, PLCB2, FZD2, NFKBIA, IL1RN, TNF, PRKCB, MAP2K3, TRAF1, IRAK2, VEGF, MAPK3, NFKB1, TNFSF13B, TLR7, SRC, CCL5, TRAF3IP2, IL1B, ATM, CEBPA, PDGFC, CXCL8, PIK3R2, CCL2, IL1A, SOCS1, IL10, RRAS
Role of IL-17A in arthritis	6,03E00	NFKB2, NFKB1, CCL7, CCL5, PIK3R2, CCL2, ATM, CXCL1, MAP2K3, NFKBIA, CXCL8, MAPK3
Altered T-cell and B-cell signaling in rheumatoid arthritis	5,88E00	NFKB1, TLR5, TNFSF13B, TLR7, IL1B, CD40, NFKB2, IL1RN, TNF, IL1A, HLA-DMB, CD80, IL10, CD86
Dendritic cell maturation	5,85E00	CCR7, NFKB1, STAT4, IL1B, PLCB2, ATM, CD40, NFKBIA, NFKB2, CD83, IL1RN, PIK3R2, TNF, IL1A, HLA-DMB, CD80, IL10, LEP, CD86, FSCN1, MAPK3
Differential regulation of cytokine production in intestinal epithelial cells by IL-17A and IL-17F	5,8E00	CCL3, IL1B, CCL5, TNF, CCL2, IL1A, CXCL1, IL10
IL-10 signaling	5,69E00	NFKB1, IL1B, SOCS3, BLVRB, NFKBIA, ELK1, BLVRA, NFKB2, IL1RN, TNF, IL1A, IL10, MAP2K3
IL-6 signaling	5,55E00	NFKB1, IL1B, SOCS3, ATM, NFKBIA, ELK1, CXCL8, NFKB2, IL1RN, TNFAIP6, PIK3R2, TNF, IL1A, SOCS1, MAP2K3, RRAS, MAPK3
Role of IL-17F in allergic inflammatory airway diseases	5,52E00	NFKB2, NFKB1, CCL7, TRAF3IP2, IL1B, RPS6KA2, CCL2, CXCL1, CXCL8, MAPK3
Differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F	5,51E00	CCL3, IL1B, CCL5, TNF, CCL2, CXCL1, IL10
Tryptophan degradation III (eukaryotic)	5,33E00	IDO2, ACAT2, HSD17B4, HADH, IDO1, KMO, KYNU
NF-κB signaling	5,15E00	EIF2AK2, NFKB1, TLR5, TNFSF13B, TLR7, IL1B, ATM, CD40, NFKBIA, PELI1, NFKB2, TGFB2, IL1RN, TNFAIP3, PIK3R2, TNF, IL1A, PRKCB, TNIP1, RRAS
Granulocyte adhesion and diapedesis	5,11E00	CCL4L1/CCL4L2, CCL3, CCL8, CCL7, CCL5, IL1B, SDC2, FPR2, CCL3L1, CCL3L3, CXCL8, CCL19, CKLF, IL1RN, TNF, CCL2, IL1A, CCL23, CXCL1, PECAM1

The complete list and detailed information are provided in Supplementary Table S3.

implicated in immunity and the complement system (CCL23, CCL4L, ISG20, IRG1, CCL5, CCL3L, CFB, CCL8, CXCL1; up to 14-fold increase); pro-inflammation (IL-1β, TNFAIP6, IL-7R); tryptophan metabolism (IDO1, INDO); and oxidative stress (SOD2); as well as transcripts of guanylate-binding proteins (GBP1 and 4) and non-coding microRNA (MIR155HG). The most repressed genes were DHRS9 (6.58-fold) and MS4A6A (8.53-fold). All significantly regulated transcripts are shown in Supplementary Table S2.

Pathways and associated diseases

According to IPA, a total of 231 canonical pathways/signaling were activated during 6 h LPS *Pg* challenge (Table 2; Supplementary Table S3). These included TREM1, NF-κB, TLR, IL-10, IL-8, HMGB1, VEGF, erythropoietin, CD40, TNFR, iNOS, Notch and leptin signaling. For example, Figure 3A shows the triggering receptor expressed on myeloid cells 1 (TREM1) signaling, which was highly induced by LPS *Pg*. Periodontitis

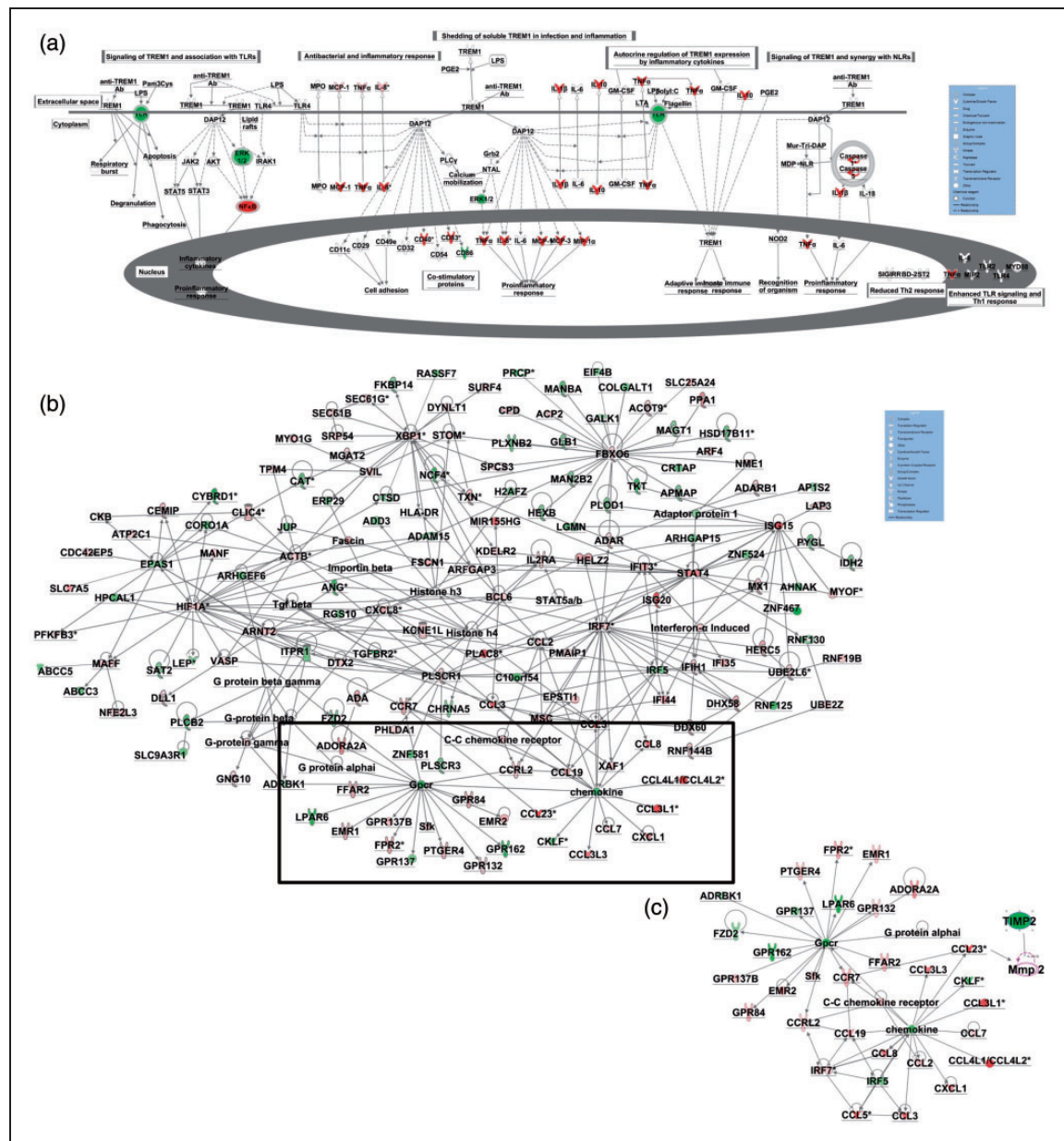


Figure 3. LPS *Pg*-related gene networks designed using information obtained from the Ingenuity Pathways Knowledge Base. 'Networks' were generated algorithmically based on their connectivity in terms of activation, expression and transcription. Direct molecular relationships between genes are represented by connecting lines between nodes/genes, as supported by published data stored in the Ingenuity Pathways Knowledge Base and/or PubMed. Red symbols indicate upregulated gene expression, whereas green symbols indicate downregulated gene expression. (a) TREM1 signaling pathway significantly activated by LPS *Pg* in human monocytes according to IPA (www.ingenuity.com). (b) Immunologically relevant genes induced by LPS *Pg* in human monocytes were merged into one network showing the relationship between LPS *Pg* induced transcripts. (c) Depiction of chemokine networking to display inter-relationships and the interplay between CCL23, MMP2 and TIMP2.

and rheumatoid arthritis share common host responses,¹² and this was reflected in several LPS *Pg*-induced pathways (Table 2).

Figure 2D shows diseases with the most pronounced association with LPS *Pg*-induced genes. These comprise immunological, dermatological, inflammatory, infectious and cardiovascular diseases, as well as cancer. Supplementary Table S4 provides a list of 500

pathologies and functional annotations regulated by LPS *Pg*. These include systemic autoimmune diseases; the quantity and function of several immune cells (neutrophils, lymphocytes, DCs, macrophages); diabetes mellitus; rheumatic diseases; atherosclerosis; obesity; the synthesis and metabolism of reactive oxygen species; lipid and fatty acid concentrations; Ag presentation; cancer; osteoclast differentiation; and bone resorption.

Upstream analysis

A total of 2149 upstream regulators were determined by IPA (R)-analysis (see Supplementary Table S5). These included various cytokines (IL-1B, TNF- α , IFNs, IL-10); transcription regulators (IRFs, STATs, NF- κ B1); transmembrane receptors (TLRs, FAS); and others (NOD2, LDL), which provide additional insights into *Pg* infection.

Networks

IPA networks were used to depict inter-relationships between diseases, functional annotations and significantly regulated transcripts (Figure 3B, C; Supplementary Figure S1A–E). Immunologically relevant genes with known chemoattractant capacities were merged into one network, demonstrating a direct relationship between these genes (Figure 3B, C). As monocytes differentiate into phagocytes, which are primarily responsible for tissue destruction; genes involved in phagocyte metabolism are networked

in Supplementary Figure S1A. Systemic inflammatory and rheumatic diseases associated with LPS *Pg* infection are networked in Supplementary Figures S1B and C, respectively. Candidates for lipid metabolism, cardiovascular disease, and cancer were also networked, and this identified distinct affected targets such as MMP8 and MIR-124 (see Supplementary Figure S1D). Consideration of genes implicated in cell development, movement and homeostasis revealed additional targets, such as heat shock proteins (HSPs; see Supplementary Figure S1E).

Protein data

To validate the transcriptome results, protein levels of pro- (TNF- α), anti-inflammatory cytokines (IL-10) and chemoattractants were determined in supernatants of monocytes challenged with LPS *Pg* for 6 h (Figure 4A–E). In accordance with transcriptional

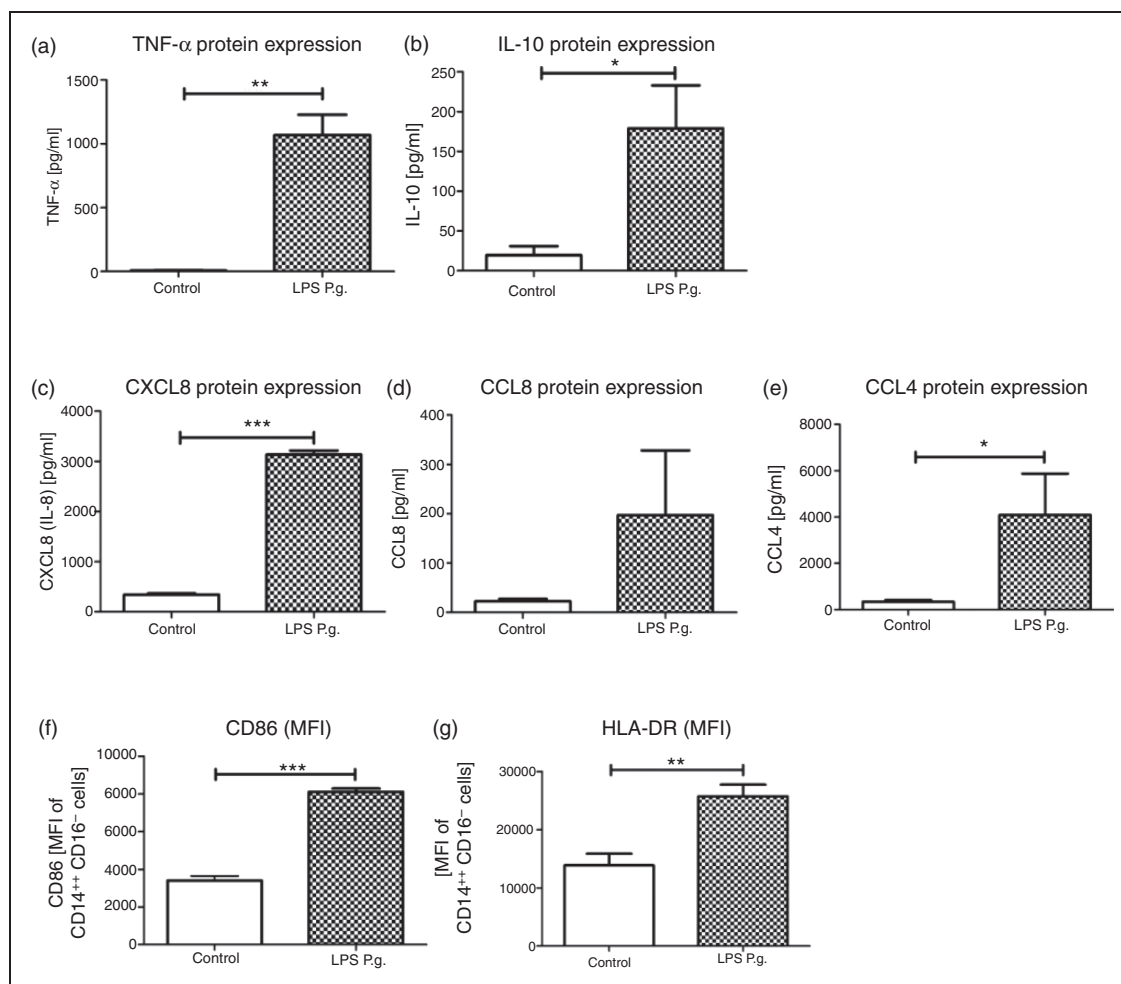


Figure 4. Protein expression of cytokines, chemokines, and markers of differentiation in human monocytes. (a) TNF- α , (b) IL-10, (c) CXCL8/IL8, (d) CCL8 and (e) CCL4 protein levels in the supernatants of human monocytes stimulated with LPS *Pg* for 6 h. Frequency and mean fluorescence intensity (MFI) of CD14⁺ monocytes expressing (f) CD86 and (g) HLA-DR/MHCII induced by LPS *Pg* challenge for 6 h. Data are shown as the mean with SEM and were analyzed using Student's *t*-test ($n = 4-7$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

data, TNF- α (1000-fold) and IL-10 (158-fold) proteins were upregulated by LPS *Pg* (Figure 4A, B). Moreover, LPS *Pg* induced a significant increase in CXCL8/IL8 protein levels (2660-fold) ($P < 0.001$, Figure 4C). In the case of CCL8, a marked protein elevation was observed (Figure 4D). The CCL4 data were consistent with our mRNA findings (15-fold) (Figure 4E). CCL23 and TIMP2 levels were below the detection limit after 6 h of LPS *Pg* challenge (data not shown).

CD14⁺ monocyte activation and differentiation

As monocytes are key players in periodontal and systemic diseases, their activation and differentiation were investigated via specific surface markers (Figure 4F, G). LPS *Pg* stimulation induced an upregulation of CD14 and repressed CD16 expression as a consequence of monocyte activation (data not shown).

Moreover, LPS *Pg* stimulation caused a significant increase in CD86 expression on classical CD14⁺⁺CD16⁻ monocytes ($P < 0.001$; Figure 4F). This was accompanied by an overexpression of HLA-DR/MHCII ($P < 0.001$; Figure 4G). Intermediate/non-classical CD14⁺⁺CD16⁺ monocytes displayed similar expression profiles (data not shown).

Discussion

CP is a highly prevalent inflammatory disease, and *Pg* is a keystone pathogen in its manifestation and severity. Extensive research evidence demonstrates that *Pg*-induced CP is associated with systemic diseases.² As monocytes constitute one of the main immune cells in the blood and play a pivotal role in systemic and periodontal inflammation,¹³ we applied a genome-wide transcriptional approach in human monocytes challenged with LPS derived from the periodontal pathogen *Pg*. In this analysis, 902 genes were significantly induced after 6 h of LPS *Pg* stimulation. Some of these genes have been associated, in previous reports, with periodontitis (IL-8, NAMPT, IL-1RN, IL-10, TNFA or CD38),^{6,14–16} thus validating the present findings. Interestingly, several new candidates, such as CCL23, ISG20, IRG1, GBP1, MIR155HG and DHRS9, were detected, accomplished by underlying pathways, upstream regulators and networks corresponding to host immune response. These findings thus support the notion that host immune response is implicated in *Pg*-induced CP and its association with systemic disease.

The top transcripts included several chemoattractants (CCL3, CCL4, CCL5, CCL8) previously associated with periodontal disease.¹⁷ These chemoattractants are responsible for immune cell recruitment and activation. CCL23 (also known as Ck β 8-1, MPIF1 and MIP3) plays a key role in immunoregulation. To our

knowledge, the present study is the first one to implicate CCL23 in *Pg*-induced immune response (9.91-fold increase). Interestingly, CCL23 is positively correlated with RA, a disorder that has also been linked to periodontal disease.^{12,18} Furthermore, investigation of a large population-based sample revealed that circulating CCL23 was associated with coronary atherosclerosis.¹⁹ Some of these effects may be attributable to increased matrix metalloproteinases (MMP)2 levels.²⁰ In addition, CCL23 has chemoattractant effects on immune cells, such as monocytes, DCs and neutrophils, as well as on osteoclast precursor cells. These chemoattractant effects promote diapedesis and bone resorption, processes that are highly implicated in periodontal destruction.^{21,22} Hence, CCL23 may constitute an interesting new target for CP and its associated disorders, even though protein levels in the present study remained unaltered after 6 h, probably because of kinetics. This hypothesis is supported by Novak et al.,²³ who found the highest CCL23 mRNA concentrations in human primary monocytes after 24 h and the highest protein levels after 4 d.

Notably, CXCL8/IL8 transcriptional and protein levels were significantly induced by LPS *Pg*. CXCL8 acts as chemoattractant for neutrophils, which are key players in periodontal health.²⁴ Interestingly, a previous report showed that subpopulations of neutrophils in patients with CP displayed enhanced responsiveness to CXCL8, thus supporting the notion of host-derived pathogen-associated periodontal destruction.²⁵

Additionally, we observed an up-regulation of pro- and anti-inflammatory transcripts accomplished by increased TNF- α and IL-10 protein. In contrast, the expression of TGF- β -related genes (TGFBR2 and TIAF1) was significantly reduced secondary to LPS *Pg* challenge. These mediators determine inflammatory processes, which favor the recruitment and activation of immune cells. Therefore, reduced or enhanced levels of these mediators predispose to an imbalanced immune response, which may aggravate CP severity. In accordance with this, SNPs in these genes have been associated with CP.⁶ Furthermore, initial experiments with 90 min of LPS *Pg* challenge revealed an increase in NLRP3 inflammasome transcription (eight-fold). Thereby, NLRP3 activation triggers IL1 β production and release via procaspase-1. This critical defense mechanism has been linked to various pathologies, including periodontitis.^{26,27}

As monocytes are confronted with periodontal pathogens and are key players in systemic disease, we focused on their surface markers. After LPS *Pg* challenge, transcriptional changes of CD38, CD40, CD86 and HLAs were found, accompanied by elevated CD86 and HLA-DR surface expression. CD86 and HLA-DR are typical mediators APC that stimulate the activation and priming of T and NK cells.²⁸ The increase in these markers indicates that monocytes were highly activated

promoting T-cell regulation and monocyte differentiation to macrophages or DCs related to periodontal and systemic disorders.²

The top genes included several of relevance to immunity and bacterial infection. Immunoresponsive gene 1 (IRG1) plays a role in innate immunity, inflammatory processes, regulation of NF- κ B signaling, oxidative stress and LPS-induced tolerance, and thus influences disorders such as sepsis and carcinoma.²⁹ IFN-stimulated exonuclease gene 20 kDa (ISG20) has antiviral and immunological capacities and is implicated in diseases of the skin.³⁰ In accordance with this, the significantly induced transcripts included several IFN-related genes, such as guanylate-binding protein (GBP)1. GBPs are a family of IFN-inducible antiviral and antimicrobial GTPases primarily responsible for bacterial growth restriction.³¹ Notably, GBP1 has been shown to inhibit the expression of MMP1, which is implicated in periodontitis.^{32,33} Dysregulated IFN-related genes might therefore promote bacterial proliferation and periodontal destruction.

MMPs and their inhibitors (TIMPs) are responsible for cell invasion, tissue remodeling and degradation. Excess MMPs activity has been associated with the progression of periodontal disease, cancer metastasis and atherosclerosis.³⁴ The present analyses identified reduced transcriptional levels of TIMP2 (3.8-fold), whereas altered TIMP2 protein levels were not detectable after 6 h of LPS *Pg* challenge. TIMP2 is an inhibitor of MMP2, which has recently been identified as candidate for periodontitis.³⁵ Notably, CCL23 induction has been linked to increased MMP2 expression, thus reinforcing the impact of TIMP2, MMP2 and CCL23 in *Pg*-induced periodontal destruction.

Furthermore, several genes implicated in oxidative stress (HIF1A, SOD2, CAT and HYOU1, also known as HSP12A) were significantly regulated by LPS *Pg*. Recent investigations have highlighted the importance of oxidative stress in periodontal disease.^{33,36,37} Therefore, hypoxic inducible factor (HIF)1 α , HSPs and redox systems such as superoxide dismutase (SOD) or catalase (CAT) appear to play a central role in these events, and the present findings support these studies.

Interestingly, overexpressed transcripts included genes involved in tryptophan catabolism, i.e. IDO1 and INDO. Both genes are implicated in NAD biosynthesis and tryptophan degradation. They regulate immunological, inflammatory and apoptotic processes, as well as T-cell proliferation and tolerance induction, all of which are associated with systemic disorders.³⁸ Current results indicate that these two genes may also be new candidates for *Pg*-induced CP.

Concerning microRNAs like MIR155HG (6.91-fold increase), current knowledge indicates that they have emerged as key negative post-transcriptional regulators referred to as 'fine-tuners' of gene expression. Thereby, each miRNA influences several target mRNAs,³⁹ and

research suggests that miRNAs are also involved in *Pg*-induced changes in innate immunity.^{40,41} MIR-155 is encoded within a region known as the B-cell integration cluster gene. Altered expression patterns have been associated with autoimmune diseases and several malignancies of B and myeloid cell origin.⁴² Recent studies have generated evidence for the impact of B cells and miRNA-155 on periodontitis.^{16,43} Moreover, our findings are validated by Tili et al.,⁴⁴ who found that putative inflammation-related targets of miR-155 include the suppressor of cytokine signaling 1 (SOCS-1), which has been linked to periodontitis.⁴⁵ We hypothesize that the application of specific human expression chips for miRNA analysis would clarify the impact of miRNAs in systemic *Pg* infection.

As for the most repressed genes, DHRS9 (6.58-fold) and MS4A6A (8.53-fold), their roles remain unclear. MS4A6A encodes specific membrane proteins with an involvement in cancer.⁴⁶ Dehydrogenase/reductase member 9 (DHRS9) is an enzyme responsible for androgen and progesterone metabolism, which might explain reports of impaired reproduction in patients with periodontal disease.⁴⁷ As steroids have anti-inflammatory properties and inhibit the immune response, altered DHRS9 expressions may exacerbate inflammatory disorders such as periodontitis.

Our data complement recent investigations, which applied microarray technology and different cell types, stimuli and exposure durations.^{48–50} For instance, Yu et al. investigated human macrophages isolated from buffy coats that were challenged with 10 μ g/ml LPS or live *Pg* for 2 h.⁴⁸ The authors detected 575 differentially regulated genes in the LPS group based on three independent macrophage experiments, and identified 13 pathways, most of which correspond to host immune defense.⁴⁸

The present study identified >900 genes, >2149 upstream regulators and 200 canonical pathways implicated in the *Pg*-induced immune response. Differentially regulated genes were associated with immunological, inflammatory and infectious disorders; insulin and leptin signaling; diabetes; arthritis; carcinogenesis; and cardiovascular diseases. This confirms that *Pg*-associated CP may contribute to systemic disorders, and that this is likely to occur via dysregulation of the host immune response.^{1,12} As the present data are based on human monocytes of relatively young males of European origin challenged with a virulence factor of *Pg*, our results might differ with that obtained from females, older individuals, people of other ethnicities, different cells or the whole bacterium. Moreover, while gene expression patterns are essential in terms of determining molecular mechanisms, they provide no insights into functional variability, as post-transcriptional and post-translational modifications can compensate changes in RNA expression. However, our data suggest that altered gene expression, or SNPs in the identified

genes, may predispose to CP and systemic disorders. In addition, we validated our transcriptional findings by evaluating the protein levels of several mediators and cell surface markers.

In conclusion, the present systematic genome-wide transcriptional approach in human monocytes explored LPS *Pg*-elicited immune responses and identified new target genes and pathways of relevance to diagnostic and therapeutic strategies. SNPs in these genes may predispose to, or aggravate, periodontal disease due to immune hypo- or hyper-responsiveness. The present findings also support the hypothesized interplay between periodontal infection and systemic diseases, and further research is therefore warranted.

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Declaration of Conflicting Interests

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References

1. Thornton-Evans G, Eke P, Wei L, et al. Periodontitis among adults aged ≥ 30 years—United States, 2009–2010. Centers for Disease Control and Prevention (CDC). *MMWR Surveill Summ* 2013; 64: 129–135.
2. Hajishengallis G. Periodontitis from microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 2015; 15: 30–44.
3. Adler CJ, Dobney K, Weyrich LS, et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 2013; 45: 450–455, 455e1.
4. Hajishengallis G and Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol Oral Microbiol* 2012; 27: 409–419.
5. Mucci LA, Björkman L, Douglass CW and Pedersen NL. Environmental and heritable factors in the etiology of oral diseases—a population-based study of Swedish twins. *J Dent Res* 2005; 84: 800–805.
6. Laine ML, Loos BG and Crielaard W. Gene polymorphisms in chronic periodontitis. *Int J Dent* 2010; 2010: 324719.
7. Zhou Q and Amar S. Identification of signaling pathways in macrophage exposed to *Porphyromonas gingivalis* or to its purified cell wall components. *J Immunol* 2007; 179: 7777–7790.
8. Tomás I, Diz P, Tobías A, et al. Periodontal health status and bacteraemia from daily oral activities: systematic review/meta-analysis. *J Clin Periodontol* 2012; 39: 213–228.
9. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004; 3: Article3.
10. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-seq and microarray studies. *Nucleic Acids Res* 2015; 43: e47.
11. Kim S, Becker J, Bechheim M, et al. Characterizing the genetic basis of innate immune response in TLR4-activated human monocytes. *Nat Commun* 2014; 5: 5236.
12. Kobayashi T and Yoshie H. Host responses in the link between periodontitis and rheumatoid arthritis. *Curr Oral Health Rep* 2015; 2: 1–8.
13. Shi C and Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol* 2011; 11: 762–774.
14. Sippert EA, de Oliveira e Silva C, Visentainer JE and Sell AM. Association of duffy blood group gene polymorphisms with IL8 gene in chronic periodontitis. *PLoS One* 2013; 8: e83286.
15. Nokhehsaim M, Keser S, Jäger A, et al. Regulation of regenerative periodontal healing by NAMPT. *Mediators Inflamm* 2013; 202530.
16. Kebschull M, Guarnieri P, Demmer RT, et al. Molecular differences between chronic and aggressive periodontitis. *J Dent Res* 2013; 92: 1081–1088.
17. Souto GR, Queiroz CM Jr, Costa FO and Mesquita RA. Relationship between chemokines and dendritic cells in human chronic periodontitis. *J Periodontol* 2014; 85: 1416–1423.
18. Rioja I, Hughes FJ, Sharp CH, et al. Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL13, CCL23, transforming growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. *Arthritis Rheum* 2008; 58: 2257–2267.
19. Castillo L, Rohatgi A, Ayers CR, et al. Associations of four circulating chemokines with multiple atherosclerosis phenotypes in a large population-based sample: results from the Dallas heart study. *J Interferon Cytokine Res* 2010; 30: 339–347.
20. Kim CS, Kang JH, Cho HR, et al. Potential involvement of CCL23 in atherosclerotic lesion formation/progression by the enhancement of chemotaxis, adhesion molecule expression, and MMP-2 release from monocytes. *Inflamm Res* 2011; 60: 889–895.
21. Youn BS, Zhang SM, Broxmeyer HE, et al. Characterization of CK β 8 and CK β 8-1: two alternatively spliced forms of human β -chemokine, chemoattractants for neutrophils, monocytes, and lymphocytes, and potent agonist at CC chemokine receptor 1. *Blood* 1998; 91: 3118–3126.
22. Votta BJ, White JR, Dodds RA, et al. CK- β [CCL23], a novel chemokine, is chemotactic for human osteoclast precursors and is expressed in bone tissues. *J Cell Physiol* 2000; 183: 196–207.
23. Novak H, Müller A, Harrer N, et al. CCL23 expression is induced by IL-4 in a STAT6-dependent fashion. *J Immunol* 2007; 178: 4334–4341.
24. Sima C and Glogauer M. Neutrophil dysfunction and host susceptibility to periodontal inflammation: current state of knowledge. *Curr Oral Health Rep* 2014; 1: 95–103.
25. Fredriksson MI. Effect of priming in subpopulations of peripheral neutrophils from patients with chronic periodontitis. *J Periodontol* 2012; 83: 1192–1199.

26. Ozaki E, Campbell M and Doyle SL. Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives. *J Inflamm Res* 2015; 8: 15–27.
27. Park E, Na HS, Song YR, et al. Activation of NLRP3 and AIM2 inflammasomes by *Porphyromonas gingivalis* infection. *Infect Immun* 2014; 82: 112–123.
28. Lichtenegger FS, Mueller K, Otte B, et al. CD86 and IL-12p70 are key players for T helper 1 polarization and natural killer cell activation by Toll-like receptor-induced dendritic cells. *PLoS One* 2012; 7: e44266.
29. Li Y, Zhang P, Wang C, et al. Immune responsive gene 1 (IRG1) promotes endotoxin tolerance by increasing A20 expression in macrophages through reactive oxygen species. *J Biol Chem* 2013; 288: 16225–16234.
30. Yao Y, Richman L, Morehouse C, et al. Type I interferon: potential therapeutic target for psoriasis? *PLoS One* 2008; 3: e2737.
31. Vestal DJ and Jeyaratnam JA. The guanylate-binding proteins: emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *J Interferon Cytokine Res* 2011; 31: 89–97.
32. Guenzi E, Töpol K, Lubeseder-Martellato C, et al. The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. *EMBO J* 2003; 22: 3772–3782.
33. Gözl L, Memmert S, Rath-Deschner B, et al. LPS from *P. gingivalis* and hypoxia increases oxidative stress in periodontal ligament fibroblasts and contributes to periodontitis. *Mediators Inflamm* 2014; 2014: 986264.
34. Sorsa T, Tjäderhane L and Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* 2004; 10: 311–318.
35. Araújo AA, Souza TO, Moura LM, et al. Effect of telmisartan on levels of IL-1, TNF- α , down-regulated COX-2, MMP-2, MMP-9 and RANKL/RANK in an experimental periodontitis model. *J Clin Periodontol* 2013; 40: 1104–1111.
36. Bullon P, Newman HN and Battino M. Obesity, diabetes mellitus, atherosclerosis and chronic periodontitis: a shared pathology via oxidative stress and mitochondrial dysfunction? *Periodontology 2000* 2014; 64: 139–153.
37. Gözl L, Memmert S, Rath-Deschner B, et al. Hypoxia and *P. gingivalis* induce HIF-1 and NF- κ B activation in PDL cells and periodontal diseases. *Mediators Inflamm* 2015; 2015: 438085.
38. Kim S, Miller BJ, Stefanek ME and Miller AH. Inflammation-induced activation of the indoleamine 2,3-dioxygenase pathway: Relevance to cancer-related fatigue. *Cancer*. Epub ahead of print 27 February 2015. DOI: 10.1002/cncr.29302.
39. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215–233.
40. Naqvi AR, Fordham JB, Khan A and Nares S. MicroRNAs responsive to *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* LPS modulate expression of genes regulating innate immunity in human macrophages. *Innate Immun* 2013; 20: 540–551.
41. Moffatt CE and Lamont RJ. *Porphyromonas gingivalis* induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells. *Infect Immun* 2011; 79: 2632–2637.
42. Elton TS, Seimon H, Elton SM and Parinandi NL. Regulation of MIR155 host gene in physiological and pathological processes. *Gene* 2013; 532: 1–12.
43. Honda T, Takahashi N, Miyauchi S and Yamazaki K. *Porphyromonas gingivalis* lipopolysaccharide induces miR-146a without altering the production of inflammatory cytokines. *Biochem Biophys Res Commun* 2012; 420: 918–925.
44. Tili E, Michaille JJ, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF- α stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol* 2007; 179: 5082–5089.
45. de Souza JA, Nogueira AV, de Souza PP, et al. Expression of suppressor of cytokine signaling 1 and 3 in ligature-induced periodontitis in rats. *Arch Oral Biol* 2011; 56: 1120–1128.
46. Satow R, Shitashige M, Kanai Y, et al. Combined functional genome survey of therapeutic targets for hepatocellular carcinoma. *Clin Cancer Res* 2010; 16: 2518–2528.
47. Hart R, Doherty DA, Pennell CE, et al. Periodontal disease: a potential modifiable risk factor limiting conception. *Hum Reprod* 2012; 27: 1332–1342.
48. Yu WH, Hu H, Zhou Q, et al. Bioinformatics analysis of macrophages exposed to *Porphyromonas gingivalis*: implications in acute vs. chronic infections. *PLoS One* 2010; 5: e15613.
49. Handfield M, Mans JJ, Zheng G, et al. Distinct transcriptional profiles characterize oral epithelium-microbiota interactions. *Cell Microbiol* 2005; 7: 811–823.
50. Zhang B, Elmabsout AA, Khalaf H, et al. The periodontal pathogen *Porphyromonas gingivalis* changes the gene expression in vascular smooth muscle cells involving the TGF β /Notch signalling pathway and increased cell proliferation. *BMC Genomics* 2013; 14: 770.