RESEARCH ARTICLE   **Open Access**

CrossMark

Porphyromonas gingivalis activates NFκB and MAPK pathways in human oral epithelial cells

Sabine Groeger[1*], Fabian Jarzina[1], Eugen Domann[2] and Joerg Meyle[1]

Abstract

Background: The bacterial biofilm at the gingival margin induces a host immune reaction. In this local inflammation epithelial cells defend the host against bacterial challenge. *Porphyromonas gingivalis* (*P. gingivalis*), a keystone pathogen, infects epithelial cells. The aim of this study was to investigate the activation of signaling cascades in primary epithelial cells and oral cancer cell lines by a profiler PCR array.

Results: After infection with *P. gingivalis* membranes the RNA of 16 to 33 of 84 key genes involved in the antibacterial immune response was up-regulated, amongst them were IKBKB (NF-κB signaling pathway), IRF5 (TLR signaling) and JUN, MAP2K4, MAPK14 and MAPK8 (MAPK pathway) in SCC-25 cells and IKBKB, IRF5, JUN, MAP2K4, MAPK14 and MAPK8 in PHGK. Statistically significant up-regulation of IKBKB (4.7 ×), MAP2K4 (4.6 ×), MAPK14 (4.2 ×) and IRF5 (9.8 ×) ($p < 0.01$) was demonstrated in SCC-25 cells and IKBKB (3.1 ×), MAP2K4 (4.0 ×) MAPK 14 (3.0 ×) ($p < 0.05$), IRF5 (3.0 ×) and JUN (7.7 ×) ($p < 0.01$) were up-regulated in PHGK.

Conclusions: *P. gingivalis* membrane up-regulates the expression of genes involved in downstream TLR, NFκB and MAPK signaling pathways involved in the pro-inflammatory immune response in primary and malignant oral epithelial cells.

Key words: Signaling pathway, MAPK, NF-κB, Oral cells, *P. gingivalis*

Background

Porphyromonas gingivalis (*P. gingivalis*), an anaerobic Gram-negative rod, is a member of the oral bacterial biofilm and considered as an important etiologic agent of gingival and periodontal inflammation [1]. *P. gingivalis* is able to invade oral epithelial and endothelial cells [2–4] and effectively induces pro-inflammatory cytokine production of monocytes, neutrophils, as well as macrophages. It is also able to modify the functions of immune cells *in vitro* and *in vivo* [5, 6].

Epithelial cells not only provide a barrier against bacterial challenge and invasion but also participate in the innate immune defense. Infection of epithelial cells by *P. gingivalis* activates signaling cascades that control transcription of target genes encoding for immune response and inflammatory reactions such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α in

monocytic and epithelial cells and interferon regulating factor (IRF) 6 in oral epithelial cells [7–9].

Pattern recognition receptors (PRRs) recognize microbial components formed as pathogen-associated molecular patterns (PAMPs). PAMPs show structural similarities between a great numbers of microorganisms, thus different PRRs usually recognize well-defined PAMPs. Toll-like receptors (TLRs) form a well-known PRR family [10]. PRRs are present on epithelial cells, neutrophils, macrophages and dendritic cells (DCs) [11]. Activation of these receptors by PAMPs initiates the innate response to microbial challenge and induces adaptive immunity to clear infections [12, 13].

Recent studies suggest that PRRs are responsible for constant surveillance of the microbial colonization by detecting conserved microbial structures such as lipopolysaccharides (LPS) [14, 15].

Intracellular invasion of pathogens is recognized by nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) which are located in the cytoplasm. Purinergic P2X receptors on the plasma membrane are

* Correspondence: Sabine.E.Groeger@dentist.med.uni-giessen.de
[1]Department of Periodontology, Justus-Liebig-University of Giessen, Giessen, Germany
Full list of author information is available at the end of the article

activated by damaged cells [16, 17]. Ligation of the purinergic receptor, P2X7, induces the assembly of the inflammasome, a protein complex of caspase-1 and an adaptor protein ASC. Activation of caspase-1 initiates the production and release of the pro-inflammatory cytokines IL-1β and IL-18. The adaptor protein, apoptosis-associated speck-like protein NLRP3 is the best studied NLR member. It contains a CARD (ASC) domain and the protease caspase-1 [18, 19].

Gingival epithelial cells (GECs) may exhibit a functional NALP3 inflammasome. Stimulation of GECs with LPS or infection with *P. gingivalis* caused induction of the IL-1β gene and accumulation of IL-1β in the cells. However, IL-1β release did not occur unless the LPS-treated or infected cells were stimulated with adenosine triphosphate (ATP). GECs showed caspase-1 activation after treatment with ATP [20]. *P. gingivalis* expresses a nucleoside-diphosphate kinase (NDK) homolog that is able to inhibit innate immune reaction caused by stimulation with extracellular ATP. Thus, *P. gingivalis* infection inhibits ATP-induced caspase-1 activation in GECs. Furthermore *P. gingivalis* NDK may modify high- mobility group protein B1 (HMGB1) release. HMGB1 is a pro-inflammatory danger signal that, in intact cells remains associated with chromatin. HMGB1 is released into the extracellular area after stimulation of uninfected GECs with ATP instead of being translocated from the nucleus into the cytosol. In comparison to wild-type *P. gingivalis* higher amounts of HMGB1 are released when cells are infected with a NDK-deficient mutant stimulated with ATP, suggesting that NDK is crucial in inhibiting the initiation of the P2X7-dependent inflammasome and HMGB1 release from infected GECs [21].

GECs belong to the first host cells which encounter with colonizing oral bacteria. The bacterial–host communication is managed by signal transduction pathways, i.e. the mitogen-activated protein kinase (MAPK) and TLR pathway that are activated by infection with *Fusobacterium nucleatum* (*F. nucleatum*) and *Streptococcus gordonii* and other bacteria of the oral biofilm [22–24].

Molecules supporting antimicrobial clearance and the control of adaptive and innate immune responses are human beta-defensins (hBDs) produced by various cell types. Investigation of the macrophage cell line RAW 264.7 revealed that treatment with synthetic hBD3-3 peptide inhibited the LPS-induced production of inducible nitric oxide synthase and nitric oxide. Furthermore this treatment inhibited the production of secretory cytokines, such as IL-6 and tumor necrosis factor (TNF)-α in cells stimulated with LPS. This inhibition was found to be concentration-dependent. Additionally, in a model of lung inflammation, hBD3-3 was shown to reduce interstitial infiltration by neutrophils. HBD3-3 was able to downregulate the nuclear factor-kappa B (NF-κB)-

dependent inflammatory response via direct suppression of the phosphorylated-nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα) degradation and downregulation of the p65 unit of activated NF-κB [25].

P. gingivalis is capable of inducing immune tolerance in antigen-presenting cells (APCs) by desensitizing them against second activation, a process that involves induction of the expression of the tolerogenic molecules immunoglobulin-like transcript 3 (ILT-3) and B7-H1 [26]. It is known that T cell activation requires a co-stimulatory signal usually provided by APCs. This additional signal regulates activation or inhibition of T cell action.

In a previous study, we demonstrated that *P. gingivalis* induces B7-H1 expression in different carcinoma cell lines (SCC-25 cells, BHY cells) as well as in primary human gingival keratinocytes [27]. The B7-H1 receptor (synonymous PD-L1) belongs to the B7-family exhibiting regulatory properties that modify cell-mediated immune reactions [28, 29]. B7-H1 ligands are induced on activated T and B cells, on endothelial and epithelial cells as well as on macrophages. Dendritic cells (DCs) and APCs exhibit constitutive B7-H1expression [30–32]. The binding receptor for B7-H1 is the CD28/CTLA-4 like programmed death-1 (PD-1) receptor which is expressed on activated T cells, B cells, monocytes and macrophages. This molecule is a member of the immunoglobulin (IG) superfamily [33]. Signals mediated by B7-H1 are essential in regulating T cell activation and tolerance [34], by inhibiting functions of activated T cells. Pro-inflammatory cytokines i.e. interferon (IFN)-γ are known to up-regulate B7-H1 expression [35, 36]. Activated T cells, B cells and monocytes show PD-1 expression [37].

B7-H1 ligand binding triggers the development of regulatory T cells (T_{reg}). This phenotype is essential in regulating peripheral tolerance by active suppression of effector T cells and inhibition of tissue damage caused by the inflammatory response [38–40]. Blockade of B7-H1 affected the inhibitory effect of T_{reg} [41]. Additionally, blockade of B7-H1/PD-1 ligation abolished T_{reg} mediated immune-regulation [42]. This was demonstrated in a mouse model expressing a phenotype with B7-H1 deficiency that caused diminished T_{reg} cell differentiation *in vivo* [43]. The underlying mechanisms are not completely understood. Using bladder cancer cells, B7-H1 up-regulation was shown to be induced by TLR4 signaling [44], and in oral Langerhans cells activation of TLR4 caused induction of B7-H1 *in vitro* [45, 46].

The aim of this study was to investigate the regulation of a selected number of genes after infection with *P. gingivalis*. The study was conducted to analyze mechanisms that are induced in epithelial cells after bacterial challenge. The analysis was performed on genes coding for

Groeger et al. BMC Immunology (2017) 18:1

Page 3 of 11

receptor activation, downstream signal transduction, apoptosis, inflammatory response, cytokines and chemokines, and antimicrobial peptides.

Materials and methods
Bacteria and growth conditions
P. gingivalis strain W83 was purchased from the American Type Culture Collection (ATCC BAA-308™, LGC Standards GmbH, Wesel, Germany) and grown at 37 °C in brain-heart-infusion broth (Difco, BD, Heidelberg, Germany) with hemine (5 µg/ml) and menadione (1 µg/ml) (Sigma-Aldrich, Munich, Germany) under anaerobic conditions using the Anaerocult A System (Merck, Darmstadt, Germany).

Cell cultures
The human squamous cell carcinoma cell line SCC-25 was purchased from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, DSMZ number ACC 617) and cultured in a medium containing Dulbecco's minimal essential medium (DMEM):Ham's F12 (1:1, vol:vol), (Invitrogen, Karlsruhe, Germany) and 20% fetal calf serum (FCS, Greiner, Frickenhausen, Germany). Primary human gingival keratinocytes (PHGK) were obtained from gingival biopsies of healthy volunteers, prepared and cultured in a serum-free medium containing DMEM:Ham's F12 (4:1, vol:vol), 10 mM HEPES (Invitrogen, Karlsruhe, Germany).

Bacterial cell fractionation
The bacteria were harvested in the late exponential growth phase (OD_{600} of 1.0) by centrifugation for 20 min at 6,500 × g and 25 °C. The bacterial pellet was re-suspended in 50 ml of 10 mM HEPES, pH 7.4, containing protease inhibitor cocktail (4 mini-tablets of Complete, EDTA-free, Roche) and DNase I/RNase A (20 µg/ml each). Bacteria were disrupted by four passages through a high-pressure cell disruption system (Model TS, 0.75 KW, Constant Systems Ltd.) at 40,000 psi. The cellular debris was removed by centrifugation at 8,000 × g for 30 min at 4 °C, and the membranes were sedimented from the cleared lysate at 150,000 × g for 2 h at 4 °C. The supernatant (cytosolic fraction) was stored, and the total membrane fraction was washed three times with 10 mM HEPES, pH 7.4. The membrane pellet was subsequently re-suspended in 10 mM HEPES, pH 7.4. The protein concentrations of all samples, i.e. cleared lysate, cytosolic fraction and total membranes, were determined using Bio-Rad's protein assay reagent. The purity of the fractions was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) using a 10% gel following staining with coomassie brilliant blue (SERVA Electrophoresis GmbH, Heidelberg, Germany).

Infection of SCC-25 cells and membrane-stimulation of SCC-25 cells and PHGK
For infection of SCC-25 cells and primary human gingival keratinocytes (PHGK), the cells were seeded in 6-well plates (1×10^6 cells/well) in antibiotic-free medium containing 1.8 mM calcium chloride and 10% FCS (Thermo Fisher Scientific, Darmstadt, Germany) and grown at 37 °C in a humidified atmosphere with 5% CO2 to 80% confluency before stimulation.

Cells were infected with whole bacterial cells as well as treated with bacterial fractions. To prepare *P. gingivalis* W83 for infection, the bacterial cells were harvested in the late exponential growth phase (OD_{600} of 1.0) by centrifugation at 25 °C for 20 min at 6,500 × g. The supernatant was discarded, and the cell pellet was re-suspended in DMEM:Ham's with 10% FCS, adjusting the bacterial cell number on the basis of spectrophotometric measurements of the optical density of the bacterial suspension at 600 nm ($OD_1 = 10^9$ cells/ml). Infection of the SCC-25 cells was performed at a multiplicity of infection (MOI) of 100 for 24 h. The bacterial membrane fractions from *P. gingivalis* W83 was used in a concentration of 50 µg/ml. A non-treated control containing cells only in culture medium was carried in every experiment. SCC-25 cells and PHGK were treated with the bacterial fractions for 24 h at 37 °C, 95% air, 5% CO_2 and 92% relative humidity and harvested by scraping in RNA protect solution (Qiagen) for RNA extraction. All analyses were performed in three independent experiments.

RNA extraction
Total RNA was extracted using RNeasy mini columns with on-column DNase treatment following the manufacturer's instructions (Qiagen). The concentration and quality of the RNA were analysed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Darmstadt, Germany). The integrity of the RNA was verified using RNA gel electrophoresis.

Human Antibacterial Response RT[2] Profiler Array
The Human Antibacterial Response RT[2] Profiler PCR Arra/cat. No. 330231 PAHS-148Z (Qiagen, Hilden, Germany) was used to profile the expression of 84 key gens involved in innate immune response to bacteria.

Synthesis of the cDNA was performed with the RT[2] first strand kit (Qiagen) according to the manufacturer's instructions at 42 °C for 15 min with a 5-min deactivation step at 95 °C in an BioRad CFX96 Real-Time System C1000 Thermal Cycler (Biorad, Munic, Germany).

The RT[2] SYBR green master mix (Qiagen) (1350 µl per 96-well plate) was mixed with 1248 µl RNase free water and 102 µl cDNA synthesis reaction template, and 25 µl PCR components were added to each well of the array. Quantitative real time polymerase chain reaction

Groeger *et al. BMC Immunology* (2017) 18:1

Page 4 of 11

(qRT-PCR) was performed in accordance with the recommendations of the manufacturer. Cycling and detection were done in a Bio Rad CFX96 real time system C1000 thermal cycler (Bio Rad).

qRT-PCR for verification of profiling

Synthesis of cDNA was performed using the Verso™ cDNA Kit (Thermo Fisher Scientific) following the manufacturer's instructions. qRT-PCR using the SYBR Green Assay was performed with SensiFast no ROX SYBR Green Mix (Bioline, Luckenwalde, Germany) according to the manufacturer's recommendations. The following primers were used: QuantiTect Primer Assay (Qiagen) Hs_NFKB1_1_SG (NF-κB1), HS_IKBKB_1_SG (IKKβ), Hs_MAP2K4_1_SG (MAP2K4), Hs_MAPK8_1_SG (MAPK8), Hs_MAPK14_1_ SG (MAPK14), Hs_IRF5_1_SG (IRF5), Hs_JUN_1_SG (Jun), Hs_IRAK3_1_SG (IRAK3), Hs_TOLLIP_1_SG (TOLLIP), and Hs-GAPDH_1_SG (GAPDH) as a housekeeping gene (patents: Roche Molecular Systems). Cycling and detection was performed in a Biorad CX96 cycler (Biorad, Munic, Germany). All samples were tested 3 × in triplicate ($n = 9$).

Data analysis

The analysis of the profiler arrays was performed using the online analysis tool of the manufacturer based on changes in gene expression for pair-wise comparison with the non-treated control using the ΔΔCt method. The results of the qRT-PCR were analyzed using the comparative CT (ΔΔCT) method. The amount of target ($2^{-\Delta\Delta CT}$) was obtained by normalizing to an endogenous reference (GAPDH) relative to non-infected control cells. The results are shown as log2 fold (x) regulation.

Statistical analysis

The results were analyzed using independent two-sample Student's *t*-test. The character of the evaluation was explorative. Probability of error was set to 5% and shown as *p*-values.

Results

SCC-25 cells treated with *P. gingivalis* W83 isolated membrane

The analysis of three experiments treating SCC-25 cells with the membrane fraction for 24 h showed up-regulation of a number of genes that play a role in different biological processes. Up-regulated were genes involved in the TLR signaling cascade, in the NF-κB pathway and the MAPK pathways. Statistically significant with a *p*- value of < 0.05 was the up-regulation of *IBKB* (4.0 ×) and *JUN* (8.7 ×). The results of this analysis are shown in Table 1. The Ct values are shown in Additional file 1: Table S4.

Table 1 Up-regulated genes in SCC-25 cells after stimulation with the membrane fraction of *P. gingivalis* W83.

Gene Symbol	Fold Regulation	Biological Function
IRAK1	2.5	TLR Signaling
IRAK3	2.2	
IRF5	3.1	
TICAM1	2.6	
TOLLIP	2.3	
TRAF6	2.8	
HSP90AA1	2.0	NLR Signaling
IKBKB*	4.0	NF-κB Pathway
NFKB1	2.6	
RELA	2.5	
Jun*	8.7	MAPK Pathway
MAP2K1	1.5	
MAP2K4	2.9	
MAPK1	2.6	
MAPK14	2.9	
MAPK8	2.4	

Mean values from 3 experiments as x-fold regulation compared to the non-infected control. * = $p < 0.05$

SCC-25 cells infected with *P. gingivalis* W83 living bacteria

Infection of SCC-25 cells with *P. gingivalis* W83 for 24 h induced up-regulation of genes also with biological functions in TLR signaling, the NF-κB pathway and MAPK downstream pathway, as well as the cytokine IL-*12A*. Statistically significant ($p < 0.05$) was the up-regulation of *IKBKB* (3.1 ×) *MAP2K4* (2.7 ×), *MAPK14* (2.7 ×) and *MAPK8* (2.6 ×). The results of this analysis are shown in Table 2. The Ct values are shown in Additional file 1: Table S4.

PHGK stimulated with *P. gingivalis* W83 membrane

The analysis of three experiments treating PHGK cells with membrane fraction for 24 h showed up-regulation of various genes as well. Up-regulated were genes that participate in the TLR signaling, in NLR signaling, apoptosis, inflammatory processes, the NF-κB pathway and the MAPK downstream signaling. Further up-regulated genes were related to inflammatory response, chemokines, apoptosis and antimicrobial peptides. Also *DMBT1*, a tumor suppressor gene that participates in various biological processes like mucosal immune response, was up-regulated. The up-regulation of *IRF5* was significant ($p < 0.05$, 14.3 ×). The results of this analysis are shown in Table 3. The Ct values are shown in (Additional file 2: Table S5).

Quantitative real time polymerase chain reaction (PCR)

Quantitative real time PCR (qRT-PCR) of RNA in SCC-25 cells after 24 h of infection with *P. gingivalis* total

Groeger et al. BMC Immunology (2017) 18:1

Page 5 of 11

Table 2 Up-regulated genes in SCC-25 cells after stimulation for 24 h with living *P. gingivalis* W 83.

Gene Symbol	Fold Regulation	Biological Function
IRAK3	2.8	TLR Signaling
IRF5	2.7	
RAC1	3.0	
TICAM1	2.5	
TOLLIP	2.2	
RELA	2.7	
CASP8	2.8	NLR signaling, Apoptosis
IKBKB *	3.1	NF-κB Pathway
NFKB1	3.6	
JUN	3.2	MAPK Pathway
MAP2K1	3.6	
MAP2K4 *	2.7	
MAPK1	3.3	
MAPK14 *	2.7	
MAPK8 *	2.6	
CCL5	2.2	Chemokines
IL12A	2.4	Cytokines
CASP1	4.9	Apoptosis

Mean values from 3 experiments as x-fold regulation compared to the non-infected control. * = $p < 0.05$

Table 3 Up-regulated genes in primary human gingival keratinocytes 24 h of infection with membrane fractions of *P. gingivalis* W83.

Gene Symbol	Fold Regulation	Biological Function
IRAK3	2.5	TLR Signaling
IRF5*	14.3	
TRAF6	2.6	
HSP90AA1	2.7	NLR Signaling
NOD1	2.3	
NOD2	2.0	
XIAP	2.4	
NAIP	2.3	
BIRC3	2.2	NLR Sign., Apoptosis
IKBKB	4.0	NF-κB Pathway
NFKB1	2.6	
NFKBIA	2.4	
MAP2K4	2.6	MAPK Signaling
MAPK14	2.3	
MAPK8	2.0	
CRP	3.8	Inflammatory Response
LBP	2.6	
LY96	2.6	
AKT1	2.1	Inflam. Resp., Apoptosis
CCL3	3.2	Chemokines
CCL5	3.0	
CXCL2	2.1	
IL-12A	2.2	Cytokines
IL-12B	3.3	
IL18	2.7	
CASP1	2.1	Apoptosis
PYCARD	3.5	
RIPK1	3.3	
BPI	3.2	Antimicrobial Peptides
CAMP	3.3	
MPO	2.3	
SLPI	3.6	
DMBT1	4.0	Mucosal Immune Response

Mean values from 3 experiments as x-fold regulation compared to the non-infected control. * = $p < 0.05$

membrane (Fig. 1) showed statistically significant up-regulation of the following genes: *IκBκB* (4.7 ×), *MAP2K4* (4.6 ×), *MAPK14* (4.2 ×) and *IRF5* (9.8 ×) ($p < 0.01$) ($n = 9$). Slightly up-regulated were *NFκB1* (4.4 ×), *MAPK8* (2.6 ×), *JUN* (3 ×), *IRAK3* (3.0 ×) and *TOLLIP* (3.5 ×) ($p > 0.05$).

Real-time RNA quantification of SCC-25 cells upon stimulation *P. gingivalis* whole bacteria (Fig. 2) showed statistically significant up-regulation of *IκBκB* (2.3 ×), *IRAK3* (3.3 ×) ($p < 0.05$), *IRF5* (4.1 ×), *MAPK8* (3.6 ×) and *MAPK* 14 (3.0 ×) ($p < 0.01$) ($n = 9$). Only slightly up-regulated were *NFκB1* (1.5 ×), *MAP2K4* (2.5 ×), *JUN* (1.7 ×) and *TOLLIP* (2.3 ×).

In primary human epithelial cells stimulation with *P. gingivalis* total membrane (Fig. 3) resulted in up-regulation of *IκBκB* (3.1 ×), *MAP2K4* (4.0 ×) *MAPK* 14 (3.0 ×) ($p < 0.05$), *IRF5* (3.0 ×) and JUN (7.7 ×) ($p < 0.01$) ($n = 9$). *NFκB1* (1.4 ×), *MAPK8* (1.8 ×), *IRAK3* (6.1 ×) and *TOLLIP* (4.7 ×) were also up-regulated as well ($n = 9$) ($p > 0.05$).

Fig. 4 shows the nuclear- factor kappa B (NF-κB) and mitogen activated protein kinase (MAPK or MKK) signaling pathways induced by activation of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain receptors (NODs). Upregulated genes in oral epithelial cells induced by *P. gingivalis* and its total membrane are indicated by red arrows.

Discussion

Periodontitis is mainly caused by an oral microbial bio-film, however, progression of the disease is regulated by the immune-inflammatory reaction and the destruction of the teeth supporting tissues [47]. *P. gingivalis* plays an essential role in the pathogenesis and progression of periodontitis. Among many different mechanisms, it has been shown that *P. gingivalis* differentially activates the NF-κB pathway. After infection with *F. nucleatum* the
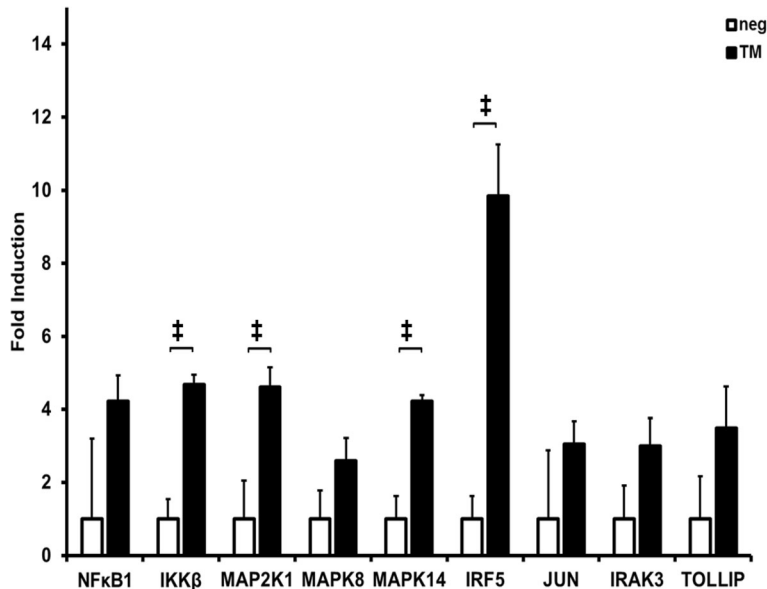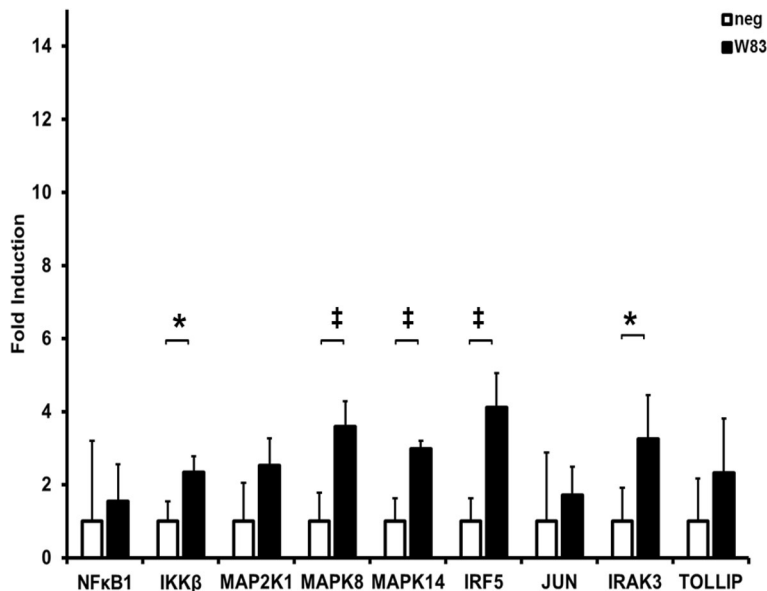
Fig. 1 Up-regulation of genes in *P. gingivalis* membrane stimulated SCC-25 cells. Up-regulation of NF-κB, IKBKB, MAP2K4, MAPK8, MAPK 14, IRF5, JUN, IRAK3 and TOLLIP in SCC-25 cells after 24 h stimulation with *P. gingivalis* total membrane fraction (= TM) analyzed by ΔΔCt method, shown as absolute fold induction of RNA expression relative to non-stimulated samples as negative control (= neg), normalized to the house keeping gene GAPDH, $n = 9$, ‡ = $p < 0.01$

oral epithelial cell line H400 responded with activation of NFκB. However, a significantly higher number of NF-kB translocations into the nucleus were detected after H400 cell infection with *F. nucleatum* suggesting that these two periodontal pathogens have different molecular influences on these cells [48]. In human monocyte-derived macrophages, *P. gingivalis* gingipains induced secretion of TNF-α and IL-8 and upon stimulation the amount of phosphorylated p38α MAPK increased [49].

Upon stimulation of primary oral epithelial cells and carcinoma cells with bacterial fractions of *P. gingivalis*, a number of genes were conjointly up-regulated. The

Fig. 2 Up-regulation of genes in *P. gingivalis* bacteria stimulated SCC-25 cells. Up-regulation of NF-κB, IKBKB, MAP2K4, MAPK8, MAPK 14, IRF5, JUN, IRAK3 and TOLLIP in SCC-25 cells after 24 h stimulation with *P. gingivalis* W83, whole bacteria (= W83), analyzed by ΔΔCt method, shown as absolute fold induction of RNA expression relative to non-stimulated samples as negative control (= neg), normalized to the house keeping gene GAPDH, $n = 9$, * = $p < 0.05$, ‡ = $p < 0.01$

Groeger *et al. BMC Immunology* (2017) 18:1

Page 7 of 11
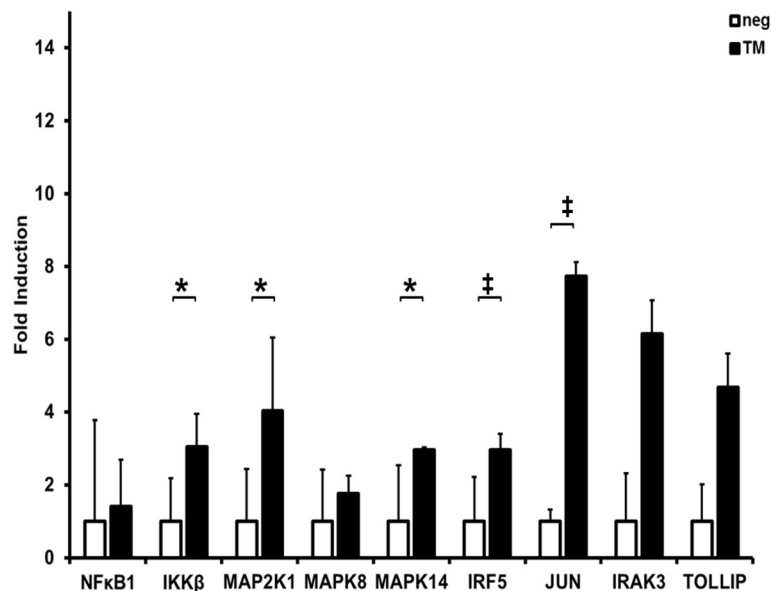
Fig. 3 Up-regulation of genes in *P. gingivalis* membrane stimulated PHGK. Up-regulation of NF-κB, IKBKB, MAP2K4, MAPK8, MAPK 14, IRF5, JUN, IRAK3 and TOLLIP in PHGK cells after 24 h stimulation with *P. gingivalis* total membrane fraction (= TM), analyzed by ΔΔCt method, shown as absolute fold induction of RNA expression relative to non-stimulated samples as negative control (= neg), normalized to the house keeping gene GAPDH, $n = 9$, * = $p < 0.05$, ‡ = $p < 0.01$

genes *IKBKB, IRAK3, IRF5, MAP2K4* (MEK4), *MAPK14* (p38), *MAPK8* (JNK1) and *NFKB1* (p50) were upregulated not only in both cell types, but also after infection with whole bacteria of *P. gingivalis* W83 as well as with the membrane fraction. The cytosolic fraction didn't induce altered gene expression (data not shown). NF-KB1 (p50) is a protein subunit of the NF-κB protein complex, a transcription factor central for a number of immunological and inflammatory reactions, including five subunit members – RelA (p65), RelB, c-Rel, p50 (NF-KB1) and p52 with functions as homodimers and heterodimers [50]. The NF-κB transcription factors are dissociated in the cytoplasm by a family of inhibitors of κB, the IκBs. The IκB kinase (IKK) complex, including IKBKB, initiates the activation and is activated as well. Further phosphorylation and disintegration of IκB protein results in activation of NF-κB [51]. Mitogen-activated protein kinases (MAPKs) are a highly conserved family of Ser/Thr protein kinases in eukaryotes that are regulating a number of cellular activities such as managing cellular responses to cell stress, and pro-inflammatory cytokines.

Epithelial cells, such as GECs, are able to respond to bacterial challenge by initiation of a deliberated signaling network. The GECs express different receptors on the cell surface or in the cytoplasm. Their activation induces innate immune reactions, including TLRs, nucleotide binding oligomerization domain receptors (NODs) and protease-activated receptors (PARs). It has been shown that surface receptors, such as TLRs and PARs, are activated when corresponding bacterial motifs or proteases are detected. Thus, activation of TLRs and PARs leads to downstream activation of NF-κB and/or MAPK pathways [52–55]. Activations of TLR and PARs by membrane fractions induce the up-regulation of downstream signaling molecules that were detected in this study. The TLR family shares downstream signaling molecules, amongst them the adaptor molecule myeloid differentiation primary-response protein kinases 88 (MyD88), a shared adaptor protein of TLRs triggers the downstream pathways like NF-κB and MAPK cascades [56]. Among the MAPK, extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 (also known as MAPK14) kinases have been intensively studied, from which JNK and p38 kinases show a higher responsiveness [57].

In human oral keratinocytes (HOKs) it was demonstrated that *P. gingivalis* LPS could activate both p38 and JNK pathways by inducing phosphorylation of IκBa and p65 transcription factors. These results indicate that induction of LPS binding protein (LBP) expression in HOKs by *P. gingivalis* LPS involves NF-κB and p38 MAPK signaling pathways[58]. Further members of the MAPK family are mitogen-activated protein kinase 4 (MEK4 or MAP2K4) and c-Jun NH2 terminal kinase 1 (JNK1 or MAPK8). After stimulation, activated TLR2 may initiate a cascade activation of MAPKs including MEK4 [59]. JNK1 is a downstream target of MEK4 [60]. In a human lung carcinoma type II epithelial cell line (A549) stimulation with LPS enhanced phosphorylation of MEK 4 and JNK1 in a time-dependent manner [61].
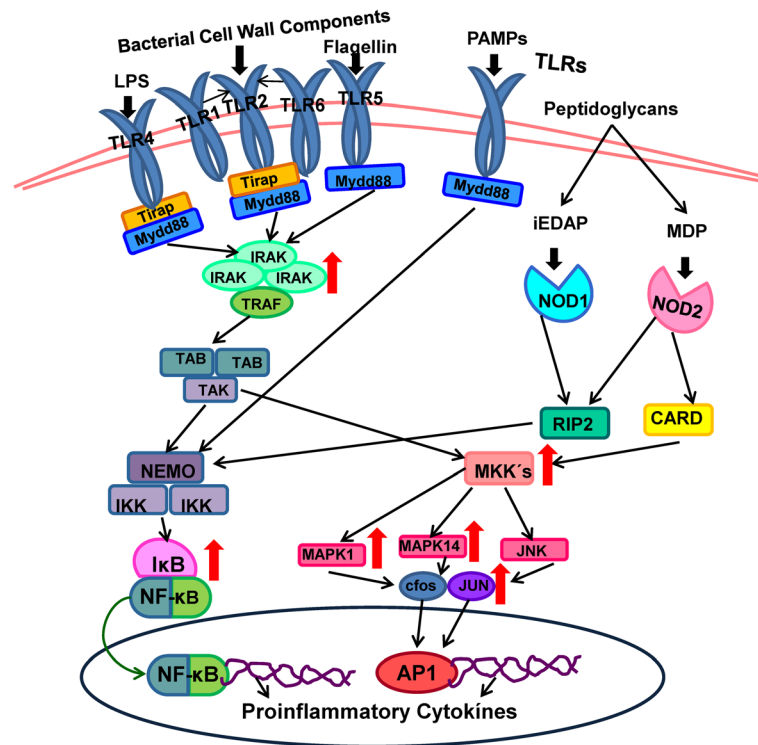
Fig. 4 Pathogen associated pattern recognition receptor activated signaling pathways. Graphics of the nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK or MKK) signaling pathways induced by activation of pathogen associated pattern recognition receptor (PAR) toll like receptors (TLR) and nucleotide-binding oligomerization domain receptors (NOD). TLRs and NODs belong to the key initiators of inflammation in host defence. Dffferent TLRs recognize differential microbial components. TLR4 detects lipopolysaccharide (LPS), TLR1/2 and TLR2/6 recognize triacylated and diacylated lipoproteins from bacterial wall components and TLR5 is activated by flagellin from the flagella of multiple bacteria. TLRs signal via the adaptor protein MyD88, leading to transforming growth factor-β-activated kinase 1 (TAK1) activation that induces NF-kB and p38/c-Jun N-terminal kinase (JNK) pathways. Recognition of NOD ligands recruit caspase activation and recruitment domain (CARD) interaction with receptor-interacting protein kinase RIP2 which leads to activation of RIP2. RIP2 mediates activation IκB kinase. The activation of IκB kinase results in the phosphorylation of inhibitor IκB which releases NF-κB and its nuclear translocation. NF-κB and p38/JNK activated activator protein 1 (AP-1) function as transcription factor for the expression of inflammatory cytokines. The red arrows indicate the upregulated genes in oral epithelial cells induced by *P. gingivalis* and its total membrane that were detected in this study

The results of our study demonstrate that *P. gingivalis* and its membrane fraction, induced RNA up-regulation of the NF-κB and p38 MAPK, MEK4-JNK1 signaling pathways. Furthermore, it was shown that a malignant oral epithelial cell line responded in a similar manner as non-transformed oral keratinocytes. These results are interesting since p38 MAPK and MEK4-JNK1 signaling pathways are known to be involved in tumor microenvironment and cancer growth control.

Head and neck squamous cell carcinoma (HNSCC) tissues express high levels of active p38 and the blockade of its signaling pathway caused significant inhibition of head and neck squamous cell carcinoma (HNSCC) proliferation [62]. Stromal fibroblasts of a variety of invasive malignant tumors express collagenase-1 (matrix metalloproteinase (MMP)-1), which was shown to correlate with the activation of c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase and phosphorylation

of c-Jun. It was also demonstrated that JNK2 is required for induction of fibroblast collagenase-3expression [63].

The data of the present study show a possible link between infection with *P. gingivalis* and oral squamous cell carcinomas, considering that periodontal disease has been associated with the risk for oral tumors [64].

Huynh et al. (2016) reported that in human oral epithelial cells interleukin regulation factor (IRF) 6 expression was strongly up-regulated upon challenge with *P. gingivalis*. IRF6 thus is acting downstream of IL-1 receptor (IL-1R)−associated kinase 1 to induce the expression of the IL-1 family cytokine IL-36 gamma responding to *P. gingivalis* [8]. The transcription factor IRF5 is a co-regulator of IFN-β [65] that exhibits a number of functions, including virus-mediated activation of interferon [66]. The results of the profiler array analysis showed up-regulation of IRF5 in SCC-25 cells by membrane fractions, as well as by whole bacteria (also in PHGK). These results

Groeger *et al. BMC Immunology* (2017) 18:1

Page 9 of 11

were confirmed by quantitative real time PCR assays. These results suggest that IRFs presumably support inflammatory processes upon infection with *P. gingivalis*.

Conclusions

In malignant and primary human oral epithelial cells, *P. gingivalis* and its membrane fraction induced up-regulation of a number of genes. These genes are involved in the downstream signaling pathway of the pro-inflammatory active transcription factor NF-κB and some members of the MAPK family. These kinases participate in the downstream signaling pathway for gene induction of pro-inflammatory cytokines and are involved in cancer proliferation and control.

Additional Files

Additional file 1: Table S4. Ct values of up-regulation of genes in *P. gingivalis* membrane and whole bacteria treated SCC-25 cells. Ct values from qRT-PCR of NF-κB, IKBKB, MAP2K4, MAPK8, MAPK 14, IRF5, JUN, IRAK3 and TOLLIP in SCC-25 cells after 24 h stimulation with *P. gingivalis* membrane fraction = TM or *P. gingivalis* whole bacteria = WB, analyzed by ΔΔCt method, shown as absolute fold induction of RNA expression relative to non-stimulated samples, normalized to the house keeping gene GAPDH, n = 9, ‡ = $p < 0.01$. (DOCX 23 kb)

Additional file 2: Table S5. Ct values of up-regulation of genes in *P. gingivalis* membrane and whole bacteria treated PHGK cells. Ct values from qRT-PCR of NF-κB, IKBKB, MAP2K4, MAPK8, MAPK 14, IRF5, JUN, IRAK3 and TOLLIP in PHGK cells after 24 h stimulation with *P. gingivalis* membrane fraction = TM or *P. gingivalis* whole bacteria = WB, analyzed by ΔΔCt method, shown as absolute fold induction of RNA expression relative to non-stimulated samples, normalized to the house keeping gene GAPDH, n = 9, ‡ = $p < 0.01$. (DOCX 19 kb)

Abbreviations
IRAK: Interleukin receptor-associated kinase; AG: Antigen; APCs: Antigen-presenting cells; DCs: Dendritic cells; ERK1/2: Extracellular signal-regulated kinase 1 and 2; *F. nucleatum*: Fusobacterium nucleatum; GECs: Gingival epithelial cells; HNSCC: Head and neck squamous cell carcinoma; HOKs: Human oral keratinocytes; IFN-γ: Interferon gamma; IG: Immunoglobulin; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; IL: Interleukin; ILT-3: Immunoglobulin-like transcript 3; IRF5/6: Interferon regulatory factor 5/6; JNK: c-Jun N-terminal kinase; LBP: LPS binding protein; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MoDCs: Monocyte derived dendritic cells; NF-κB: Nuclear factor-kappaB; NOD: Nucleotide-binding oligomerization domain; *P. gingivalis*: Porphyromonas gingivalis; PAMPs: Pathogen-associated molecular patterns; PARs: Protease-activated receptors; PD-1: Programmed death-1; PD-L1: Programmed death receptor ligend 1; PHGK: Primary human gingival keratinocytes; PRRs: Pattern recognition receptors; QRT-PCR: Quantitative real time PCR; SCC-25 cells: Squamous cell carcinoma-25 cells; TCR: T-cell receptor; TLR: Toll-like receptor; TOLLIP: Toll-interacting protein; T_{reg}: Regulatory T cells; VCAM 1: Vascular cell adhesion protein 1

Author details
[1]Department of Periodontology, Justus-Liebig-University of Giessen, Giessen, Germany. [2]Institute for Medical Microbiology - German Center for Infection Research, DZIF Partner Site Giessen-Marburg-Langen - Justus-Liebig-University of Giessen, Giessen, Germany.

References
1. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10(10):717–25.
2. Darveau RP, Cunningham MD, Bailey T, Seachord C, Ratcliffe K, Bainbridge B, Dietsch M, Page RC, Aruffo A. Ability of bacteria associated with chronic inflammatory disease to stimulate E-selectin expression and promote neutrophil adhesion. Infect Immun. 1995;63(4):1311–7.
3. Deshpande RG, Khan M, Genco CA. Invasion strategies of the oral pathogen porphyromonas gingivalis: implications for cardiovascular disease. Invasion Metastasis. 1998;18(2):57–69.
4. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135–87.
5. Dobrovolskaia MA, Medvedev AE, Thomas KE, Cuesta N, Toshchakov V, Ren T, Cody MJ, Michalek SM, Rice NR, Vogel SN. Induction of in vitro reprogramming by Toll-like receptor (TLR)2 and TLR4 agonists in murine macrophages: effects of TLR "homotolerance" versus "heterotolerance" on NF-kappa B signaling pathway components. J Immunol. 2003;170(1):508–19.
6. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol. 1995;13:437–57.
7. Hajishengallis G, Sojar H, Genco RJ, DeNardin E. Intracellular signaling and cytokine induction upon interactions of Porphyromonas gingivalis fimbriae with pattern-recognition receptors. Immunol Invest. 2004;33(2):157–72.
8. Huynh J, Scholz GM, Aw J, Kwa MQ, Achuthan A, Hamilton JA, Reynolds EC. IRF6 Regulates the Expression of IL-36 gamma by Human Oral Epithelial Cells in Response to Porphyromonas gingivalis. J Immunol. 2016;196(5):2230–8.
9. Sandros J, Karlsson C, Lappin DF, Madianos PN, Kinane DF, Papapanou PN. Cytokine responses of oral epithelial cells to Porphyromonas gingivalis infection. J Dent Res. 2000;79(10):1808–14.
10. Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol. 2001;1(2):135–45.
11. Medzhitov R, Janeway Jr C. Innate immune recognition: mechanisms and pathways. Immunol Rev. 2000;173:89–97.
12. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84.

Groeger *et al. BMC Immunology* (2017) 18:1

Page 10 of 11

13. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785–97.

14. Bourhis LL, Werts C. Role of Nods in bacterial infection. Microbes Infect. 2007;9(5):629–36.

15. Medzhitov R, Janeway Jr C. The Toll receptor family and microbial recognition. Trends Microbiol. 2000;8(10):452–6.

16. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, Devera ME, Liang X, Tor M, Billiar T. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev. 2007;220:60–81.

17. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol. 2009;27:229–65.

18. Martinon F, Gaide O, Petrilli V, Mayor A, Tschopp J. NALP inflammasomes: a central role in innate immunity. Semin Immunopathol. 2007;29(3):213–29.

19. Said-Sadier N, Ojcius DM. Alarmins, inflammasomes and immunity. Biomed J. 2012;35(6):437–49.

20. Yilmaz O, Sater AA, Yao L, Koutouzis T, Pettengill M, Ojcius DM. ATP-dependent activation of an inflammasome in primary gingival epithelial cells infected by *Porphyromonas gingivalis*. Cell Microbiol. 2010;12(2):188–98.

21. Johnson L, Atanasova KR, Bui PQ, Lee J, Hung SC, Yilmaz O, Ojcius DM. *Porphyromonas gingivalis* attenuates ATP-mediated inflammasome activation and HMGB1 release through expression of a nucleoside-diphosphate kinase. Microbes Infect. 2015;17(5):369–77.

22. Handfield M, Mans JJ, Zheng G, Lopez MC, Mao S, Progulske-Fox A, Narasimhan G, Baker HV, Lamont RJ. Distinct transcriptional profiles characterize oral epithelium-microbiota interactions. Cell Microbiol. 2005;7(6):811–23.

23. Hasegawa Y, Mans JJ, Mao S, Lopez MC, Baker HV, Handfield M, Lamont RJ. Gingival epithelial cell transcriptional responses to commensal and opportunistic oral microbial species. Infect Immun. 2007;75(5):2540–7.

24. Kinane DF, Galicia JC, Gorr SU, Stathopoulou PG, Benakanakere M. P. gingivalis interactions with epithelial cells. Front Biosci. 2008;13:966–84.

25. Lee JY, Suh JS, Kim JM, Kim JH, Park HJ, Park YJ, Chung CP. Identification of a cell-penetrating peptide domain from human beta-defensin 3 and characterization of its anti-inflammatory activity. Int J Nanomedicine. 2015;10:5423–34.

26. Cohen N, Morisset J, Emilie D. Induction of tolerance by *Porphyromonas gingivalis* on APCS: a mechanism implicated in periodontal infection. J Dent Res. 2004;83(5):429–33.

27. Groeger S, Domann E, Gonzales JR, Chakraborty T, Meyle J. B7-H1 and B7-DC receptors of oral squamous carcinoma cells are upregulated by *Porphyromonas gingivalis*. Immunobiology. 2011;216(12):1302–10.

28. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5(12):1365–9.

29. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192(7):1027–34.

30. Chen Y, Zhang J, Guo G, Ruan Z, Jiang M, Wu S, Guo S, Fei L, Tang Y, Yang C, et al. Induced B7-H1 expression on human renal tubular epithelial cells by the sublytic terminal complement complex C5b-9. Mol Immunol. 2009;46(3):375–83.

31. LaGier AJ, Pober JS. Immune accessory functions of human endothelial cells are modulated by overexpression of B7-H1 (PDL1). Hum Immunol. 2006;67(8):568–78.

32. Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, Shin T, Tsuchiya H, Pardoll DM, Okumura K, et al. Expression of programmed death 1 ligands by murine T cells and APC. J Immunol. 2002;169(10):5538–45.

33. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. Embo J. 1992;11(11):3887–95.

34. Wang S, Chen L. T lymphocyte co-signaling pathways of the B7-CD28 family. Cell Mol Immunol. 2004;1(1):37–42.

35. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8(8):793–800.

36. Subudhi SK, Zhou P, Yerian LM, Chin RK, Lo JC, Anders RA, Sun Y, Chen L, Wang Y, Alegre ML, et al. Local expression of B7-H1 promotes organ-specific autoimmunity and transplant rejection. J Clin Invest. 2004;113(5):694–700.

37. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol. 1996;8(5):765–72.

38. Kronenberg M, Rudensky A. Regulation of immunity by self-reactive T cells. Nature. 2005;435(7042):598–604.

39. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–87.

40. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008;9(3):239–44.

41. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4 + CD25high regulatory cells in human peripheral blood. J Immunol. 2001;167(3):1245–53.

42. Kitazawa Y, Fujino M, Wang Q, Kimura H, Azuma M, Kubo M, Abe R, Li XK. Involvement of the programmed death-1/programmed death-1 ligand pathway in CD4 + CD25+ regulatory T-cell activity to suppress alloimmune responses. Transplantation. 2007;83(6):774–82.

43. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206(13):3015–29.

44. Qian Y, Deng J, Geng L, Xie H, Jiang G, Zhou L, Wang Y, Yin S, Feng X, Liu J, et al. TLR4 signaling induces B7-H1 expression through MAPK pathways in bladder cancer cells. Cancer Invest. 2008;26(8):816–21.

45. Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T, Berge S, Novak N. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. J Allergy Clin Immunol. 2008;121(2):368–74. e1.

46. Liu J, Hamrouni A, Wolowiec D, Coiteux V, Kuliczkowski K, Hetuin D, Saudemont A, Quesnel B. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. Blood. 2007;110(1):296–304.

47. Van Dyke TE. The management of inflammation in periodontal disease. J Periodontol. 2008;79(8 Suppl):1601–8.

48. Milward MR, Chapple IL, Wright HJ, Millard JL, Matthews JB, Cooper PR. Differential activation of NF-kappaB and gene expression in oral epithelial cells by periodontal pathogens. Clin Exp Immunol. 2007;148(2):307–24.

49. Grenier D, Tanabe S. *Porphyromonas gingivalis* gingipains trigger a proinflammatory response in human monocyte-derived macrophages through the p38alpha mitogen-activated protein kinase signal transduction pathway. Toxins (Basel). 2010;2(3):341–52.

50. Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. J Immunol. 2000;164(2):966–72.

51. Qin H, Wilson CA, Lee SJ, Zhao X, Benveniste EN. LPS induces CD40 gene expression through the activation of NF-kappaB and STAT-1alpha in macrophages and microglia. Blood. 2005;106(9):3114–22.

52. Darmoul D, Gratio V, Devaud H, Laburthe M. Protease-activated receptor 2 in colon cancer: trypsin-induced MAPK phosphorylation and cell proliferation are mediated by epidermal growth factor receptor transactivation. J Biol Chem. 2004;279(20):20927–34.

53. Fyfe M, Bergstrom M, Aspengren S, Peterson A. PAR-2 activation in intestinal epithelial cells potentiates interleukin-1beta-induced chemokine secretion via MAP kinase signaling pathways. Cytokine. 2005;31(5):358–67.

54. Toubi E, Shoenfeld Y. Toll-like receptors and their role in the development of autoimmune diseases. Autoimmunity. 2004;37(3):183–8.

55. Yu Y, Zeng H, Lyons S, Carlson A, Merlin D, Neish AS, Gewirtz AT. TLR5-mediated activation of p38 MAPK regulates epithelial IL-8 expression via posttranscriptional mechanism. Am J Physiol Gastrointest Liver Physiol. 2003;285(2):G282–90.

56. Hennessy EJ, Parker AE, O'Neill LA. Targeting Toll-like receptors: emerging therapeutics? Nat Rev Drug Discov. 2010;9(4):293–307.

57. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev. 2004;68(2):320–44.

58. Ding PH, Wang CY, Darveau RP, Jin LJ. Nuclear factor-kappaB and p38 mitogen-activated protein kinase signaling pathways are critically involved in *Porphyromonas gingivalis* lipopolysaccharide induction of lipopolysaccharide-binding protein expression in human oral keratinocytes. Mol Oral Microbiol. 2013;28(2):129–41.

59. Dillon S, Agrawal A, Van Dyke T, Landreth G, McCauley L, Koh A, Maliszewski C, Akira S, Pulendran B. A Toll-like receptor 2 ligand stimulates Th2 responses *in vivo*, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. J Immunol. 2004;172(8):4733–43.

60. Flood DG, Finn JP, Walton KM, Dionne CA, Contreras PC, Miller MS, Bhat RV. Immunolocalization of the mitogen-activated protein kinases p42MAPK and

Groeger *et al. BMC Immunology* (2017) 18:1

Page 11 of 11

JNK1, and their regulatory kinases MEK1 and MEK4, in adult rat central nervous system. J Comp Neurol. 1998;398(3):373–92.

61. Chuang CY, Chen TG, Tai YT, Chen TL, Lin YH, Tsai CH, Chen RM. Toll-like receptor 2-mediated sequential activation of MyD88 and MAPKs contributes to lipopolysaccharide-induced sp-a gene expression in human alveolar epithelial cells. Immunobiology. 2011;216(6):707–14.

62. Leelahavanichkul K, Amornphimoltham P, Molinolo AA, Basile JR, Koontongkaew S, Gutkind JS. A role for p38 MAPK in head and neck cancer cell growth and tumor-induced angiogenesis and lymphangiogenesis. Mol Oncol. 2014;8(1):105–18.

63. Westermarck J, Li S, Jaakkola P, Kallunki T, Grenman R, Kahari VM. Activation of fibroblast collagenase-1 expression by tumor cells of squamous cell carcinomas is mediated by p38 mitogen-activated protein kinase and c-Jun NH2-terminal kinase-2. Cancer Res. 2000;60(24):7156–62.

64. Tezal M, Grossi SG, Genco RJ. Is periodontitis associated with oral neoplasms? J Periodontol. 2005;76(3):406–10.

65. Steinhagen F, McFarland AP, Rodriguez LG, Tewary P, Jarret A, Savan R, Klinman DM. IRF-5 and NF-kappaB p50 co-regulate IFN-beta and IL-6 expression in TLR9-stimulated human plasmacytoid dendritic cells. Eur J Immunol. 2013;43(7):1896–906.

66. Barnes BJ, Moore PA, Pitha PM. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon alpha genes. J Biol Chem. 2001;276(26):23382–90.