Porphyromonas gingivalis Gingipains Selectively Reduce CD14 Expression, Leading to Macrophage Hyporesponsiveness to Bacterial Infection

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Key Words

CD14 · Gingipains · Macrophages · *Porphyromonas gingivalis* · Toll-like receptors · TNFα

Abstract

Cysteine proteases (gingipains) from *Porphyromonas gingivalis* are key virulence factors in chronic periodontitis. Innate immune receptors CD14, Toll-like receptor (TLR) 2 and TLR4 are important in *P. gingivalis* recognition. We examined the ability of gingipains to cleave CD14, TLR2 and TLR4, and the consequences for the cellular response to bacterial challenge. Macrophages were exposed to Arg (RgpA and RgpB)- and Lys (Kgp)-gingipains, and residual expression of TLR2, TLR4 and CD14 was determined by flow cytometry. The cellular response to live bacteria following exposure to purified gingipains was evaluated by TNFα production and bacterial phagocytosis. RgpA and Kgp decreased CD14 detection in a concentration ($p = 0.0000002$)- and time ($p = 0.03$)-dependent manner, whereas RgpB had no significant effect. TLR2 and TLR4 expression were unaffected. Reduction in CD14 expression was more efficient with Lys-gingipain than with Arg-gingipain. A reduced CD14 surface level correlated with decreased TNFα secretion and bacterial phagocytosis following challenge with live *P. gingivalis*, but the response to heat-killed bacteria was unaffected. Therefore, gingipains

reduce CD14 expression without affecting expression of the bacterial-sensing TLRs. Reduced CD14 expression depends on the gingipain hemagglutinin/adhesion site and results in macrophage hyporesponsiveness to bacterial challenge. Further studies are needed to determine if reduced CD14 expression is linked to periodontitis induced by *P. gingivalis*.

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Introduction

Porphyromonas gingivalis is one of the major pathogens associated with periodontal disease [1]. Several virulence factors have been identified that enable *P. gingivalis* to induce chronic inflammation and alveolar bone loss in animal models of periodontal disease. These include fimbriae, lipopolysaccharides (LPSs), toxic metabolic products and proteases [2]. Two types of cysteine proteases, referred to as gingipains, account for 85% of the proteolytic activity of *P. gingivalis*. Rgp proteases are specific for arginine-Xaa bonds and Kgp protease is specific for lysine-Xaa bonds [3]. Two distinct but related genes encode

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for Rgp proteases, *rgpA* and *rgpB*. The proteins RgpA (95 kDa) and RgpB (50 kDa) share a nearly identical catalytic domain (93% sequence identity) but differ in that RgpB does not contain a hemagglutinin/adhesion domain. Both RgpA and Kgp are noncovalent complexes containing separate catalytic and hemagglutinin/adhesin domains. The catalytic subunits of RgpA and Kgp share 23% identity whereas their hemagglutinin/adhesion domains are over 97% identical [4]. Gingipains cleave a wide range of proteins and thereby provide the bacteria with essential nutrients. In addition, gingipains interfere with the host recognition and response to infection by cleaving extracellular matrix proteins, antibacterial peptides, cytokines such as TNF α [5], cell surface receptors and immunoglobulins [6].

Toll-like receptors (TLRs) represent a conserved family of receptors involved in the detection of pathogen-associated molecular patterns (PAMPs) and the cellular response to bacterial invasion [7]. TLRs recognize bacterial PAMPs such as LPS recognized by TLR4 [8], lipopeptides recognized by TLR2 [9] and unmethylated CpG oligonucleotides recognized by TLR9 [10]. CD14 is a glycosphosphatidyl inositol-anchored membrane protein that functions as a coreceptor for the recognition of bacterial molecules by several TLRs [11, 12]. Multiple bacterial pathogens evade innate immune responses by interfering with robust TLR-mediated recognition that would normally lead to rapid bacterial clearance [13, 14]. Several groups have found that TLR2 is required for a full cytokine response to infection with *P. gingivalis* [15, 16] raising the question of whether *P. gingivalis* evades clearance by reducing recognition through TLR2. Previously, gingipains were shown to reduce CD14 surface expression in human monocytes while not affecting TLR4 expression [17]. The aim of the present study was to examine the ability of Arg (RgpA, RgpB) and Lys (Kgp) gingipains to reduce the expression of the murine macrophage receptors TLR2, CD14 and TLR4, and to determine the effect of changes in receptor expression levels on the macrophage response to infection with live *P. gingivalis*.

Materials and Methods

Animals

Six- to 12-week-old female BALB/c mice (Harlan, Jerusalem, Israel) were used. The study was carried out in the specific pathogen-free unit of the animal facility. The mice were maintained on a 12-hour light/dark cycle and received distilled water and food ad libitum. The experimental protocols were approved by the Internal Review Board of the Hadassah-Hebrew University Medical Center.

Materials

RAW 246.7 (ATCC) and *cd14*^{-/-} murine macrophage (BEI Resources) cell lines were maintained in cell culture plates with DMEM supplemented with fetal calf serum (FCS; 10%), 4 mM L-glutamine, 1 mM sodium pyruvate and penicillin-streptomycin (1%). Cells were used between passages 4–10. FITC (Sigma-Aldrich) was prepared in NaHCO₃ buffer (pH = 9) at 0.1 mg/ml. Purified anti-mouse CD16/32, FITC-conjugated anti-mouse CD14 (Sa2-8), PE-conjugated anti-mouse TLR4 (MTS510), PE-conjugated anti-mouse TLR2 (6C2) and isotype-matched controls were purchased from eBioscience (San Diego, Calif., USA) and used according to the manufacturer's instructions. A mouse TNF α ELISA MAXTM standard kit was purchased from Biolegend (San Diego, Calif., USA).

Mouse Peritoneal Macrophage Isolation

Each mouse was injected intraperitoneally with 2 ml 4% thio-glycollate (DIFCO) [18]. Four days later, mice were sacrificed and the peritoneal cavity was washed five times with 3 ml PBS plus heparin (5 units/ml). Peritoneal exudate cells (PECs) were incubated in DMEM, 10% FCS, 4 mM L-glutamine, 1 mM sodium pyruvate and penicillin-streptomycin (1%). The following day, cells were harvested, washed with PBS and suspended in 0.2 M HEPES buffer containing 10 mM cysteine and 5 mM CaCl₂ at 37°C for 10 min (in order to activate gingipains).

Bacteria

P. gingivalis strain 381 (ATCC), W83 (wild type, WT), *rgpA*⁻ and *kgp*⁻ deficient strains were used in this study (W83, *rgpA*⁻ and *kgp*⁻ strains were kindly provided by C.A. Genco, Boston University, Boston, Mass., USA). Bacteria were grown in Wilkins media (Oxoid, Basingstoke, UK) under anaerobic conditions (85% N₂, 5% H₂ and 10% CO₂) at 37°C. After 2–3 days, the bacteria were harvested and supernatants from each strain were collected and stored at –80°C for further use. Protease activity of bacterial supernatants was tested as previously described [19, 20]. To test phagocytosis, *P. gingivalis* 381 was labeled with 0.1 mg/ml FITC (Sigma-Aldrich) in NaHCO₃ buffer (pH 9) for 20 min at room temperature, as previously described [16].

Arg- and Lys-Gingipain Purification

RgpB was purified from strain H66 culture fluid as previously described [21] using a combination of gel filtration and ion-exchange chromatography. Kgp and RgpA proteinase-adhesin complexes were isolated by the sequential use of gel filtration, arginine-Sepharose chromatography and anion exchange chromatography on Mono Q [22]. Purified protease activity was tested as previously described [19, 20].

Macrophage Receptor Expression following Exposure to Gingipains

Macrophages (PECs or RAW 264.7 cells) were incubated individually with culture supernatants from W83 WT, *rgpA*⁻ or *kgp*⁻ *P. gingivalis*, or with purified RgpA, RgpB or Kgp at the concentrations and time periods indicated in the figures. After incubation, the cells were washed with 0.5 ml FACS buffer (98% PBS + 2% FCS), and receptor expression was determined by FACS. Macrophage Fc receptors were blocked with purified anti-mouse CD16/32 (4°C, 15 min) and cells were loaded with FITC/PE-conjugated monoclonal antibodies or isotype-matched rat IgG con-

trols (4°C, 45 min). Flow-cytometric analyses were performed using a BD LSRII (Becton Dickinson, Mountain View, Calif., USA). The mean fluorescence intensity (MFI) of viable cells was analyzed by gating on the basis of forward and side scatter characteristics. MFI results are shown as the mean $\pm$ SEM for each group.

Macrophage Stimulation

In some experiments, macrophages were treated with 1 μ M of purified Kgp for 30 min. Cells were washed three times in PBS containing 2% FCS in order to inactivate the exogenously added gingipain prior to challenging them with live or heat-killed and sonicated (HKS) *P. gingivalis* at increasing multiplicity of infection (MOI), or with the purified TLR ligands *Escherichia coli* LPS (Sigma Aldrich, Rehovot, Israel), *P. gingivalis* LPS or Pam₃CSK₄ (Invivogen, San Diego, Calif., USA). Supernatants were collected and TNFa levels were determined by ELISA according to the manufacturer's instructions (mouse TNFa ELISA MAXTM standard; Biolegend). In addition, cells were stained with FITC-conjugated anti-mouse CD14 monoclonal antibody (or isotype control) in order to evaluate the level of detectable CD14 prior to and at the end of the 3-hour incubation with *P. gingivalis*. Cell viability at the end of the 3-hour incubation was determined by propidium iodide staining.

P. gingivalis Phagocytosis Assay

Macrophages were pre-incubated with either purified RgpA or Kgp (1 μ M) for 30 min. Cells were washed and incubated with FITC-labeled *P. gingivalis* (MOI 10) for 15–120 min. Extracellular labeling was quenched using trypan blue (1.25 mg/ml) for 1 min before determining fluorescence (Tecan fluorescence plate reader; Tecan, Männedorf, Switzerland).

Statistical Analysis

Experiments in this study were conducted at least twice. Experimental values are given as means $\pm$ SEM of macrophage MFI for TLR2/TLR4/CD14 staining. In order to compare different exposure times and concentrations, we used the Kruskal-Wallis test. This was done for each innate immune receptor and each protease separately. Multiple pairing analyses were done with the Mann-Whitney and Bonferroni post hoc test for significance. To simultaneously estimate the effects of time and protease concentration, three-way ANOVA was used. Multiple paired comparisons were done according to Scheffé. This analysis was done for each innate immune receptor separately. TNFa secretion levels by stimulated macrophages were compared using Student's *t* test. All statistical analyses were two tailed and a *p* value of 0.05 or less was considered statistically significant.

Results

P. gingivalis Culture Supernatant Reduces CD14 Surface Expression, but Not That of TLR2 or TLR4

Detectable macrophage CD14 surface expression was reduced following exposure to culture supernatants of W83, *rgpA*[−] and *kgp*[−] *P. gingivalis* at all concentrations tested (*p* < 0.01; fig. 1a). However, although W83 supernatant was equally active at all concentrations tested, both *rgpA*[−] and *kgp*[−] supernatants were less active at the lowest

concentrations tested, suggesting that both gingipains are required for optimal activity. Nevertheless, CD14 levels were least affected by the *kgp*[−] strain, suggesting that Kgp is responsible for more of the activity than RgpA (fig. 1a, d). Following treatment with the W83, *rgpA*[−] and *kgp*[−] supernatants, the surface expression of TLR4 (fig. 1b) and TLR2 (fig. 1c) remained unchanged at even the highest concentration. Taken together, these results suggest that both Arg- and Lys-gingipains are able to specifically cleave CD14, but neither gingipain nor other *P. gingivalis* proteases affect macrophage TLR4 or TLR2 expression.

TLR2, TLR4 and CD14 Surface Expression following Macrophage Exposure to Purified Gingipains

We next tested the dose- and time-dependent effect of purified gingipains on the expression of CD14, TLR4 and TLR2 on murine macrophages. Overall, purified gingipains RgpA and Kgp reduce CD14 expression in a time-, concentration- and protease-type-dependent manner (*p* < 0.05). At the shortest time period tested (5 min exposure followed by washing), and the two lowest concentrations of gingipain (0.1 and 0.3 μ M), only Lys-gingipain (Kgp) significantly reduced CD14 expression (fig. 2a), further suggesting that Kgp more efficiently reduces CD14 expression compared to Rgp. At higher concentrations of gingipain or longer exposure times, Arg-gingipain (RgpA) also significantly reduced CD14 surface expression (fig. 2a–c). Finally, the Rgp adhesin domain is required for efficient reduction in surface CD14, as RgpB, which lacks the adhesin domain, was inactive except for a modest effect at the longest exposure time (1 h) and highest concentration (1 μ M; fig. 2c). Similar to the culture supernatants, purified gingipains did not reduce surface-detectable expression of TLR2 or TLR4 (fig. 2d, e). For figures 1 and 2, comparable results were obtained using primary PECs and the murine macrophage cell line RAW 264.7.

Kgp-Mediated Reduction of CD14 Expression Correlates with a Diminished Cytokine Response to Infection with Live *P. gingivalis*

We next determined whether diminished surface expression of CD14, in the presence of unchanged levels of TLR2 and TLR4, would impact on the cytokine response to *P. gingivalis*. Macrophages were exposed to Kgp (1 μ M for 30 min), washed three times in medium containing serum and then stimulated for 3 h with either live or HKS *P. gingivalis*, *P. gingivalis* LPS, *E. coli* LPS or the synthetic lipopeptide Pam₃CSK₄. Three hours following exposure to Kgp and washing, the CD14 level remained reduced by 60% compared to control cells not exposed to protease

Fig. 1. *P. gingivalis* culture supernatant cleaves CD14 but not TLR2 or TLR4. CD14 (a), TLR4 (b) and TLR2 (c) surface expression detected following murine macrophage (PEC) exposure to W83 (WT), *rgpA*⁻ and *kgp*⁻ *P. gingivalis* culture supernatants. Culture supernatants were collected from bacteria at 6×10^6 CFU/ml. PECs were incubated with supernatants at increasing concentrations (10, 20 and 50%) or with Wilkins medium as a control. Following

incubation for 1 h, supernatants were discarded and macrophage CD14, TLR4 and TLR2 surface expression was detected using fluorescent antibodies. MFI was evaluated by flow cytometry. The values represent means $\pm$ SEM. ** $p < 0.01$ vs. untreated. d Representative histogram of macrophage CD14 surface expression following exposure to 10% culture supernatant of WT, *rgpA*⁻ or *kgp*⁻ *P. gingivalis*.

(fig. 3a). In addition, macrophage viability was not affected by exposure to Kgp or the further 3-hour activation (data not shown). As shown in figure 3b, Kgp pretreatment led to a significant decrease in TNF α secretion by cells responding to infection with live *P. gingivalis* at MOIs of 10 and 50 ($p < 0.05$), although the diminished response was not observed at MOI 100. The response to *P. gingivalis* LPS and *E. coli* LPS was also diminished by Kgp pretreatment ($p < 0.05$). However, the response to HKS *P. gingivalis*, where additional PAMPs are presumably revealed, and the response to TLR2/1 activation by Pam₃CSK₄, was unaffected by Kgp pretreatment (fig. 3b).

Similar results were obtained under serum-free conditions, suggesting that soluble bovine CD14 in the medium cannot compensate for the reduced membrane CD14 expression (data not shown). These results suggest that live *P. gingivalis* may dampen early cellular responses to infection by reducing surface CD14 levels.

TNF α Secretion by WT or CD14^{-/-} Macrophages following Stimulation with Live P. gingivalis

To confirm that CD14 is specifically required for a full cytokine response to live *P. gingivalis* challenge, we compared TNF α production by WT versus *cd14*^{-/-} macro-

Fig. 2. Purified Kgp and RgpA selectively cleave CD14. Macrophage CD14 (a–c), TLR4 (d) and TLR2 (e) surface expression following PEC exposure to purified gingipains. Macrophages were treated with RgpA, RgpB and Kgp (0.1, 0.3 and 1 μM for 5, 30 and 60 min). CD14, TLR4 and TLR2 were detected using fluorescent antibodies following protease removal and MFI was evaluated by

flow cytometry. Results represent means of three independent experiments. The MFI of live-treated cells was compared to the MFI of viable untreated cells, which was set as 100%. Cell viability was on average 65% and did not differ between protease-treated and untreated cells. * $p < 0.05$ vs. untreated cells.

Fig. 3. Macrophages exposed to Kgp are hyporesponsive to live *P. gingivalis* (*P. g.*). **a** CD14 surface expression (RAW 264.7 cells) following exposure to Kgp for 30 min and further 3 h of incubation with no stimulation. CD14 expression by cells not exposed to Kgp is defined as 100%. **b** TNFα secretion by macrophages pretreated with Kgp and then activated. RAW 264.7 cells were treated with 1 μM of Kgp for 30 min. Cells were washed extensively and then challenged with live *P. gingivalis* or HKS *P. gingivalis*, (at MOI = 10, 50 and 100), *P. gingivalis* LPS (1 μg/ml), *E. coli* (*E. c.*) LPS (10 ng/ml) or Pam₃CSK₄ (PAM, 10 μg/ml) for 3 h. Values represent

means ± SEM. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, vs. untreated cells. **c** TNFα secreted by WT (RAW 264.7 cells) or *cd14*^{-/-} macrophages (M) following challenge. Macrophages were exposed to live *P. gingivalis*, HKS *P. gingivalis*, *E. coli* LPS (10 ng/ml) or Pam₃CSK₄ (10 μg/ml) concentrations for 3 h. TNFα secretion was determined by ELISA. Values represent means ± SEM. * $p < 0.05$, ** $p < 0.001$, WT vs. *cd14*^{-/-} macrophages. **d** Representative histogram of macrophage CD14 surface expression following exposure to 1 μM gingipain.

phages (fig. 3c). Consistent with the activity of Kgp in reducing surface CD14, macrophages that do not express CD14 are hyporesponsive to challenge with live *P. gingivalis* ($p < 0.001$). In contrast to Kgp pretreatment, even at MOI 100 *cd14*^{-/-} cells secrete significantly less TNFα than WT cells ($p < 0.05$), presumably since there is a com-

plete absence of CD14 in this system. Importantly, the response to *E. coli* LPS was reduced in *cd14*^{-/-} macrophages compared to WT, and the responses to HKS *P. gingivalis* and Pam₃CSK₄ were similar between *cd14*^{-/-} and WT cells, consistent with the impact or lack of impact of Kgp on the response to activation with these factors.

Fig. 4. Phagocytosis of *P. gingivalis* by macrophages is reduced following pretreatment with purified gingipains. Kgp- or RgpA-treated macrophages (M) were incubated with FITC-labeled *P. gingivalis* for 15–120 min. Extracellular fluorescence was quenched with trypan blue (1.25 mg/ml) prior to fluorescence measurement. Values represent the means of relative fluorescence units (RFU) $\pm$ SD of one representative experiment out of three repeats with similar results. Purified Kgp and RgpA significantly reduced phagocytosis at 15 and 30 min (***) $p < 0.001$ and at 60 min (** $p < 0.01$ for Kgp; * $p < 0.05$ for Rgp). The level of phagocytosis by *cd14*^{-/-} cells was similar to RgpA-pretreated WT cells at all time points.

These results suggest that Kgp mediates reduced CD14 expression that correlates with a reduced macrophage response to *P. gingivalis* infection.

Macrophage Pretreatment with Gingipains Reduces Phagocytosis of *P. gingivalis*

We next asked whether diminished CD14, in the presence of unchanged levels of TLR2 and TLR4, impacts upon phagocytosis of *P. gingivalis*. Untreated cells phagocytosed *P. gingivalis* in a time-dependent manner, reaching peak activity at 30 min (fig. 4). Pretreatment with purified Kgp and RgpA significantly reduced phagocytosis at 15 and 30 min ($p < 0.001$) and at 60 min ($p < 0.01$ for Kgp; $p < 0.05$ for Rgp; fig. 4). Phagocytosis of *P. gingivalis* by *cd14*^{-/-} macrophages was similar to that of WT cells pretreated with RgpA, suggesting that Kgp affects additional receptors involved in phagocytosis.

Discussion

Our results show that the purified gingipains RgpA and Kgp, as well as supernatants from WT *P. gingivalis* W83, and the single mutants *rgpA*⁻ and *kgp*⁻, cleave sur-

face-expressed CD14 in a concentration- and a time-dependent manner. Reduced CD14 expression was dependent on the presence of a hemagglutinin/adhesion domain since the Arg-gingipain RgpB, which contains a nearly identical catalytic domain to RgpA but lacks the adhesion domain, was not active. The hemagglutinin/adhesion site binds fibrinogen, fibronectin and laminin to interact with cell membrane phospholipids thus increasing the protease affinity to its substrate [23]. Furthermore, the reduced CD14 expression was significantly more sensitive to Lys-gingipain activity in comparison to Arg-gingipain activity. Importantly, Kgp and RgpA reduced CD14 expression even at 0.1 μ M, a concentration that is well within the recently reported gingipain concentrations found in gingival crevicular fluid (GCF; $\leq 1.5 \mu$ M) [24]. Though the GCF levels are only directly relevant to the CD14-positive cells that have migrated through the tissue to the gingival sulcus [25], the levels measured in the GCF provide some indication of what can be expected in vivo. In addition, CD14 in vivo surface expression was reported to be greater in clinically healthy gingival pockets than in periodontitis patients, suggesting that greater CD14 surface expression correlates with periodontal health [26].

Our findings regarding CD14 expression following macrophage exposure to gingipains are consistent with results from previous studies on human monocytes and macrophages [17, 27]. Both human monocyte CD14 and murine macrophage CD14 are cleaved more efficiently by gingipains containing the hemagglutinin/adhesion domain. However, in contrast to human monocyte CD14, murine macrophage CD14 expression is more rapidly and efficiently reduced by Lys-gingipain than Arg-gingipain. This may be explained by the presence of 4 more arginine residues in human CD14 in comparison to the murine protein [28]. Analysis of solvent accessible lysine and arginine residues in the murine CD14 crystal structure did not provide an explanation for the greater influence of Lys-gingipain relative to Arg-gingipain over CD14 expression since there are 9 solvent-exposed residues of each amino acid (<http://www.rcsb.org/pdb/ex-plore/remediatedSequence.do?structureId=1WWL>).

Of significance, previous reports have shown TLR2, rather than TLR4, to be critical for the host response to infection with *P. gingivalis*. In the absence of TLR2, the in vitro and in vivo cytokine response to *P. gingivalis* is diminished [15, 16]. In addition, TLR2 activation is also central to the evasion strategy of *P. gingivalis* since TLR2-deficient mice are able to rapidly clear infection, as are TLR2-deficient macrophages in vitro [29]. Therefore, it

was critical to determine the effect of gingipains on this pattern recognition receptor since it was not reported previously. In contrast to CD14, gingipains did not reduce detectable surface expression of TLR2 or TLR4, and their expression was consistently unaffected by any of the gingipains in any of the conditions tested. In retrospect, the fact that TLR2 expression remains unchanged is not surprising, given that multiple reports have shown that TLR2, and not TLR4, is critical for the responsiveness of cells to *P. gingivalis* LPS [30] and to the whole bacteria [16].

CD14 is a coreceptor for multiple TLRs [11, 12]; however, its relative importance for the cellular response depends on the particular TLR ligand. We found that reduced CD14 expression mediated by Kgp did not affect the macrophage response to the TLR2/TLR1 ligand Pam₃CSK₄, but the response to infection with live *P. gingivalis* and *E. coli* LPS was significantly reduced. Furthermore, the response of CD14-deficient cells to infection with *P. gingivalis*, but not to challenge with Pam₃CSK₄, was also reduced compared to WT macrophages. Although some studies have found CD14 to physically bind triacylated lipopeptides such as Pam₃CSK₄ and induce physical proximity to the TLR2/TLR1 complex [31], our results are consistent with other reports that CD14 is dispensable for the cellular response to Pam₃CSK₄ [32, 33]. The macrophage response to HK5 *P. gingivalis* did not require CD14, presumably because multiple TLR and non-TLR ligands are exposed by heat and sonication. Importantly, pre-exposure to both Arg- or Lys-gingipains resulted in reduced phagocytosis of *P. gingivalis* by macrophages. Phagocytosis ability of *cd14*^{-/-} macrophages resembled that of WT macrophages pretreated with

RgpA, pointing to a possible role of CD14 in bacterial elimination by macrophages. This emphasizes the possibility that the bacteria evade elimination by the host through reducing CD14 expression on macrophages, which in turn may result in reduced cytokine responses and a lower ability to phagocytose bacteria.

Taken together, our findings suggest that gingipains selectively influence the surface expression of innate immune receptors important for the recognition of live *P. gingivalis*, leaving surface immune receptors linked to bacterial persistence intact. The accumulation of gingipains [34] in inflammatory periodontal lesions can reduce innate receptor expression and thereby alter the responsiveness of macrophages ahead of their encounter with the bacteria. Further studies will examine whether *P. gingivalis* signaling via TLR2 in the absence of CD14 leads to improved bacterial persistence and greater pathogenicity.

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