Induction of Distinct TLR2-Mediated Proinflammatory and Proadhesive Signaling Pathways in Response to Porphyromonas gingivalis Fimbriae

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The oral pathogen *Porphyromonas gingivalis*, as well as its purified fimbriae, are known to activate TLR2 and induce proinflammatory and proadhesive effects. The TLR2 proinflammatory pathway induces NF-κB-dependent inflammatory cytokines, whereas the TLR2 proadhesive pathway is characterized by inside-out signaling that transactivates β2 integrin adhesive activities. In this article, using dominant-negative or pharmacological approaches, we show that the two pathways bifurcate and proceed independently downstream of TLR2. Whereas the proinflammatory pathway is dependent on the adaptor molecules Toll/IL-1 receptor domain-containing adaptor protein (also known as Mal) and MyD88, the proadhesive pathway is Toll/IL-1 receptor domain-containing adaptor protein/MyD88-independent and proceeds through PI3K-mediated signaling. Although the Ser/Thr kinase Akt is a major downstream target of PI3K and was activated by *P. gingivalis* fimbriae in a TLR2- and PI3K-dependent way, Akt was shown not to play a role in the proadhesive pathway. In contrast, another PI3K downstream target, cytohesin-1, was shown to mediate *P. gingivalis* fimbriae-induced activation of β2 integrin for ICAM-1 binding. Therefore, *P. gingivalis* fimbriae activate two distinct TLR2 pathways mediating proinflammatory or proadhesive effects. The delineation of these signaling pathways may provide appropriate targets for selectively inhibiting or enhancing specific activities, depending on whether they undermine or promote the host defense. *The Journal of Immunology*, 2009, 182: 6690–6696.

The oral-like receptors play a central role in the innate recognition of microbial pathogens and the induction of the inflammatory response aiming to control pathogen spread (1, 2). TLR2, in association with TLR1 or TLR6, recognizes a variety of pathogens (3) including *Porphyromonas gingivalis*, an oral pathogen associated with periodontal disease and implicated in atherosclerosis (4–7). TLR signaling is triggered upon recruitment of Toll/IL-1 receptor (TIR) domain-containing adaptor molecules to the cytoplasmic domains of activated TLRs (8). Initiation of TLR2 signaling, for example, involves recruitment of the TIR domain-containing adaptor protein (TIRAP; also known as Mal) and MyD88 to the TLR2 cytoplasmic domain, and downstream signaling events activate NF-κB leading to induction of proinflammatory and host-defense genes (8).

Evidence from animal models of experimental periodontitis or atherosclerosis suggests that the fimA-encoded fimbriae of *P. gingivalis* constitute an important virulence factor of this pathogen (9, 10). Due to their considerable length (up to 3 μm from the bacterial cell surface; Ref. 11), the fimbriae may be the first *P. gingivalis* molecule to interact with host structures. Although traditionally recognized as a colonization factor (11, 12), *P. gingivalis* fimbriae also interact with TLR2 and modulate innate immune responses (7, 13, 14). Therefore, understanding the mode whereby *P. gingivalis* fimbriae interact with different types of host cells (15–18) may lead to a better understanding of host-*P. gingivalis* interactions and their impact on disease pathogenesis.

From our laboratory has described molecular events underlying *P. gingivalis*-induced activation of monocytes/macrophages (6, 14, 19–21), which play an important role in the innate host response in periodontitis and other chronic infections (22–24). The binding of *P. gingivalis* fimbriae to CD14 greatly facilitates TLR2 activation by whole bacterial cells or purified fimbriae, thereby initiating of a core intracellular signaling cascade for NF-κB activation and induction of proinflammatory cytokines (6, 14, 16). In addition, following interaction with the CD14/TLR2 recognition complex, *P. gingivalis* or its purified fimbriae induce PI3K-mediated inside-out signaling for activating the ligand-binding capacity of complement receptor-3 (CR3; CD11b/CD18), (20, 21). In terms of biological significance, *P. gingivalis*-activated TLR2 inside-out signaling leads to enhanced CR3-dependent monocyte adhesion to endothelial ICAM-1 and transendothelial migration (20). Intriguingly, however, *P. gingivalis* has co-opted this TLR2/CR3 proadhesive pathway for CR3 binding and entry into macrophages (19) in a way that promotes the survival and virulence of this pathogen (25, 26).

Although the above discussed *P. gingivalis*-induced proinflammatory and proadhesive pathways mediate distinct biological effects, it is not currently known whether the proadhesive signaling pathway downstream of TLR2 proceeds independently of the proinflammatory pathway, i.e., by using different adaptors and/or signaling molecules. This issue was addressed in this study. The
objective to characterize and delineate TLR2 downstream signaling pathways may provide appropriate targets for selectively inhibiting those signaling cascades (e.g., the TLR2 proadhesive pathway) that are exploited by *P. gingivalis* to enhance its survival within the host. Thus, because the innate antimicrobial response to *P. gingivalis* appears to depend on the TLR2/NF-κB pathway (18), it would be inappropriate to design and apply intervention strategies that inhibit TLR2 signaling altogether. The data presented in this article show that TLR2 signaling can indeed bifurcate into two distinct pathways: The standard TLR2 proinflammatory pathway that is dependent upon the signaling adaptors TIRAP and MyD88, and a novel TLR2-mediated but TIRAP/MyD88-independent proadhesive pathway, which stimulates CR3 adhesive activity via Rac1, PI3K, and cytohesin-1.

**Materials and Methods**

**Reagents**

mAbs to human CD11b (CBRM1/5) or mouse CD11b (M1/70), and Ig isotype controls were purchased from eBiSciences. PMA, wortmannin, LY294002, and LY30351 were from Sigma-Aldrich. Recombinant human or mouse ICAM-1 was obtained from the R&D Systems. FimA fimbriae were chromatographically purified from *P. gingivalis* strain 381, as previously described (21), or from strain ATCC 33277, as described by Yoshimura et al. (27). The strain 33277 fimbriae were provided by Dr. F. Yoshimura, Aichi-Gakuin University, Nagoya, Japan. The final fimbrial preparations were free of any contaminating substances on silver-stained SDS-PAGE, and tested negative for endotoxin (<0.7 ng/ml protein) according to quantitative Limulus amebocyte lysate assay (BioWhittaker). Fimbriae from either strain (99.7% identity at the FimA amino acid level) were indistinguishable in the functional assays presented in this study. All reagents were used at effective concentrations determined in preliminary experiments or in previous publications (6, 20, 26).

**Cell culture**

Human monocytic THP-1 cells stably transfected with human CD14 (THP-1/CD14) (29) were provided by Dr. P. S. Tobias (The Scripps Research Institute, La Jolla, CA) and were cultured at 37°C and 5% CO2 atmosphere, in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS (Invitrogen Life Technologies), 2 mM l-glutamine, 10 mM HEPES, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.05 mM 2-ME (complete RPMI). THP-1/CD14 cells were transfected with pDeNy-hTLR2tirless, TRIF-related adaptor molecule (TRAM) (pDeNy-hTRAM), TRIF-related adaptor molecule TRAM (pDeNy-hTRAM), or with empty vector controls (Invivogen). A plasmid expressing a dominant-negative signaling mutant of human MyD88 (MyD88-DN) (40) did not affect the ability of the fimbriae to induce the CR3 activation-specific reporter gene assay. The CBRM1/5 epitope induction assay was used to monitor the activation state of human CR3 (CD11b/CD18), as we have previously described (21). The assay is based on the property of the CBRM1/5 mAb to detect a conformational change on human CD11b that signifies the high-affinity binding state of CR3 (37). Activation of mouse CR3 was assessed by monitoring its binding activity for soluble ICAM-1, a ligand that binds activated but not resting CR3 (20, 38). Specifically, biotinylated sICAM-1 was allowed to bind to mouse macrophages for 30 min at 37°C. Subsequently, the cells were washed and incubated on ice with FITC-labeled streptavidin. After washing, binding was determined by measuring cell-associated fluorescence (in relative fluorescence units) on a microplate fluorescence reader (FL600, Bio-Tek) with excitation/emission wavelength settings of 485/530 nm. Background fluorescence was determined in cells treated with medium only and FITC-streptavidin.

**Statistical analysis**

Data were evaluated by ANOVA and the Dunnett multiple-comparison test using the InStat program (GraphPad Software). Where appropriate (comparing two or more groups of data), Student’s t tests were also performed. A p value <0.05 was taken as the level of significance. Experiments were performed using triplicate samples and were performed twice or more for verification.

**Results**

The TLR2 proadhesive pathway is MyD88 independent

Although *P. gingivalis* fimbria-activated TLR2 mediates both proinflammatory and proadhesive effects, it is unknown whether these two types of activities involve common or distinct signaling adaptors. Because MyD88 is a central signaling adaptor for TLR2 proinflammatory signaling (39), we investigated whether treatments that block MyD88 could also inhibit TLR2 inside-out signaling for CR3 activation (monitored by induction of the CBRM1/5 epitope), which signifies the high-affinity binding conformation of CR3 (Ref. 37). We found that transfection of THP-1/CD14 cells with a plasmid expressing a dominant-negative signaling mutant of human MyD88 (MyD88-DN) (40) did not affect the ability of the fimbriae to induce the CR3 activation-specific CBRM1/5 epitope, in contrast to transfection with TLR2-DN, which served as positive control (Fig. 1A). However, in the same cells, MyD88-DN significantly inhibited NF-κB activation by fimbriae, as also observed with TLR2-DN (*p* < 0.05; Fig. 1A).
Similar results were obtained using a cell permeable peptide, which inhibits dimerization of the MyD88 TIR domains (31). This MyD88 inhibitory peptide had no effect on the ability of P. gingivalis fimbriae to induce the CR3 activation-specific CBRM1/5 epitope in monocyteic THP-1/CD14 cells, although it significantly suppressed fimbria-induced activation of NF-κB in the same cells (p < 0.05; Fig. 1B). Therefore, MyD88 signaling is not required for CR3 activation by P. gingivalis fimbriae, although it mediates activation of NF-κB by the same agonist.

These conclusions were confirmed in the mouse model using WT or MyD88-deficient macrophages. The cells were monitored for their adhesive activity for soluble (s)ICAM-1, which binds activated but not resting CR3 (38). We found that P. gingivalis fimbriae could activate CR3-dependent binding of sICAM-1 equally well in WT or MyD88-deficient macrophages, although, as expected, this activity was abrogated in TLR2-deficient macrophages (Fig. 2A). In a control experiment, the activity of a TLR4 agonist, Escherichia coli LPS, was not influenced by TLR2 deficiency but was abrogated by MyD88 deficiency (Fig. 2A). In an additional control experiment, the ability of PMA (a cell-permeable agonist that bypasses receptor interactions; Ref. 19) to activate CR3 binding of sICAM-1 was not affected by either MyD88 or TLR2 deficiency (Fig. 2A). That the inducible binding of sICAM-1 was indeed dependent upon CR3 was confirmed by the specific blocking effect of anti-CD11b mAb (Fig. 2B). However, deficiency of either TLR2 or MyD88 abrogated proinflammatory cytokine induction by P. gingivalis fimbriae, although, as expected, not by Poly(I:C) which is a MyD88-independent TLR3 agonist (Fig. 2C). As an additional control, the cytokine-inducing ability of E. coli LPS was significantly inhibited by MyD88 deficiency but not by TLR2 deficiency (Fig. 2C). Taken together, the results from Fig. 1 and 2 show that human or mouse MyD88 mediates P. gingivalis fimbria-induced proinflammatory but not proadhesive signaling. In contrast, TLR2 is essential in both pathways.

Role of TIRAP in P. gingivalis fimbria-induced proadhesive or proinflammatory activities

Unlike MyD88 which mediates signaling downstream of most TLRs (except for TLR3), TIRAP (or Mal) is involved only in TLR2 and TLR4 signaling (8, 41). The role of TIRAP is to facilitate the recruitment of MyD88 to the cytoplasmic domain of the activated TLR, and is thus believed to function as a sorting adaptor (42). Although TIRAP would likely be involved in P. gingivalis fimbria-induced TLR2 proinflammatory signaling, it is unknown whether it could function as an adaptor, or play any other role, in the TLR2 proadhesive pathway. To examine this possibility, we first determined the ability of THP-1/CD14 cells to induce the CR3 activation-specific CBRM1/5 epitope in response to P. gingivalis.
gingivalis fimbriae, upon transfection with a TIRAP mutant (P125H) which acts as dominant negative inhibitor (TIRAP-DN) (43). Transfection with TLR2-DN served as a positive control, whereas transfections with empty vector or a dominant negative version (C117H) of the TRAM, which is exclusively involved in TLR4 signaling (44, 45), served as negative controls. We found that TIRAP-DN failed to suppress CBRM1/5 epitope induction by P. gingivalis fimbriae (as seen with the negative controls), although TLR2-DN readily inhibited the same activity (p < 0.05; Fig. 3). However, as expected, both TLR2-DN and TIRAP-DN (but not TRAM-DN) inhibited fimbria-induced NF-κB activation (Fig. 3).

The notion that TIRAP is involved in the TLR2 proinflammatory but not the proadhesive pathway was confirmed using mouse macrophages. Indeed, a TIRAP inhibitory peptide, which blocks TIRAP-dependent signaling (32, 33), significantly (p < 0.05) diminished the ability of P. gingivalis fimbriae to induce IL-6 production, although it had no effect on the ability of fimbria to stimulate CR3 adhesive activity, as measured by sICAM-1 binding (Fig. 4). Treatments with a control peptide had no effect on either assay (Fig. 4). Therefore, TIRAP signaling has no effect on P. gingivalis fimbria-induced proadhesive activities, although it readily inhibits P. gingivalis fimbria-induced proinflammatory activities. These findings, taken together with those from Figs. 1 and 2, indicate that the proadhesive pathway is TIRAP- and MyD88-independent.

Akt is activated in a TLR2/P13K-dependent way but is not involved in the proadhesive pathway

P13K plays an essential role in the P. gingivalis fimbria-induced TLR2 proadhesive signaling pathway (20, 21). Because the Ser/Thr kinase Akt was previously implicated in inside-out proadhesive signaling (46) and is one of the most likely downstream targets of P13K for a variety of cellular functions (47), we examined possible involvement of Akt in the fimbria-activated proadhesive pathway.

We first examined whether Akt is activated by P. gingivalis fimbriae in a TLR2- and P13K-dependent way. Indeed, stimulation of THP-1/CD14 cells by fimbriae led to activation (phosphorylation) of Akt, although this activity was significantly (p < 0.05) suppressed in cells transfected with TLR2-DN or pretreated with the P13K inhibitor, LY294002 (but not with its inactive analog, LY305351) (Fig. 5A). None of the treatments influenced total Akt levels (Fig. 5A). We then determined whether transfection of THP-1/CD14 cells with a dominant negative inhibitor of Akt signaling (Akt-DN) could inhibit induction of the CR3 activation-specific CBRM1/5 epitope by P. gingivalis fimbriae. Empty vector and TLR2-DN were used as negative and positive controls, respectively. Akt-DN failed to inhibit CBRM1/5 induction, in contrast to TLR2-DN, which displayed dose-dependent inhibitory action (Fig. 5B). We observed a similar lack of inhibitory effects on CBRM1/5 epitope induction, using a range of concentrations (25–125 μM) of a cell permeable peptide that blocks Akt kinase activity (34) or a pharmacological inhibitor of Akt (5–10 μM; 1L-6-Hydroxymethyl-chiro-inositol2-((R)-2-O-methyl-3-O-octadecylcarbonate)) (data not shown). Therefore, a different P13K downstream effector may link TLR2 to CR3 activation, and we set out to identify it.

FIGURE 3. TIRAP-dependent and independent proinflammatory and proadhesive activities of P. gingivalis fimbriae. Human THP-1/CD14 cells were transfected with dominant negative (DN) point mutants of human TIRAP (TIRAP-DN) or TRAM (TRAM-DN; negative control), or a TIR domain-deficient mutant of TLR2 that acts as a dominant negative inhibitor (TLR2-DN; positive control), at the indicated μg amounts of plasmid DNA per 2 × 10⁵ cells. The cells were stimulated with P. gingivalis fimbriae (1 μg/ml) and assayed for induction of the CR3 activation-specific CBRM1/5 epitope (after staining with FITC-labeled CBRM1/5 mAb) or for NF-κB-dependent transcription of a luciferase reporter gene. CBRM1/5 induction is reported in relative fluorescent units (RFU) and NF-κB activation in relative luciferase activity (RLA). The discontinuous horizontal lines indicate CBRM1/5 epitope induction or NF-κB activation in resting cells, which were <1% of the values of fimbria-stimulated cells. Data are means ± SD (n = 3) from one of two experiments that yielded consistent results. The asterisks indicate statistically significant (p < 0.05) differences compared with empty vector control treatments.

FIGURE 4. Role of TIRAP in P. gingivalis fimbria-induced proadhesive or proinflammatory activities in mouse macrophages. Mouse macrophages were pretreated for 1h with 10 μM of a cell-permeable TIRAP inhibitory peptide or inactive control. The cells were subsequently left unstimulated or were stimulated with P. gingivalis fimbriae (1 μg/ml) and assayed for sICAM-1 binding or induction of IL-6 release. Data are means ± SD (n = 3) from one of two independent experiments that yielded consistent findings. Asterisks indicate statistically significant (p < 0.05) differences compared with medium-treated (no peptide) controls.
inositol-3,4,5-trisphosphate (PIP3) generated by PI3K (48). Moreover, cytohesin-1 is also implicated in induction of integrin adhesive capacity (36, 48). We have thus investigated whether cytohesin-1 participates in the *P. gingivalis* fimbria-induced pathway for CR3 activation, using an established antisense strategy for inhibiting cytohesin-1 expression (35). Treatment of THP-1/CD14 cells with antisense S-oligos to cytohesin-1 mRNA (but not with control sense S-oligos) significantly inhibited the ability of fimbria-stimulated cells to induce the CBRM1/5 epitope and bind sICAM-1 (*p* < 0.05; Fig. 6). However, the antisense S-oligo treatments had no significant effect on NF-κB activation by *P. gingivalis* fimbriae (Fig. 6). Therefore, cytohesin-1 appears to mediate *P. gingivalis* fimbria-induced activation of the proadhesive but not of the proinflammatory pathway.

**Discussion**

As more information becomes increasingly available on the intricacies of TLR signaling, it is appreciated that the control of adverse host responses in infection-driven inflammatory diseases would require highly selective and precisely targeted intervention. This in turn requires precise delineation of TLR signaling pathways in response to a given pathogen, such as *P. gingivalis*. It is generally thought that TLR4 signaling is more complex than TLR2 signaling, because TLR4 activation initiates two divergent signaling cascades: The TIRAP/MyD88 pathway which activates NF-κB, and the TRAM/TRIF (TIR-domain-containing adapter-inducing IFN-β) pathway, which additionally triggers activation of the IFN regulatory factor-3 (8). In this study, we have presented evidence that TLR2 activation also initiates two divergent signaling pathways.

Specifically, TLR2 activation by *P. gingivalis* fimbriae induces a TIRAP/MyD88-dependent proinflammatory pathway and a TIRAP/MyD88-independent but PI3K-dependent proadhesive pathway, as illustrated in the Fig. 7 model. The former pathway induces production of NF-κB-dependent proinflammatory cytokines and the antimicrobial molecule NO, within hours upon cell stimulation with *P. gingivalis* (6, 18). The proadhesive pathway is characterized by inside-out signaling which, within minutes, transactivates the adhesive capacity of CR3 (CD11b/CD18) (21), a β2 integrin which can binds both host and microbial molecules (49). More recently, other agonists were also shown to activate the TLR2 proadhesive pathway (50). In particular, peptidoglycan and the PamCysSerLys4 lipopeptide were demonstrated to activate TLR2- and CD11b/CD18-dependent monocyte adhesion to ICAM-1, although intermediate signaling molecules were not examined (50).

Our demonstration that the TLR2/PI3K proadhesive pathway proceeds independently of MyD88 and TIRAP is consistent with earlier findings by another group that the cytoplasmic domain of TLR2 contains two PI3K binding motifs (YXXM/W) that could serve as docking sites for PI3K (51). Taken together, these findings suggest that PI3K may be recruited to *P. gingivalis* fimbriae-activated TLR2 in a MyD88- and TIRAP-independent way. MyD88, however, was required for LPS-induced binding of soluble ICAM-1 by CR3, in line with a previous report that activation of the αMβ2 integrin (CR3) by LPS involves MyD88-mediated signaling (52). Therefore, *P. gingivalis* fimbriae acting through TLR2 may stimulate a different inside-out signaling pathway from that activated by LPS acting through TLR4. This difference may be attributable to the inability of TLR4 to directly recruit PI3K to its cytoplasmic tail which lacks PI3K binding motifs (51). Our previous work has also shown that the small GTPase Rac1 acts upstream of PI3K and plays a positive regulatory role in the TLR2 proadhesive pathway (20) (Fig. 7), presumably through

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**FIGURE 5.** Akt is activated by *P. gingivalis* fimbriae in a TLR2/PI3K-dependent way but is not involved in CR3 activation. THP-1/CD14 cells were transfected or not with dominant negative inhibitors of TLR2 (TLR2-DN) (A and B) or Akt (Akt-DN) (B) at the indicated μg of plasmid DNA per 2 × 10⁵ cells. A, Untransfected THP-1/CD14 cells were pretreated with medium only, LY294002 (PI3K inhibitor), or the inactive analog LY30351 (both at 20 μM). Cells were then activated with *P. gingivalis* fimbriae and assayed for total and phosphorylated Akt (A), or for induction of the CBRM1/5 epitope, measured in relative fluorescence units (RFU) (B). The discontinuous horizontal line in B indicates CBRM1/5 epitope induction in resting cells which was <10% of the activity of fimbria-stimulated cells. Data are means ± SD (n = 3), from one of three (A) or two (B) independent experiments yielding consistent results. Asterisks indicate statistically significant (*p* < 0.05) differences compared with corresponding control treatments.

**FIGURE 6.** Cytohesin-1 is involved in *P. gingivalis* fimbria-induced proadhesive but not proinflammatory activities. THP-1/CD14 cells were treated with antisense S-oligos (AS) to cytohesin-1 mRNA or with control sense S-oligos (S), at the indicated concentrations (μM). Following stimulation with *P. gingivalis* fimbriae (1 μg/ml), the cells were assayed for induction of the CBRM1/5 epitope, binding of sICAM-1, or for NF-κB-dependent transcription of a luciferase reporter gene. CBRM1/5 induction and sICAM-1 binding are reported in relative fluorescent units (RFU) and NF-κB activation in relative luciferase activity (RLA). Untransfected cells displayed <5% of the activities of fimbria-stimulated cells in all three assays. Results are means ± SD (n = 3) from one of two independent experiments that yielded consistent findings. Asterisks indicate statistically significant (*p* < 0.05) inhibition of activities compared with cells that were not treated with anti-sense S-oligos.
its ability to bind PI3K and augment its activity (53). Despite these earlier developments, the downstream effector of PI3K in the \( P. \) gingivalis fimbria-activated proadhesive pathway had remained elusive.

In this study, we identified cytohesin-1, rather than Akt, as the downstream PI3K effector in the TLR2 proadhesive pathway. Interestingly, although PMA induces \( \beta_2 \) integrin activation through a distinct pathway involving protein kinase C, this pathway also uses cytohesin-1 as an effector (54, 55). Moreover, the ability of LPS to activate the ligand-binding capacity of leukocyte function-associated Ag-1 (CD11a/CD18) is similarly dependent on cytohesin-1 activity (35). Therefore, cytohesin-1 appears to functionally integrate distinct signaling pathways for \( \beta_2 \) integrin activation. Earlier studies have established that the inside-out signaling regulation of integrin binding affinity involves targeting of intracellular proteins to the cytoplasmic tails of the integrin, thereby causing high-affinity conformational changes on its ligand-binding domain (reviewed in Ref. 56). Because cytohesin-1 can use PI3K-generated PIP3 for membrane docking and likely to function immediately downstream of PI3K, it appears that cytohesin-1 may be a more feasible target for intervention. More studies are warranted to determine the usefulness of a cytohesin-based approach as a strategy to counteract immune evasion by \( P. \) gingivalis.

### Disclosures

The authors have no financial conflict of interest.

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In addition to a better understanding of the signaling pathways activated by *P. gingivalis* or its purified fimbriae, our current findings may be significant from a translational viewpoint. In this regard, delineation of signaling pathways may provide potential therapeutic targets for selectively enhancing or inhibiting pathway activities, depending on whether they promote or undermine host defense. If TLR2/NF-\( \kappa \)B-mediated immune response controls *P. gingivalis* infection, whereas exploitation of TLR2-transactivated CR3 favors *P. gingivalis* persistence, as supported by our recent studies (18, 25, 26, 59), it would make sense to inhibit TLR2 signaling in a way that would inhibit specifically the proadhesive pathway. Indeed, inhibition of the TLR2 proadhesive pathway may prevent CR3-mediated uptake of *P. gingivalis*, an internalization route that suppresses the ability of macrophages to clear *P. gingivalis* infection (25, 26). So far, we have identified three targets in the proadhesive pathway downstream of TLR2: Rac1, PI3K, and cytohesin-1. Considering that Rac1 and PI3K are involved in a great number of essential physiological functions (60, 61), it appears that cytohesin-1 may be a more feasible target for intervention.


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