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Porphyromonas gingivalis interactions with complement receptor 3 (CR3): Innate immunity or immune evasion?

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1. ABSTRACT

Porphyromonas gingivalis is a major oral pathogen associated with periodontal disease. In this paper, we review the mechanism whereby this organism induces a proadhesive signaling pathway for activation of complement receptor 3 (CR3; CD11b/CD18) and discuss its biological significance on the basis of published findings by our lab and other investigators. The proadhesive pathway is initiated when *P. gingivalis* fimbriae bind CD14 and activate Toll-like receptor 2 (TLR2)- and phosphatidylinositol 3-kinase-mediated signaling leading to induction of the high-affinity conformation of CR3 in leukocytes. Although this TLR2 proadhesive signaling pathway may normally be involved in enhancing leukocyte-endothelial cell interactions and transendothelial migration, intriguing evidence suggests that *P. gingivalis* has co-opted this pathway for enhancing the interaction of its cell surface fimbriae with CR3. Indeed, activated CR3 interacts with *P. gingivalis* fimbriae and induces downregulation of interleukin-12 p70, a key cytokine involved in intracellular bacterial clearance. Moreover, the interaction of activated CR3 with *P. gingivalis* leads to the internalization of the pathogen by macrophages. Since CR3 does not readily activate microbicidal mechanisms and constitutes a "preferred receptor" for certain intracellular pathogens, possible exploitation of CR3 by *P. gingivalis* for evading innate immune clearance becomes a plausible hypothesis.

2. INTRODUCTION

The β_2 integrins are heterodimeric receptors comprising a common β subunit (CD18) non-covalently linked to a unique α subunit (CD11a, b, c, or d), and display diverse cellular functions, including the ability to orchestrate critical processes in immunity and inflammation (1, 2). In this context, integrins mediate cell-cell, cell-extracellular matrix, and cell-pathogen interactions (2, 3). Integrins, in general, constitute a critical link between the cells and the extracellular matrix, functioning both as anchoring sites and as central elements for sensing, processing, and transducing received information. In fact, the term "integrin" was proposed in order to characterize a protein complex that was thought to be integral to the transmembrane association between the extracellular matrix and the cytoskeleton (4).

To integrate the intracellular and extracellular environments, integrins employ inside-out and outside-in bidirectional signaling (3, 5, 6). Inside-out signaling refers to the regulation of the integrin adhesive capacity from within the cell by means of signals generated by other receptors. In this regard, the induction of their high-affinity binding state by inside-out signaling is known as "integrin activation". Once activated, integrins bind ligands or counter-receptors resulting in stimulation of downstream signaling pathways, referred to as outside-in signaling (3, 5, 6). Integrin activation primarily involves two mechanisms;

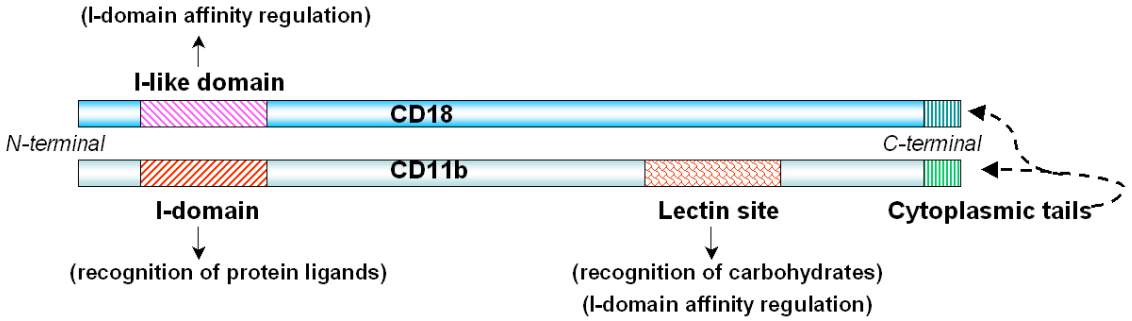

Figure 1. Schematic representation of key features of the CD11b/CD18 (CR3).

changes in the conformation of individual receptors resulting in increased affinity for the ligand (affinity regulation) as mentioned above, or changes in lateral mobility resulting in receptor clustering and increased avidity for the binding substrate (avidity regulation) (3, 6). At least theoretically, a third mechanism could involve quantitative upregulation of integrin receptors on the cell surface. However, this mechanism was shown not to be essential for enhanced adhesive activity (7). Although both affinity regulation and avidity regulation appear to be important mechanisms, available evidence supports the notion that the former may be more critical. Indeed, the formation of integrin clusters in adherent cells resulting in enhanced avidity takes place after the initial contacts with ligands have been made, a process requiring affinity upregulation of individual receptors (3).

CD11b/CD18, also known as complement receptor 3 (CR3) or Mac-1 (Figure 1), is the most prevalent integrin expressed by phagocytic leukocytes such as monocytes and neutrophils (8, 9). This integrin has the capacity to recognize and interact with a wide variety of structurally unrelated molecules derived from either the host (*e.g.*, complement C3 fragment [iC3b], intercellular adhesion molecule-1 [ICAM-1], fibrinogen, factor X, or platelet glycoprotein Ibα) or pathogens (*e.g.*, *Bordetella pertussis* filamentous hemagglutinin and *Leishmania* gp63) (9-12). The ligand binding promiscuity of CD11b/CD18 suggests that it may possess pattern recognition capability, a relatively common attribute of innate immune receptors (13). Moreover, CD11b/CD18 has been shown to cluster with other pattern-recognition receptors (PRRs), such as CD14 and Toll-like receptors (TLRs), in membrane microdomains (lipid rafts) of activated cells (14-16).

The CD11b/CD18-mediated adhesive interactions are important for leukocyte adhesion and transendothelial migration, induction of inflammatory mediators, and phagocytosis (2, 6) and are thus under tight regulation through inside-out signaling pathways. The low-affinity conformation of CD11b/CD18 is energetically favored and is thus the default conformation in the resting state (3, 17). The shift to the high-affinity binding state is rapid, transient, and requires external energy input, which can be effected by inside-out signals generated upon ligation of various other surface receptors by certain agonists including immune complexes, chemokines, or chemotactic peptides (18-20). The mechanisms whereby integrin affinity is regulated at the molecular level has been the subject of intense investigation. Recent progress suggests that the generated inside-out signals lead to the targeting of cytoplasmic factors to the integrin cytoplasmic tails, which act through induction of long-range structural rearrangements involving the CD18 I-like domain and culminating in conformational changes of the ligand-binding or I-domain of CD11b (6, 17, 21) (Figure 1). According to this model, therefore, the CD18 I-like domain regulates the activity of the CD11b I-domain. In addition, a polysaccharide-recognition region ("lectin site") which is located *C*-terminal to the I-domain (22) (Figure 1) also appears to regulate the function of CD11b/CD18, though via a different mechanism. Specifically, ligand binding to the lectin site induces a conformational change of the I-domain resulting in upregulation of the binding and functional activity of CD11b/CD18 (23-25). The induction of the high-affinity conformation of CD11b/CD18 can be detected by certain monoclonal antibodies (mAbs), such as the CBRM1/5 which binds to the I-domain of CD11b in its activated but not in its resting state (26).

We will now refer to CD11b/CD18 as CR3 which more appropriately denotes its phagocytic properties, being of great relevance in this review. Although CR3 appears to play important roles in innate immune defense against microbial pathogens, it is puzzling, if not paradoxical, that CR3 constitutes a "preferred" receptor exploited by phylogenetically unrelated pathogens as a safe portal of entry within macrophages (27-32). In this paper, we will summarize the state of knowledge regarding the interactions of CR3 with the oral pathogen *Porphyromonas gingivalis*, and attempt to elucidate their biological significance in terms of innate host defense or potential exploitation of this receptor for microbial immune evasion. We will begin with a synopsis of the virulent properties of *P. gingivalis* fimbriae, which mediate *P. gingivalis* binding to CR3, followed by a description of the molecular signaling mechanisms underlying fimbria-CR3 interactions.

3. *P. GINGIVALIS* VIRULENCE AND THE ROLE OF FIMBRIAE

P. gingivalis and several other gram-negative oral anaerobic bacteria have been strongly correlated with periodontitis, an infection-driven chronic inflammatory

disease that affects the integrity of the tooth-supporting tissues (33, 34). Moreover, *P. gingivalis* is among several microbial pathogens that have been implicated in contributing to the development of atherosclerosis (35-38). This oral pathogen has been intensively investigated at the molecular level and its virulence has been attributed to several potential virulence factors, including hemagglutinins, cysteine proteinases, LPS, and fimbriae (39, 40). The *fimA* gene-encoded major fimbriae of *P. gingivalis* (41) are adhesive hair-like appendages (0.3 to 1.6 μm long) on the bacterial cell surface (42). The fimbriae have been studied in great molecular detail and their role in virulence has been established in rodent models of periodontitis or atherosclerosis (43, 44). Indeed, FimA-deficient mutants were found to be relatively avirulent compared to wild-type strains of *P. gingivalis* in inducing periodontal bone loss (43) or in accelerating atherosclerotic plaque formation (44).

The virulence potential of fimbriae is attributable to their capacity to interact with various dental or epithelial substrates, extracellular matrix proteins, other bacteria, as well as host immune cells (reviewed in (45)). Fimbrial peptide mapping studies suggest that the promiscuous binding reactivity of *P. gingivalis* fimbriae depends on a multitude of adhesion epitopes, although the overall hydrophobicity and polymeric nature of fimbriae may considerably promote the avidity of binding interactions (reviewed in (46)). These attributes enable *P. gingivalis* to colonize, invade, and degrade periodontal tissue through induction of bone-resorbing cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (43, 47, 48). Although fimbriae are just but one of several proinflammatory molecules of *P. gingivalis*, wild-type strains of this pathogen induce significantly stronger cytokine responses than non-fimbriated isogenic mutants (16, 36). In this regard, the fact that fimbriae extend to a significant distance from the bacterial cell wall suggests that they are likely the first *P. gingivalis* molecule to interact with host cell receptors and initiate intracellular signaling cascades.

4. CR3 IS A CELLULAR RECEPTOR FOR *P. GINGIVALIS* FIMBRIAE

CR3 was the first pattern-recognition receptor implicated in the induction of proinflammatory cytokines in response to purified *P. gingivalis* fimbriae (49). Specifically, Takeshita *et al* showed that fimbriae bind mouse macrophage CD11b/CD18 resulting in induction of TNF-α and IL-1β production (49), consistent with the notion that β_2 integrins mediate induction of proinflammatory signaling for activation of the transcription factor nuclear factor-κB (NF-κB) (50). Strikingly, it was also shown that the cytoplasmic tails of β_2 integrins, though necessary for transducing phagocytic signals, are not required for NF-κB activation upon integrin binding of bacterial lipopolysaccharide (51). These intriguing findings raised the possibility that *P. gingivalis* fimbria-induced NF-κB activation and proinflammatory cytokine release may similarly depend on a downstream signal transducer distinct from the cytoplasmic tails of either CD11 or CD18. This hypothetical scenario was reminiscent of the ability of the tailless CD14 receptor to bind LPS and initiate intracellular signaling through subsequent LPS interactions with the TLR4/MD2 complex, which functionally associates with CD14 (52). These considerations, in conjuction with the notion proposed by Ingalls *et al*, that CD14 and CR3 may share a common signal-transducing element (53), had prompted us to investigate possible cooperative interactions of CR3 with TLRs in response to *P. gingivalis* fimbriae.

Although in early studies we showed that the ability of native fimbriae from *P. gingivalis* to induce cytokine induction could be inhibited by anti-TLR2 or anti-β_2 integrin antibodies (38, 54), it was only after a series of subsequent reports that we established that *P. gingivalis* fimbriae interact with both TLR2 and CR3 (CD11b/CD18) in an tightly regulated mode (16, 55-57). Below we describe the studies which established that CR3 engages in cooperative interactions with the CD14/TLR2 signaling in response to *P. gingivalis* fimbriae.

5. COOPERATIVE INTERACTIONS OF CR3 WITH THE CD14/TLR2 COMPLEX

The observations that a mAb to the ligand-binding domain of CD11b inhibits the ability of *P. gingivalis* fimbriae to bind human monocytes (57) and to induce NF-κB activation (38) indicated that CR3 is involved in the direct recognition of *P. gingivalis* fimbriae. Interestingly, however, the ability of fimbriae to bind monocytes was completely abrogated by anti-CD14 mAb alone (57). This finding prompted us to hypothesize that CR3 may recognize *P. gingivalis* fimbriae in a CD14-dependent mode. This intriguing notion was nevertheless consistent with the fact that integrins require prior activation to efficiently bind their ligands (3, 6). In the absence of known integrin activators in our experimental model of *P. gingivalis* fimbriae-monocyte interactions, we hypothesized that CR3 activation is induced by the fimbriae themselves through a hitherto undescribed inside-out signaling pathway.

We thus investigated the possibility that *P. gingivalis* fimbriae function both as an activator and ligand of CR3 in monocytes. We first established that *P. gingivalis* fimbriae induce the high-affinity conformation of CR3, detected by the CBRM1/5 reporter mAb (57). The induction of the CBRM1/5 neoepitope by fimbriae was evident at 10 min after stimulation, peaked at 30 min, and slowly declined thereafter (57), consistent with the transient nature of CR3 activation (3). No significant quantitative upregulation of CR3 expression by fimbriae was observed (57). Therefore, the mechanism whereby *P. gingivalis* fimbriae activate CR3 involves affinity upregulation, although it is unknown whether fimbriae induce avidity upregulation.

As expected, the ability of *P. gingivalis* fimbriae to induce the CBRM1/5 epitope correlated with increased cell binding activity of fimbriae (56). To directly demonstrate that *P. gingivalis* fimbriae function both as an activator and

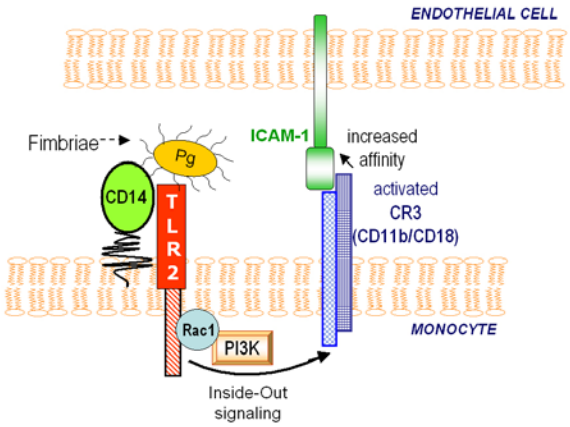

Figure 2. *P. gingivalis* fimbria-induced TLR2 inside-out signaling activates the adhesive activity of monocytes for endothelial ICAM-1. The CD14/TLR2 signaling complex is activated by *P. gingivalis* fimbriae leading to Rac1- and PI3K-mediated signaling which activates the ligand-binding capacity of CR3 (CD11b/CD18). Activated CR3 can thereby bind endothelial ICAM-1, thus promoting monocyte-endothelial cell interactions for monocyte transedothelial migration (60).

ligand of CR3, we modified the direct binding assay. Specifically, the assay was dissected into two steps: These included an activation step at 37°C, which would serve to induce the ligand-binding capacity of CR3, followed by a binding step, which was performed on ice in the presence of metabolic inhibitors (56). The experimental conditions of the binding step would allow ligand binding to pre-activated CR3, but would not promote further CR3 activation, since this requires significantly higher temperatures and energy input (58). Accordingly, human monocytes were pretreated at 37°C for 10 min with unlabeled fimbriae (activation step) and then biotinylated fimbriae were added to medium only- or fimbria-pretreated monocytes for 1-h incubation on ice (binding step). We found that fimbria-pretreated cells bind added labeled fimbriae at significantly higher levels than medium only-pretreated cells, attributable to the presence of activated CR3 in the former group (56). These results indicated that *P. gingivalis* fimbriae activate the ligand binding capacity of CR3, which can thereby efficiently recognize fimbriae. We subsequently set out to define the signaling pathway utilized by *P. gingivalis* fimbriae to activate CR3.

As alluded to above, the ability of anti-CD14 mAb to completely abrogate the ability of *P. gingivalis* fimbriae to bind CR3, provided us with a clue as to the inside-out signaling pathway involved in fimbria-induced CR3 activation. We first showed that the same anti-CD14 mAb inhibits also the ability of *P. gingivalis* fimbriae to activate CR3 (57), thereby implicating CD14 in the signaling pathway. However, CD14 was unlikely to serve as a signal transducing receptor for CR3 activation, since CD14 lacks a cytoplasmic domain (59). We have thus suspected the involvement of TLR-dependent inside-out signaling downstream of CD14. Indeed, the capacity of fimbriae to induce CR3 activation in monocytes, as well as in neutrophils, was found to be significantly inhibited by a mAb to TLR2, but not to TLR4 or unrelated surface molecules (57). Moreover, fimbria-induced CR3 activation in monocytes and neutrophils was significantly inhibited by pharmacological inhibitors of phosphatidylinositol 3-kinase (PI3K) but not of protein kinase C or of the p38 mitogen-activated protein kinase (57). According to these findings, therefore, the inside-out signaling pathway involved the sequential participation of CD14→ TLR2 → PI3K → CR3 (Figure 2). The same antagonists (anti-CD14, anti-TLR2, and PI3K inhibitors) moreover inhibited CR3-dependent adhesion of *P. gingivalis* fimbria-stimulated monocytes to ICAM-1(60). The role of CD14 and TLR2 was confirmed using Chinese hamster ovary (CHO) cells engineered to express a recombinant inside-out signaling system. Specifically, cotransfection of human CD14 and TLR2 (but not a signaling-deficient mutant of TLR2) into CR3-expressing CHO cells rendered them capable of upregulating the adhesive capacity of CR3 in response to *P. gingivalis* fimbriae (56). Consistent with these findings, mouse macrophages deficient in CD14 or TLR2 adhered poorly to immobilized ICAM-1 upon their stimulation with fimbriae, in stark contrast to wild-type or TLR4-deficient macrophages (60).

Although both *P. gingivalis* fimbriae and *N*-formyl-Met-Leu-Phe, a prototypical integrin activator, activate CR3, the intracellular signaling pathways involved are quite distinct. Indeed, we found that the fimbria-stimulated proadhesive pathway is sensitive to the action of *Clostridium difficile* toxin B but is not affected by pertussis toxin or the *Clostridium botulinum* C3 exoenzyme, whereas the *N*-formyl-Met-Leu-Phe-stimulated pathway is sensitive to all three toxins (60). Interestingly, *C. difficile* toxin B inhibits the small-molecular-weight GTPases, Rho, Rac, and Cdc42, whereas C3 exoenzyme specifically inhibits only Rho (61). We thus hypothesized that the fimbria-stimulated pathway involves participation of Rac or Cdc42, but not of Rho GTPase. Experiments using dominant-negative signaling mutants of various GTPases, demonstrated that Rac1 constitutes a component of the *P. gingivalis* fimbria-stimulated proadhesive pathway, functioning downstream of TLR2 and upstream of PI3K (60) (Figure 2). These findings establish a novel TLR2 function in transmodulating the CR3-dependent adhesive activity in response to the fimbriae of *P. gingivalis*. In this regard, CD14 plays an essential TLR2 coreceptor function, whereas Rac1 and PI3K act as signaling intermediates downstream of TLR2 (Figure 2).

The findings presented above are consistent with the notion that CD14, TLR2, and CR3 function in cooperation rather than independently. For example, CR3 cannot effectively bind fimbriae in the absence of TLR2 signaling (56), whereas effective TLR2 signaling by fimbriae requires the participation of CD14 (16, 56). We were thus prompted to hypothesize that CD14, TLR2, and CR3 physically interact for innate recognition of *P. gingivalis* fimbriae, perhaps in membrane lipid rafts which serve as major platforms for cellular signaling (62, 63) or for host cell invasion by several pathogens (64). Although CD14 is constitutively found in lipid rafts, TLRs and

integrins are recruited there upon activation with appropriate ligands (15, 63). Using fluorescence resonance energy transfer (FRET), a biophysical technique which can determine sterical co-association of molecules (63, 65), we found that *P. gingivalis* fimbriae induce the recruitment of TLR2 into lipid rafts where it associates with resident CD14 (56). Moreover, TLR2 associates with CR3 in fimbria-stimulated but not in resting cells (16). We also found that methyl-β-cyclodextrin, a cholesterol-sequestering agent that disrupts lipid raft organization, inhibits fimbria-induced assembly of CD14/TLR2 signaling complexes and activation of the high-affinity state of CR3, although the methyl-β-cyclodextrin inhibitory effect is reversed by exogenous addition of cholesterol (56). Taken together, these findings suggest that lipid raft function is essential for fimbria-induced CD14/TLR2 cluster formation and ensuing inside-out signaling for CD11b/CD18 activation.

6. BIOLOGICAL SIGNIFICANCE OF CR3-*P. GINGIVALIS* INTERACTIONS

The biological significance of CR3 interactions with *P. gingivalis* is uncertain at the moment. It could be speculated that the ability of the CD14/TLR2 signaling complex to upregulate the ligand-binding capacity of CR3 for efficient binding of fimbriae may contribute to the host innate defense. This may involve CR3-mediated induction of cytokines, such as TNF-α and IL-1β (16, 66). However, it should be noted that cytokine induction is an immunological "double-edged sword" which may also have pathophysiological consequences in periodontal disease if their production is prolonged and excessive (67, 68). An additional plausible mechanism whereby *P. gingivalis* fimbria-induced activation of CR3 may play a role in periodontal disease pathogenesis involves inflammatory cell recruitment. Neutrophils are receiving considerable attention as effectors of inflammation-induced tissue damage in periodontitis (68). CR3 is abundantly expressed by neutrophils and plays a role in their migration to sites of extravascular inflammation (69, 70). It is thus plausible that *P. gingivalis* fimbriae promote the recruitment of neutrophils through activation of CR3, thereby amplifying neutrophil-elicited inflammation. In this context, fimbriae could act in cell-associated form or perhaps more effectively as free fimbriae shed from the cell surface or as a component of outer membrane vesicles released from the bacterial cell surface (40).

CR3 and other β_2 integrins have also been implicated as effectors of pathological inflammation in atherosclerosis and other chronic or acute disease states, and thus constitute potential targets for therapeutic intervention (71). Specifically, activated CR3 promotes formation of leukocyte-platelet complexes that in turn promote vascular inflammation in atherosclerosis (11, 72). Moreover, the interactions of CR3 with fibrinogen and ICAM-1 mediate adhesion of neutrophils or monocytes to sites of fibrinogen deposition and the endothelium, respectively, and contribute to cardiovascular inflammation (10, 70, 73). In this context, the adhesion of monocytes to the arterial endothelium and their subsequent migration into the subendothelial area is a hallmark of early atherogenesis (74). The transmigratory process is mediated by interacting sets of cell adhesion molecules, including the CR3 - ICAM-1 pair, which has been experimentally implicated in atherosclerosis and other inflammatory conditions (73, 75-77). Several bacterial pathogens such as *Chlamydia pneumoniae, Helicobacter pylori*, and *Porphyromonas gingivalis* are thought to contribute to vascular inflammation and have been implicated as contributory factors in the development or acceleration of atherosclerosis (36, 38, 74, 78). In this regard, viable *P. gingivalis* has been localized in atherosclerotic plaques (37). It is plausible, therefore, that the *P. gingivalis* fimbria-induced TLR2 signaling for CR3 activation may constitute a mechanistic basis linking *P. gingivalis* to inflammatory atherosclerotic processes, by promoting monocyte recruitment into subendothelial areas. Indeed, we have shown that purified fimbriae or fimbriated *P. gingivalis* stimulate monocyte adhesion to endothelial ICAM-1 and transmigration across endothelial cell monolayers (60) (Figure 2). Moreover, *P. gingivalis*-stimulated monocytes display enhanced transendothelial migration compared to monocytes stimulated with fimbria-deficient isogenic mutants (60). This novel fimbria-dependent mechanism is consistent with earlier findings by others that the presence of wild-type but not of non-fimbriated *P. gingivalis* is associated with periodontal disease and increased atherosclerotic plaque formation in orally infected hyperlipidemic mice, although both strains are detectable in the blood and aortic arch tissue (44).

In a different context, however, the ability of CD14/TLR2 to detect *P. gingivalis* fimbriae and initiate inside-out signaling for CR3 activation could be a potentially protective mechanism, which can contribute to monocyte recruitment to sites of *P. gingivalis* infection or to promote CR3-mediated phagocytosis of *P. gingivalis*. Interestingly, however, phagocytosis does not necessarily lead to pathogen killing as will be discussed below. Our initial report that *P. gingivalis* fimbriae stimulate TLR2 inside-out signaling for CR3 activation (57) was published concomitantly (April 2005) with a study by an independent group which demonstrated that mycobacterial lipoarabinomannan also stimulates this proadhesive pathway (79). Strikingly, this pathway is exploited by mycobacteria for promoting their uptake by monocytes/macrophages through activated CR3 (79) leading to their survival and intracellular persistence (32). The exploitability of CR3 by mycobacteria and certain other intracellular pathogens may, at least partly, be due to the notion that this receptor is not linked to vigorous microbicidal mechanisms (27-31, 80, 81). Consistent with this, the *in vivo* phagocytic uptake of *Bordetella pertussis* through the Fcγ receptor III (CD16) facilitates its clearance in contrast to CR3-mediated uptake (82). It is intriguing to speculate that pathogen-induced TLR2 inside-out signaling for CR3 activation may be a universal pathway exploited by intracellular pathogens. Whether *P. gingivalis* can similarly induce its uptake through CR3 resulting in intracellular persistence, rather than post-phagocytosis killing, is currently under investigation in our lab. However, we have already shown that the receptors

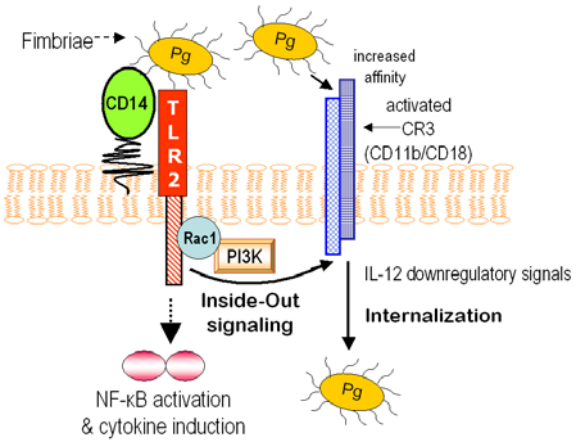

Figure 3. Potential immune evasion mechanism of *P. gingivalis* through CR3 exploitation. *P. gingivalis* fimbriae interact with the CD14/TLR2 complex and induce Rac1/PI3K-mediated inside-out signaling which activates the high-affinity conformation of CR3 within minutes (57). TLR2 stimulation is also essential for NF-κB activation and cytokine induction (16, 55). Upon CR3 activation, fimbriated *P. gingivalis* can readily interact with this β_2 integrin leading to IL-12 downregulation (55). Moreover, the interaction of activated CR3 with *P. gingivalis* leads to uptake of the pathogen by macrophages (56). Since CR3 is exploited for cell entry and parasitism by several intracellular pathogens (27, 28, 30, 31, 80-82), it is plausible that *P. gingivalis* may similarly exploit TLR2 inside-out signaling and the ensuing interaction with CR3 for evading or attenuating immune clearance.

involved in the inside-out signaling pathway for CR3 activation (*i.e.*, CD14, TLR2, and CR3) play important roles in mediating internalization of fimbriated *P. gingivalis* by mouse macrophages (56) (Figure 3). The involvement of TLR2 in mediating *P. gingivalis* internalization by macrophages is interesting, given that TLR2 is not known to be a phagocytic receptor; however, this finding is consistent with the role of TLR2 in stimulating the adhesive activity of CR3 (56). Although the fate of *P. gingivalis* within macrophages is uncertain at the moment, *P. gingivalis* can invade, through interactions of its fimbriae with β1 integrins (45, 83), into gingival epithelial cells where the pathogen can replicate and persist at least *in vitro* (40).

We have generated additional evidence that is consistent with the intriguing notion that *P. gingivalis* fimbriae may exploit CR3 function. Specifically, we found that the binding of *P. gingivalis* fimbriae to activated CR3 results in CR3-mediated inhibition of bioactive (p70) IL-12 production (55), a major cytokine involved in intracellular bacterial clearance (84). Specifically, we have found that the ability of mouse macrophages to elicit IL-12 p70 in response to *P. gingivalis* fimbriae is upregulated by CD11b deficiency but is eliminated by TLR2 deficiency (55). These findings may suggest that fimbriae interact with CR3 and inhibit TLR2-dependent induction of IL-12 p70, thereby potentially undermining effective innate immunity.

From the host viewpoint, the property of CR3 to inhibit IL-12 may serve a physiological role. Indeed, the phagocytosis of apoptotic cells by macrophages is heavily dependent on CR3 and is associated with inhibition of IL-12 p70, since apoptotic cells are not normally recognized as a danger to the immune system (85, 86). It appears, therefore, that *P. gingivalis* may have co-opted a natural anti-inflammatory CD11b/CD18-dependent mechanism to evade innate immunity. This mechanism may not be unique to this pathogen, since the interaction of *Bordetella pertussis* filamentous hemagglutinin (FHA) with CR3 also leads to IL-12 p70 inhibition, as well as to IL-10 induction (87). The FHA, however, uses a different CD11b/CD18 activation mechanism. Rather than interacting with CD14/TLR2, FHA interacts with a signal transduction complex comprising the αvβ3 integrin and the integrin-associated protein CD47 (88, 89). Nevertheless, similarly to CD14/TLR2 (57), inside-out signaling generated by αvβ3/CD47 is relayed to CR3 via PI3K (89). *Histoplasma capsulatum* is another organism capable of inducing CR3-dependent downregulatory signals for inhibition of IL-12 p70 production in macrophages (90).

If monocytes/macrophages fail to kill intracellular pathogens, an alternative course of defensive action is to undergo apoptosis, in which case the pathogen is deprived of its nutritional niche and the spread of infection is prevented (91, 92). In principle, such apoptotic mechanism could be triggered upon *P. gingivalis* infection. However, *P. gingivalis* fimbriae inhibit caspase-3-mediated apoptosis of human monocytic cells through activation of an extracellular signal-regulated kinase (ERK)-dependent pathway (93). Although the biological significance of this finding is uncertain, it is consistent with the hypothesis that *P. gingivalis* may be capable of infecting and persisting within monocytes/macrophages.

7. CONCLUSIONS

We and others have speculated that microbial virulence proteins have evolved to interact with and possibly exploit the pattern recognition system, in ways that promote the potential of the pathogens for survival and persistence in the host (16, 55, 56, 94-97). In this regard, although activation of TLR2 inside-out signaling by *P. gingivalis* fimbriae leads to enhanced CR3-dependent monocyte adhesion and transendothelial migration (60), subsequent work by our group has shown that *P. gingivalis* has co-opted this proadhesive pathway for CR3 binding and entry within macrophages (56). Interestingly, CR3 is not linked to vigorous microbicidal mechanisms and this receptor is thus exploited by certain pathogens for intracellular persistence (27, 28, 30, 31, 80-82). Moreover, we have shown that the interaction of *P. gingivalis* fimbriae with CR3 leads to downregulation of IL-12 p70 (55), which plays a key role in intracellular bacterial clearance (84). These findings strongly suggest that the TLR2/CR3 proadhesive pathway may be exploited by *P. gingivalis*. Intriguingly, a recent *in vivo* study in the mouse periodontitis model demonstrated that TLR2-deficient mice display increased resistance to *P. gingivalis*-induced

periodontal bone loss, compared to wild-type controls (98). Although the precise underlying mechanisms are uncertain, at least partly, they may involve the ability of *P. gingivalis* to co-opt TLR2 signaling for activation and exploitation of CR3. Whether CR3 is indeed utilized by *P. gingivalis* as an immune evasion strategy remains to be established experimentally.

8. ACKNOWLEDGMENTS

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Abbreviations: CR3: complement receptor 3, FRET: fluorescence resonance energy transfer, ICAM-1: intercellular adhesion molecule-1, IL-1β: interleukin-1β, mAb: monoclonal antibody, NF-κB: nuclear factor-κB, PI3K: phosphatidylinositol 3-kinase, PRR: pattern-recognition receptor, TLR: Toll-like receptor, TNF-α: tumor necrosis factor-α

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