Peptide Mapping of Bacterial Fimbrial Epitopes Interacting with Pattern Recognition Receptors*

Received for publication, July 6, 2005, and in revised form, August 3, 2005. Published, JBC Papers in Press, August 29, 2005, DOI 10.1074/jbc.M507326200

George Hajishengallis1, Pukar Ratti, and Evlambia Harokopakis

From the Center of Excellence in Oral and Craniofacial Biology and Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70119

The fimbrae of the oral pathogen Porphyromonas gingivalis induce Toll-like receptor 2 (TLR2)-dependent macrophage activation upon their recognition by CD14 and the β2 integrin CD11b/CD18. To map functional epitopes of fimbrae that interact with these pattern recognition receptors (PRRs), we examined 20 synthetic peptides covering the entire length of the 41-kDa fimbrillin subunit. Using direct or competitive inhibition assays for receptor binding or cell activation, the CD14 binding activity of fimbrae was localized to residues 69–90 and was essential for TLR2-dependent cytokine induction. The CD11b/CD18 binding activity of fimbrae was localized to two neighboring epitopes defined by residues 166–185 and 206–225. Unlike epitope 69–90 that constitutively bound CD14, the CD11b/CD18 binding activity of epitopes 166–185 and 206–225 was inducible by integrin activators. The CD11b/CD18 binding activity played a contributory role to TLR2-dependent induction of tumor necrosis factor-α by fimbrae but was involved in specific down-regulation of interleukin-12. Cell activation by a combination of fimbrillin peptides corresponding to the CD14 and CD11b/CD18 binding activities resulted in higher tumor necrosis factor-α responses than would be expected from a simply additive effect, attributable to CD14-dependent inside-out signaling leading to enhanced binding interactions with CD11b/CD18. These data suggest that P. gingivalis fimbrae display a modular structure that interacts through discrete epitopes and in a regulated mode with distinct PRRs, which in turn differentially modulate the state of cell activation. Elucidation of pathogen interactions with PRRs at the molecular level may glean insight into host defense mechanisms as well as into microbial strategies that subvert innate immunity.

Porphyromonas gingivalis is a Gram-negative bacterium that has been strongly associated with periodontal disease (1) and has more recently been implicated in systemic inflammatory conditions such as atherosclerosis (2–4). Studies in animal models of periodontitis or atherosclerosis have established the fimbrae (filamentous appendages on the cell surface) of P. gingivalis as a major virulence factor of this pathogen (5, 6). In vitro mechanistic studies have shown that fimbrae display adhesive properties that enable P. gingivalis to bind diverse extracellular substrates (7, 8) or to interact with a variety of cell types (9–12). The multifunctional adhesive capacity of P. gingivalis fimbrae may result from versatile structural motifs, which in turn may offer pattern recognition substrates for the innate host defense. Indeed pattern recognition receptors (PRRs)2 of the innate immune system can detect the presence of fimbrae and respond by inducing release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) (13–15).

Monocytes/macrophages constitute a major source of TNF-α production, and their number increases in periodontal inflammation compared with healthy periodontal tissue (16, 17). These cells express multiple PRRs, including CD14 and CD11b/CD18, which play an important accessory role in Toll-like receptor (TLR)-dependent innate immune and inflammatory responses (18–20). It is thought that these PRRs function as TLR-associated co-receptors that detect microbial pathogens or components thereof and present them to TLRs for activation of signaling pathways (19, 21). In our efforts to determine the PRRs and the mechanisms involved in innate immune recognition of P. gingivalis fimbrae, we have found that CD14 directly binds fimbrae (22) and mediates their ability to stimulate TLR2-dependent TNF-α release (14, 23). The β2 integrin CD11b/CD18 is also involved in P. gingivalis fimbra-induced cell activation (23), although it does not constitutively recognize this bacterial molecule (22). However, the ligand binding activity of CD11b/CD18 is stimulated through an inside-out signaling pathway that is activated by fimbrae and involves the participation of CD14, TLR2, and phosphatidylinositol 3-kinase (PI3K) (22).

Although CD14 and CD11b/CD18 function as constitutive and inducible binding receptors for fimbrae, respectively, it is unknown which fimbrial segments interact with which receptor. The objective of the current study was to identify fimbrial epitopes that interact with CD14 or CD11b/CD18 leading to cell activation. Our approach was based on the use of a series of synthetic peptides covering the entire length of the fimbrillin (FimA) subunit of P. gingivalis fimbrae. Similar FimA peptides were used previously to identify fimbrial domains interacting with fibronectin (8) or salivary proteins (7). We found that CD14 and CD11b/CD18 recognize distinct fimbrial domains; CD14 constitutively interacts with a domain corresponding to amino acid residues (aa) 69–90, whereas CD11b/CD18 inducibly interacts with fimbrial segments defined by aa 166–185 and 206–225. Moreover CD14 was found to be indispensable for cell activation by fimbrial peptide or intact protein, whereas fimbrae-CD11b/CD18 interactions are involved in both positive and negative regulation of innate immune responses. It appears, therefore, that P. gingivalis fimbrae interact through discrete domains with CD14 and CD11b/CD18, which in turn differentially modulate the activation state of monocytes/macrophages.

* This work was supported by United States Public Health Service Grant DE015254 from the NIDCR, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Oral Health and Systemic Disease Research Center, Dept. of Periodontics and Endodontics, University of Louisville School of Dentistry, Rm. 206, 501 S. Preston St., Louisville, KY 40202. Tel.: 502-852-5276; Fax: 502-852-4052; E-mail: ghajali01@louisville.edu.

2 The abbreviations used are: PRR, pattern recognition receptor; TNF-α, tumor necrosis factor-α; TLR, toll-like receptor; PI3K, phosphatidylinositol 3-kinase; aa, amino acid residue(s); CHO, Chinese hamster ovary; mAb, monoclonal antibody; NF-κB, nuclear factor κB; fMLP, N-formyl-Met-Leu-Phe; BSA, bovine serum albumin; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; PI(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; FITC, fluorescein isothiocyanate; CR3, complement receptor 3.
EXPERIMENTAL PROCEDURES

FimA Peptides—Synthetic peptides (17–22 aa) covering the entire length of *P. gingivalis* fimbriin (FimA) (TABLE ONE), in standard or biotinylated form were synthesized by SynPep (Dublin, CA) or the Peptide Synthesis Core Laboratories of the Louisiana State University Health Sciences Center, New Orleans, LA. For control purposes, the reverse amino acid sequences were also synthesized for selected FimA peptides that gave positive results in cell activation assays (*i.e.* peptides 5, 11, and 13). All peptides were >95% pure (by high pressure liquid chromatography) and tested negative for endotoxemia activity in the *Limulus* amebocyte lysate assay (BioWhittaker, Walkersville, MD). Native fimbriae were used as a positive control in the peptide experiments and were purified from *P. gingivalis* 381 as described previously (22) based on the method developed by Lee et al. (24).

Cells—Monocytes were purified from the peripheral blood of healthy human volunteers as described previously (22). Briefly monocytes were separated from lymphocytes upon centrifugation of peripheral blood over Nycodenz™ 1.068 (Axis-Shield, Oslo, Norway). Incidental non-monocytes were removed by magnetic depletion using a mixture of biotin-conjugated monoclonal antibodies (mAbs) and magnetic microbeads coupled to anti-biotin mAb (monocyte isolation kit II; Miltenyi Biotec, Auburn, CA). Purified monocytes were cultured in 96-well polystyrene culture plates at 37 °C and 5% CO2 in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.05 mM 2-mercaptoethanol (complete RPMI). The human monocytic THP-1 cell line (ATCC TIB-202) was differentiated into a macrophage phenotype after a 3-day incubation in complete RPMI supplemented with 10 ng/ml phorbol myristate acetate (25). Differentiated THP-1 cells were used for cytokine induction assays (see below). To determine the binding of biotinylated FimA peptides to cell surface CD14 (see below), we used undifferentiated THP-1 cell lines stably transfected with human CD14 (THP-1/CD14) or with empty vector (THP-1/RSV) (26). Both clones were kindly provided by Dr. R. J. Ulevitch (The Scripps Research Institute, La Jolla, CA). Mouse macrophages were isolated from the peritoneal cavity of *C57BL/6* wild-type mice (The Jackson Laboratory, Bar Harbor, Maine) upon thioglycollate-induction elicitation (30). The cells were cultured in complete RPMI as above except for the use of autologous serum from CD14-deficient mice in assays involving comparison of CD14-deficient macrophages against wild-type controls. Chinese hamster ovary (CHO) cells stably transfected with human complement receptor 3 (CR3) (CD11b/CD18) or CR1 (CD35) were kindly provided by Dr. Douglas T. Golenbock (University of Massachusetts Medical School, Worcester, MA) (31). Both cell lines were cultured in Ham’s F-12 nutrient mixture (Invitrogen) supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin.

Cytokine Induction Assays—Human monocytes, differentiated THP-1 cells, or mouse macrophages (*1.5 × 10^6/well*) were stimulated with FimA peptides (10–500 µg/ml) or native fimbriae (1–10 µg/ml) for 16 h at 37 °C. For control purposes, cells were treated with medium supplemented with the appropriate concentration of MeSO (vehicle control for peptide stimulation). Culture supernatants were collected at the end of the experiment and stored at −80 °C until assayed for TNF-α, interleukin (IL)-6, or IL-12 p70 responses using ELISA kits (eBioscience, San Diego, CA). None of the peptides or native protein affected cell viability as determined by trypsin blue exclusion. In assays involving peptide interactions with activated human CD11b/CD18, cells were pretreated for 30 min with 10 µg/ml VIM12 (Caltag, Burlingame, CA), an activating mAb to CD11b (32). In certain experiments, cell activation by FimA peptides was performed in the absence or presence of blocking mAbs to CD14 (MEM-18; Caltag), CD11b (2LPM19c; DakoCytomation, Carpinteria, CA), or immunoglobulin isotype-controlled experiments (IgG1; e-Bioscience).

Nuclear Factor κB (NF-κB) Activation Assay—Activation of the transactivating p65 subunit of NF-κB was determined by means of a NF-κB/p65 transcription factor assay kit (Active Motif, Carlsbad, CA) (33). This is an ELISA-based procedure in which the detecting antibody recognizes an epitope of NF-κB p65 that is accessible only when NF-κB is activated and bound to its target DNA (containing the NF-κB consensus binding site 5’-GGGACTTTCC-3’) attached to 96-well plates. Preparation and ELISA were carried out according to protocols supplied by the manufacturer. Monocytes were incubated with FimA peptides or native fimbriae as described above for cytokine induction assays except for the time used for stimulation; the optimal stimulation time (90 min) and the amount of total protein used in the ELISA (7.5 µg) were determined in earlier experiments (33, 34).

**PI3K Activation Assay**—PI3K activity was measured as enzymatic production of PI[3(4,5)P3 from PI(4,5)P2 substrate by means of a PI3K ELISA kit following the instructions of the manufacturer (Echelon Biosciences, Salt Lake City, UT). Briefly PI3K was immunoprecipitated from cell lysates using anti-PI3K antibody and protein-A agarose beads, and the bead-bound enzyme was subsequently incubated with 100 pmol of PI(4,5)P2 substrate in kinase reaction buffer (4 mM MgCl2, 20 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 25 µM ATP) for 2 h at room temperature. The generation of PI(3,4,5)P3 product was determined in competitive ELISA. Specifically the reaction product was incubated with PI(3,4,5)P3 detector protein for 1 h at room temperature in the dark, and the mixture was then added to a PI(3,4,5)P3-coated microplate for competitive binding. After a 30-min incubation at room temperature in the dark, the plate was washed. The manufacturer’s peroxidase-linked secondary reagent was then added to colorimetrixically probe plate-bound PI(3,4,5)P3, detector protein. The colorimetric signal is inversely proportional to the amount of PI(3,4,5)P3 produced by PI3K activity, which was calculated from a calibration curve generated using known concentrations of PI(3,4,5)P3 standard.

**CD11b/CD18 Activation Assay**—The CBRM1/5 epitope induction assay was used to monitor the activation state of CD11b/CD18 as we have described previously (22). The assay is based on the property of the CBRM1/5 mAb to detect a conformational change on CD11b that signifies the high affinity binding state of CD11b/CD18 (35). The CBRM1/5 mAb was determined to detect a conformational change on CD11b that signifies the high affinity binding state of CD11b/CD18 (35).

**CD14 Binding Assay**—The binding of biotinylated FimA peptides (100 µg/ml) or native fimbriae (0.25 µg/ml) to plate-immobilized CD14 was determined as described previously (22) based on the method developed by Cunningham et al. (36). Briefly 96-well microtiter plates were coated overnight with 2 µg/ml CD14 or CD40 as receptor control (R&D Systems, Minneapolis, MN). Nonspecific binding sites were blocked with 5 mg/ml bovine serum albumin (BSA). Biotinylated fimbriae or peptides were allowed to bind to the plates for 30 min at 37 °C. After washing, bound biotinylated molecules were detected with peroxidase-conjugated streptavidin followed by addition of tetramethylbenzidine chromogenic substrate. The optical density signal at 450 nm was read in a Bio-Tek Instruments (Winooski, VT) microplate reader.

**Binding of FimA Peptides to Primary Cells or Cell Transfectants**—Biotinylated FimA peptides (100 µg/ml) were allowed to bind to monocytes (*1.5 × 10^6 cells/well*) for 30 min at 37 °C in complete RPMI as described previously (22). To activate the ligand binding capacity of CD11b/CD18, the cells were pretreated for 30 min with 10 µg/ml

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RESULTS

Screening of FimA Peptides for Induction of Cell Activation—A series of synthetic peptides (17–22 aa) covering the entire length of P. gingivalis fimbrillin (TABLE ONE) were examined for their ability to activate human primary monocytes or differentiated (macrophage-type) THP-1 cells. In a preliminary screening of the peptides for TNF-α induction in monocytes, only FimA peptide 5 (aa 69–90) induced TNF-α release at levels (283 ± 36 pg/ml) substantially higher than background levels (≤20 pg/ml) in unstimulated cells. However, we thought that FimA peptides that could potentially induce TNF-α release through interaction with CD11b/CD18 could not demonstrate this effect under the experimental conditions used. This is because CD11b/CD18 requires the presence of 100 ng/ml phorbol myristate acetate for 30 min at 37°C. Unbound peptides were removed by washing, and the cells were then incubated on ice with FITC-labeled streptavidin in the dark. After washing, the binding of peptides was determined by measuring cell-associated fluorescence as described above.

Statistical Analysis—Data were evaluated by analysis of variance and the Dunnett multiple comparison test using the InStat program (GraphPad Software, San Diego, CA). Where appropriate (comparisons involving two groups only), two-tailed t tests were performed. Statistical differences were considered significant at the level of p < 0.05.

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CD14 or THP-1/RSV (Fig. 2B). The data from Fig. 2 demonstrate that peptide 5 defines a FimA region (aa 69–90) that is involved in binding interactions with CD14. These CD14 interactions are specific in the sense that peptide 5 does not bind CD40, BSA, or CD14-non-expressing cells; moreover CD14 binding interactions are not shared by other FimA peptides (peptides 11 and 13) that induce cell activation.

We next determined whether the ability of FimA peptide 5 to activate human monocytes is similarly dependent upon CD14. Indeed we found that the ability of peptide 5 to induce activation of NF-κB is significantly inhibited by anti-CD14 but not by anti-CD11b or IgG1 isotype control. Moreover a control peptide of the same amino acid composition but in reverse sequence failed to activate monocytes (Fig. 3). Similar results were obtained when differentiated THP-1 cells were used in lieu of primary monocytes (not shown). These data demonstrate that CD14 mediates cellular activation in response to a specific FimA sequence defined by aa 69–90.

**Competitive Inhibition by FimA Peptide 5 of Native Fimbriae-CD14 Interactions**—If the binding of native fimbriae to CD14 involves a region defined by FimA peptide 5 (aa 69–90), as suggested above, peptide 5 should be able to competitively inhibit the binding of native fimbriae to immobilized CD14. To investigate this possibility, biotinylated native fimbriae were allowed to bind to CD14 in the presence of increasing concentrations of peptide 5 or its reverse sequence peptide control (Fig. 4A). We observed a partial but statistically significant (p < 0.05) dose-dependent inhibition of native fimbriae by peptide 5 but not by the control peptide (Fig. 4A). Maximal inhibition (about 53%) was observed at a concentration of peptide 5 that was 500-fold higher than that of native fimbriae (Fig. 4A). At this concentration (125 μg/ml), no other FimA peptide could inhibit the binding of biotinylated fimbriae to CD14.
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FIGURE 2. Binding of FimA peptides to CD14. A, microtiter wells were coated with CD14 or CD40 (receptor control) or were left untreated. After blocking uncoated sites with BSA, 100 μg/ml biotinylated FimA peptides (peptides 5, 11, and 13; TABLE ONE) were allowed to bind to the immobilized receptors for 30 min at 37 °C. Bound peptides were colorimetrically detected by ELISA using streptavidin-conjugated peroxidase. Background binding was determined in cells treated with medium only (no peptide) and streptavidin-peroxidase (SA-HRP control). B, THP-1/CD14 and CD14-nonzexpressing THP-1/RSV cells were incubated with biotinylated FimA peptides (100 μg/ml) for 30 min at 37 °C. Peptide binding was measured as cell-associated fluorescence (relative fluorescence units (RFU)) after cell staining with streptavidin-FITC. Background binding was determined in cells treated with medium only (no peptide) and streptavidin-FITC (SA-FITC control). Data are shown as means ± S.D. (n = 3) from one of two independent experiments that yielded similar results. Asterisks indicate statistically significant (p < 0.05) differences in peptide binding to plate-immobilized CD14 (A) or to CD14-expressing cells (B) compared with corresponding controls.

(4B). Unlabeled native fimbriae at 100-fold excess resulted in potent competitive inhibition (85%) of the labeled molecule (Fig. 4B). These data suggest that peptide 5 defines a CD14-binding region that is shared with the native molecule.

Because the CD14-binding peptide 5 is a relatively weak cytokine-inducing agonist (Fig. 1), it may function as an antagonist of native fimbriae with regard to induction of inflammatory responses. Indeed, inducing agonist (Fig. 1), it may function as an antagonist of native fimbriae with regard to induction of inflammatory responses. Indeed, inducing agonist (Fig. 1), it may function as an antagonist of native fimbriae with regard to induction of inflammatory responses.

Interactions of FimA Peptides with CD11b/CD18—The FimA peptides 11 and 13 failed to induce monocyte activation unless the cells were pretreated with VIM12 mAb (Fig. 1). Because VIM12 is known to activate the ligand binding capacity of CD11b/CD18 (32), we hypothesized that FimA peptides 11 and 13 bind to activated CD11b/CD18. If this is true, the ability of these peptides to bind VIM12-pretreated monocytes should be inhibitable by 2LPM19c, a mAb that blocks the CD11b ligand-binding domain (38). In a preliminary experiment, we confirmed that biotinylated FimA peptides 11 and 13 do not bind medium only- or IgG1 isotype control-pretreated monocytes but readily bind VIM12-treated monocytes (not shown). We then examined whether the binding of these peptides to VIM12-pretreated monocytes is inhibited by 2LPM19c (Fig. 6). We found that the binding of both peptides was potently inhibited by 2LPM19c but not by MEM-18 (a CD14-specific mAb) or by IgG1 isotype control (Fig. 6A). Conversely the binding of the CD14-interacting FimA peptide 5 was inhibited by MEM-18 but not by 2LPM19c (Fig. 6A). A similar binding pattern was observed when FimA peptides 11 and 13 were allowed to interact with monocytes exposed to fMLP (10−7 M) (Fig. 6B), a physiologically relevant agonist that we have shown to induce the high affinity conformation of CD11b/CD18 (22). We next demonstrated that the ability of FimA peptides 11 and 13 to induce TNF-α release in activated monocytes is similarly inhibited by 2LPM19c but not by MEM-18, whereas the converse was true for the FimA peptide 5 (Fig. 6C).

To conclusively show that peptides 11 and 13 bind to CD11b/CD18, we examined their binding to CHO cells transfected with CD11b/CD18 (CR3) ("CHO/CR3 cells"). CHO cells transfected with CD35 (CR1) ("CHO/CR1") were used as negative controls. We found that both peptides 11 and 13 bound CHO/CR3 but not CHO/CR1 (Fig. 6D). In contrast, FimA peptide 12, which corresponds to the intervening region (TABLE ONE), did not exhibit significant binding to CHO/CR3 compared with CHO/CR1 cells (Fig. 6D). Peptide 5 was used as control in this assay and did not bind either cell line (Fig. 6D). The Fig. 6 results collectively suggest that the regions defined by...
peptides 11 and 13 (residues 166–185 and 206–225, respectively) are important for the ability of P. gingivalis fimbriae to interact with CD11b/CD18.

**Competitive Inhibition by CD11b/CD18-interacting FimA Peptides of the Cell Binding Activity of Native Fimbriae**—We then showed that FimA peptides 11 and 13, but not control peptides displaying reverse amino acid sequences, inhibited partially but significantly ($p < 0.05$) the ability of biotinylated native fimbriae to bind monocytes (Fig. 7A). Inhibition was somewhat more effective when the two inhibitor peptides were combined, although the combination of their reverse amino acid sequences, inhibited partially but significantly ($p < 0.05$) the ability of native fimbriae to induce TNF-$\alpha$ by FimA peptides 5, 11, and 13 using an independent approach, we determined the ability of macrophages from wild-type mice or mice deficient in CD11b to respond to the peptides.

**Relative Contribution of CD14 and CD11b/CD18 in Cytokine Induction by Fimbriae and Synthetic Peptides**—To confirm the PRR specificities of FimA peptides 5, 11, and 13 using an independent approach, we determined the ability of macrophages from wild-type mice or mice deficient in CD11b to respond to the peptides. A second objective was to determine the relative importance of CD14 and CD11b/CD18 (the expression of which is undetectable in CD11b-deficient mice [28]) in fimbria-induced cytokine release. As expected, the ability of FimA peptide 5 to induce TNF-$\alpha$ was completely abrogated in CD14-deficient cells (Fig. 8A) but was unaffected in CD11b-deficient cells (Fig. 8C). The converse was true for FimA peptides 11 and 13, i.e., they could induce TNF-$\alpha$ release in CD14-deficient but not in CD11b-deficient macrophages (Fig. 8, A
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and C). As with the human monocyte model (Fig. 6B), mouse macrophages incubated with FimA peptides 11 and 13 also received fMLP (10^{-7} M) to activate CD11b/CD18 and enable the cells to respond to these peptides (fMLP alone did not induce detectable TNF-α release; not shown). Although native fimbriae induced high levels of TNF-α in wild-type macrophages, the production of this cytokine was undetectable in CD14-deficient cells (Fig. 8A) and partially reduced (by about 40%) in CD11b-deficient cells (Fig. 8C). A similar pattern was observed for IL-6 (i.e. induction of IL-6 was abrogated in CD14-deficient but only partially reduced in CD11b-deficient cells; data not shown). Surprisingly, however, we detected low levels (40 ± 9 pg/ml) of IL-12 p70 in the supernatants of CD11b-deficient macrophages stimulated with native fimbriae, whereas no IL-12 p70 was detectable in similarly stimulated wild-type or CD14-deficient cells. To better investigate this observation, the experiment was repeated in the presence of interferon-γ (1 µg/ml) to prime the cells for IL-12 p70 induction. Although fimbriae induced a modest IL-12 p70 response in interferon-γ-primed wild-type macrophages (≈210 pg/ml; Fig. 8B and D), the response was increased about 6-fold in CD11b-deficient cells (Fig. 8D) but was abolished in CD14-deficient cells (Fig. 8B). In interferon-γ-primed wild-type cells, FimA peptide 5 (but not peptides 11 and 13) induced a weak but detectable IL-12 p70 response, which remained unaltered in CD11b-deficient cells (Fig. 8, D and inset) but was abolished in CD14-deficient cells (Fig. 8B). Taken together, these data confirm that CD14 and CD11b/CD18 respond to different fimbrial epitopes and suggest that these PRRs exert differential effects on cell activation by P. gingivalis fimbriae.

Role of TLR2 in Cell Activation by FimA Peptides—The ability of P. gingivalis fimbriae to induce activation of human monocytes is inhibited by anti-TLR2 mAb (22, 23, 34). This was confirmed in the current study because fimbriae lost at least 90% of their cytokine-inducing capacity in TLR2-deficient macrophages in comparison with wild-type controls (Fig. 8, E–G). Moreover TLR2-deficient macrophages completely failed to respond to the CD14-interacting FimA peptide 5 (Fig. 8, E–G). In contrast, the CD11b/CD18-interacting peptides (peptides 11 and 13) maintained about 30–40% of their ability to induce release of TNF-α (Fig. 8E) or IL-6 (Fig. 8F) in TLR2-deficient macrophages compared with wild-type controls. The remaining FimA peptides (TABLE ONE) were not tested in TLR2-deficient macrophages because preliminary experiments showed that they were unable to induce detectable cytokine responses in wild-type macrophages (not shown). These data suggest that peptides 5, 11, and 13 define fimbrial epitopes that induce TLR2-dependent cell activation, although only peptide 5 is entirely dependent on TLR2 signaling for cytokine induction.

Induction of Inside-out Signaling by FimA Peptide 5—Peptide 5 (aa 69–90) is capable of binding CD14 and activating TLR2 signaling (Figs. 2, 8, and 9). We thus determined whether peptide 5 can furthermore activate the inside-out signaling pathway that is downstream of CD14/TLR2 and leads to PI3K-dependent activation of CD11b/CD18 as we have shown earlier for native fimbriae (22). Peptide 5 was indeed found to activate PI3K and to induce the high affinity conformation of CD11b/CD18, although with considerably lower potency (≈16%) compared with native fimbriae (TABLE TWO). A control peptide (displaying the reverse amino acid

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**FIGURE 6.** Interactions of FimA peptides with activated CD11b/CD18 (CR3). Primary human monocytes were pretreated with CD11b/CD18 activators, VIM12 (A and C) or fMLP (B), in the absence or presence of blocking mAbs to CD11b (2LPM19c) or CD14 (MEM-18) or IgG1 isotype control (all antibodies at 10 µg/ml). In A and B, the cells were either left without further treatment or were incubated for 30 min at 37 °C with biotinylated FimA peptides 11 and 13 (100 nM) for 1 h at 4 °C in the absence or presence of blocking mAbs to CD11b (2LPM19c) or CD14 (MEM-18) or IgG1 isotype control (all antibodies at 10 µg/ml). Peptide binding was measured as cell-associated fluorescence after cell staining with streptavidin-FITC. Background binding was determined in cells treated with medium only and streptavidin-FITC (SA-FITC control). In C, the cells were treated exactly as in the experiment shown in A except that non-biotinylated peptides were used, and incubation was carried out for 16 h to assay induction of TNF-α release. In D, biotinylated FimA peptides were examined for binding to CHO/CR3 cells or to CD11b/CD18-nexpressing controls (CHO/CR1). Data are presented as means ± S.D. (n = 3) of typical experiments that were performed twice and yielded similar results. In A–C, asterisks indicate statistically significant (p < 0.05) inhibition by mAb treatment of the ability of FimA peptides to bind (A and B) or activate (C) monocytes. In D, asterisks indicate significant (p < 0.05) binding of FimA peptides to CHO/CR3 compared with CHO/CR1 control cells. ctrl, control; RFU, relative fluorescence units.
sequence compared with peptide 5) and peptides 11 and 13 were completely inactive in this regard (TABLE TWO). The lack of activity by peptides 11 and 13 is consistent with the requirement for integrin activators (VIM12, fMLP, or phorbol myristate acetate) to facilitate their interactions with CD11b/CD18. We then examined whether the ability of FimA peptide 5 to activate CD11b/CD18 is sufficient for a synergistic effect with peptide 11 or 13 on cell activation. Such a reductionist system would, at least in principle, provide the necessary PRR-interacting FimA epitopes for reconstructing a native fimbrial type effect. Addition of peptide 5 together with peptide 11 or 13 (or both) to the monocyte culture system resulted in higher TNF-α responses than what would be expected from simply an additive effect (Fig. 9A), although the obtained responses were at least 4-fold lower than when monocytes were activated with peptide 11 and/or 13 in the presence of potent integrin activators such as VIM12 mAb (not shown). Similarly the ability of peptide 5 to enhance the binding of peptide 11 or 13 to monocytes was only about 12–17% of the enhancement seen in the presence of VIM12 (Fig. 9B).

The data from TABLE TWO and Fig. 9 collectively suggest that a fimbrillin region defined by residues 69–90 plays a role in the ability of fimbriae to induce inside-out signaling for CD11b/CD18 activation.

**DISCUSSION**

In this study we mapped functional epitopes of the *P. gingivalis* fimbriae interacting with PRRs involved in monocyte/macrophage activation. Our findings indicate that fimbriae interact through distinct epitopes and in a regulated mode with CD14 and CD11b/CD18, which in turn mediate differential downstream effects. Specifically a fimbrial epitope defined by aa 69–90 of the fimbrillin subunit interacts with CD14, whereas the epitopes defined by aa 166–185 and 206–225 interact with CD11b/CD18. The CD14 binding activity of fimbriae was entirely dependent on TLR2 for downstream signaling and cell activation. In contrast, the CD11b/CD18 binding activity was partially dependent on TLR2 for downstream signaling and cell activation and mediated both positive (TNF-α induction) and negative regulation (IL-12 p70 induction) of the macrophage activation state. We do not know which signaling receptor is responsible for the residual TNF-α-inducing activity of CD11b/CD18-interacting FimA peptides in TLR2-deficient macrophages. However, this finding suggests that CD11b/CD18 may not simply act as a co-receptor for TLR2 activation but may additionally induce TNF-α through outside-in signals transduced by its own cytoplasmic tails. Alternatively another TLR may be involved. However, we have ruled out involvement of TLR4 because TLR4-deficient macrophages respond normally to native fimbriae or FimA peptides.

It has been rather puzzling how a given PRR, such as CD14, is capable of interacting with a variety of chemically diverse ligands (e.g. lipopolysaccharide, lipoteichoic acid, bacterial lipoproteins, etc.) that do not exhibit apparent structural similarities. In this regard, it was recently proposed that the ligand promiscuity of PRRs could at least partially be explained by hydrophobic interactions, which are relatively less dependent on exact molecular fit than other types of chemical interactions (39). Crystallographic analysis of CD14 has revealed a horseshoe-shaped structure with a deep hydrophobic pocket, the top of which is surrounded by a flexible rim and multiple external grooves that are thought to participate in ligand binding (40). Most TLR agonists contain either lipid moieties (e.g. lipopolysaccharide, lipoteichoic acid, and

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**FIGURE 7.** CD11b/CD18-interacting FimA peptides (peptides 11 and 13) inhibit the cell binding activity of native fimbriae. Human monocytes (A and B) or CHO/CR3 cells (C) were incubated for 30 min at 37 °C with biotinylated native fimbriae (0.5 μg/ml) in the absence or presence of 200 μg/ml FimA peptides (peptides 11, 13, or 5), control peptides displaying reverse amino acid sequences (rsq), or combinations of peptides as indicated. The binding of biotinylated fimbriae was measured as cell-associated fluorescence after cell staining with streptavidin-FITC. Data are presented as means ± S.D. of triplicate determinations from one set of experiments that was performed two (A and C) or three times (B) with similar findings. Asterisks indicate statistically significant (p < 0.05) inhibition of the ability of native fimbriae for cell binding.

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3 G. Hajishengallis, P. Ratti, and E. Harokopakis, unpublished observations.
lipoproteins) or extensive hydrophobic peptide regions (e.g., bacterial flagellin and fimbriae), whereas the TLR themselves contain leucine-rich repeat regions that are believed to engage in hydrophobic interactions (39). Interestingly, the adhesive capacity of *P. gingivalis* for host cells and extracellular matrix has been linked to its surface hydrophobicity, which in turn has been correlated with its fimbriation (41, 42). The polymeric nature of fimbriae, due to the repeated protein subunits, is expected to contribute to their potential for PRR interactions. Theoretically, if fimbrillin displays a certain epitope with low binding affinity for a PRR, the multiplicity of such epitopes along the polymeric mole-

![Figure 8](image)

**FIGURE 8.** Cytokine induction by fimbriae and synthetic peptides in PRR-deficient macrophages. Mouse macrophages from wild-type mice or mice deficient in CD14 (A and B), CD11b (C and D), or TLR2 (E–G) were incubated with medium only, FimA peptides (pept., 100 µg/ml), or native fimbriae (1 µg/ml). After 16 h, culture supernatants were assayed for induction of TNF-α, IL-6, or IL-12 p70. In B, D, and G, cells additionally received interferon-γ (1 µg/ml) to prime them for induction of IL-12 p70 release. Results are shown as means ± S.D. of triplicate determinations from one set of experiments performed twice with similar findings. The inset in D shows the same peptide-induced responses with more clarity. Asterisks indicate statistically significant (p < 0.05) differences in cytokine induction by the same agonist in PRR-deficient macrophages versus wild-type controls.
Mapping of Fimbrial Epitopes Interacting with Host Receptors

TABLE TWO

| FimA peptide tested | Activity (means ± S.D.; n = 3) compared with native fimbriae in assay for |  |
|---------------------|-------------------------------------------------------------------------|---|
|                     | PI3K activation<sup>a</sup> | CD11b/CD18 activation<sup>b</sup> |
| Peptide 5 (aa 69–90) | 15.6 ± 3.5<sup>c</sup> | 13.4 ± 4.5<sup>c</sup> |
| Control peptide 5 (reverse) | 3.8 ± 3.3 | 3.3 ± 2.1 |
| Peptide 11 (aa 166–185) | 1.6 ± 1.8 | 2.8 ± 2.3 |
| Peptide 13 (aa 206–225) | 2.5 ± 3.2 | 1.7 ± 1.6 |

<sup>a</sup> Assayed as enzymatic production of PI(3,4,5)P3 from PI(4,5)P2 substrate. Induction of the lipid kinase activity of PI3K by native fimbriae resulted in production of 36.7 ± 4.2 pmol of PI(3,4,5)P3.

<sup>b</sup> Assayed as induction of an activation-specific epitope probed by FITC-labeled CBRM1/5 mAb. The value obtained upon induction using native fimbriae was 76,768 ± 9,895 relative fluorescence units.

<sup>c</sup> Statistically significant (p < 0.05) differences compared to reverse sequence control or peptides 11 and 13.

### FIGURE 9

A. human monocytes were incubated with vehicle control (vc) or synthetic FimA peptides (pept., 100 μg/ml) either alone or in combinations, and culture supernatants were collected after 16 h to assay induction of TNF-α release. B, the ability of biotinylated peptide 11 or 13 to bind monocytes was assayed in the absence or presence of non-biotinylated peptide 5 or VIM12 mAb (integrin activator control). Binding was measured as cell-associated fluorescence after cell staining with streptavidin-FITC. Background binding was determined in cells treated with medium only and streptavidin-FITC (SA-FITC control). Results are shown as means ± S.D. (n = 3) from one set of experiments performed twice with similar results. Asterisks in B indicate statistically significant (p < 0.05) increase of the binding activity of peptide 11 or 13 in the presence of integrin activators. RFU, relative fluorescence units.

of FimA peptide 5 enables it to act (when in excess) as a specific antagonist of native fimbria-induced TNF-α responses (Fig. 5A) by competitively blocking binding to CD14 (Fig. 4A). This might be a useful molecular strategy to control excessive fimbria-induced periodontal inflammation without completely inhibiting the innate immune defense.

In terms of evolution, PRRs are thought to have evolved to recognize conserved pathogen-associated molecular patterns such as lipopolysaccharide and lipoteichoic acid (43). Pathogen-associated molecular patterns and virulence factors are not equivalent terms and generally represent distinct microbial molecules. Unlike the relatively conserved nature of pathogen-associated molecular patterns, genes encoding microbial virulence proteins may mutate to prevent innate immune recognition. However, if exploitation of PRRs is essential for the survival of the pathogen (e.g. for colonization), the virulence factors involved are expected to display some relatively invariant motifs that are potentially recognizable by PRRs. The fimbriae of *P. gingivalis* function as a major adhesin and constitute an important virulence factor of this pathogen (5, 6). It seems unlikely that PRRs have evolved to specifically recognize this fimbrial structure, which is not shared by any other bacteria (44). Rather it seems more plausible to speculate that *P. gingivalis* fimbriae have evolved to recognize and interact with host receptors for the benefit of the pathogen. For example, the fimbriae promote the uptake of *P. gingivalis* into host epithelial cells (12, 45) where the pathogen can survive and replicate (46). Other pathogens, such as *Mycobacterium tuberculosis*, exploit CD11b/CD18 as a mechanism for entrance and intracellular parasitism (47). It is currently unknown whether *P. gingivalis* can similarly induce its uptake by monocytes through fimbriae-CD11b/CD18 interactions resulting in survival rather than postphagocytosis killing. If this is true, the fimbria-induced inside-out signaling pathway leading to CD11b/CD18 activation (22) may represent a strategy for access into a nutritionally rich and immunologically safe site for the pathogen. Although inflammation is a potentially protective host response, induction of fimbria-induced periodontal inflammation could also benefit *P. gingivalis* through acquisition of crucial nutrients derived from serum exudate into the crevicular area of the teeth. Moreover as discussed in greater detail below, we have obtained initial evidence that the interaction of *P. gingivalis* fimbriae with CD11b/CD18 leads to down-regulation of biologically active IL-12 (p70), a major cytokine in mediating bacterial clearance (48).

In contrast to CD14, which is essential for TLR2-dependent cytokine release by native fimbriae (or by the FimA peptide 5), CD11b/CD18 plays a contributory role but is not required for induction of TNF-α release by native fimbriae (Fig. 8B). Interestingly in the absence of this receptor (i.e. in CD11b-deficient macrophages), fimbriae induce
Mapping of Fimbrial Epitopes Interacting with Host Receptors

FIGURE 10. Epitopes of \textit{P. gingivalis} fimbriin (FimA) interacting with PRRs. CD14 recognizes a fimbriin region defined by aa 69–90. Following activation through an inside-out signaling pathway dependent upon CD14, TLR2, and PI3K, the CD11b/CD18 integrin is induced to recognize a fimbriin region involving two neighboring epitopes defined by aa 166–185 and 206–225.

increased levels of IL-12 \textit{p}70 (Fig. 8D). This suggests that binding to CD11b/CD18 down-regulates IL-12 \textit{p}70. Furthermore we have found that the ability of lipopolysaccharide to induce IL-12 \textit{p}70 in human monocytes is suppressed in the presence of the CD11b/CD18-interact-

In summary, we have defined two different epitopes from the same microbial molecule interacting with two distinct PRRs, leading to different downstream effects. Specifically \textit{P. gingivalis} fimbriae seem to display a modular structure that enables them to interact with CD14 and CD11b/CD18. Synthetic peptides representing fimbrial epitopes that interact with CD14 or CD11b/CD18 appear to mimic (alone or in combination) immunomodulatory effects induced by native fimbriae. Because CD14 and CD11b/CD18 differentially regulate cell activation, the modular structure of fimbriae may allow the development of molecular approaches that would selectively interfere with interactions that favor the pathogen.

Acknowledgment—We thank Dr. Seth Pincus for critical review of the manuscript.

REFERENCES

1. Zambon, J. J., Grossi, S., Dunford, R., Harazsathy, V. L., Preus, H., and Genco, R. J. (1994) in Molecular Pathogenesis of Periodontal Disease (Genco, R. J., Hamada, S., Lehrer, I. R., McGhee, I. R., and Mergenhangen, S., eds) pp. 3–12, American Society for Microbiology, Washington, D. C.

2. Harazsathy, V. L., Zambon, J. J., Trewisan, M., Zeid, M., and Genco, R. J. (2000) \textit{J. Periodontol.} 71, 1554–1560

3. Chun, Y. H., Chun, K. R., Olguin, D., and Wang, H. L. (2005) \textit{J. Periodontal Res.} 40, 87–95

4. Devsariex, M., Demmer, R. T., Rundek, T., Boden-Alkala, B., Jacobs, D. R., Jr., Sacco, R. L., and Papapanou, P. N. (2005) \textit{Circulation} 111, 576–582

5. Malek, R., Fisher, J. G., Calea, A., Stinson, M., van Oss, C. J., Lee, J. Y., Cho, M. I., Genco, R. J., Evans, R. T., and Dyer, D. W. (1994) \textit{J. Bacteriol.} 176, 1052–1059

6. Gibson, F. C., III, Hong, C., Chou, H. H., Yumoto, H., Chen, J., Lien, E., Wong, J., and Genco, C. A. (2004) \textit{Circulation} 109, 2801–2806

7. Amano, A., Sharma, A., Lee, J. Y., Soja, H. T., Raj, P. A., and Genco, R. J. (1996) \textit{Infect. Immun.} 64, 1631–1637

8. Soja, H. T., Lee, J.-Y., and Genco, R. J. (1995) \textit{Biochem. Biophys. Res. Commun.} 216, 785–792

9. Weinberg, A., Belton, C. M., Park, Y., and Lamont, R. J. (1997) \textit{Infect. Immun.} 65, 313–316

10. Deshpande, R. G., Khan, M. B., and Genco, C. A. (1998) \textit{Infect. Immun.} 66, 5337–5343

11. Iovani, R., and Cutler, C. W. (2004) \textit{Infect. Immun.} 72, 1725–1732

12. Giacosa, M. B., Papapanou, P. N., Lamster, I. B., Rong, L. L., D’Agati, V. G., Schmidt, A. M., and Lulla, E. (2004) \textit{FEBS Microbiol. Lett.} 241, 95–101

13. Ogawa, M., Arai, Y., Hashimoto, M., and Uchida, H. (2002) \textit{Eur. J. Immunol.} 32, 2543–2550

14. Hajishengallis, G., Martin, M., Sojar, H. T., Sharma, A., Schifferle, R. E., DeNardin, E., Russell, M. W., and Genco, R. J. (2002) \textit{Clin. Diagn. Lab. Immunol.} 9, 403–411

15. Zhou, Q., Desta, T., Fenton, M., Graves, D. T., and Ammar, S. (2005) \textit{Infect. Immun.} 73, 935–943

16. Muthukuru, M., Iovani, R., and Cutler, C. W. (2005) \textit{Infect. Immun.} 73, 687–694

17. Delma, A. J., and Van Dyke, T. E. (2005) \textit{Periodontol.} 2000 30, 55–76

18. Perera, P. Y., Mayadas, T. N., Takeuchi, O., Akira, S., Zaks-Zilberman, M., Goyert, S. M., and Vogel, S. N. (2003) \textit{Immunol.} 166, 574–581

19. Triantafillou, M., Brandenburg, K., Gutschmann, T., Seydel, U., and Triantafillou, K. (2002) \textit{Crit. Rev. Immunol.} 22, 251–268

20. Underhill, D. M. (2003) \textit{Eur. J. Immunol.} 33, 1767–1775

21. Akira, S., and Takekda, K. (2004) \textit{Nat. Rev. Immunol.} 4, 499–511

22. Harokopakis, E., and Hajishengallis, G. (2005) \textit{Eur. J. Immunol.} 35, 1201–1210

23. Hajishengallis, G., Sharma, A., Russell, M. W., and Genco, R. J. (2002) \textit{Ann. Periodontol.} 7, 72–78

24. Lee, J.-Y., Soja, H. T., Amano, A., and Genco, R. J. (1995) \textit{Protein Expr. Purif.} 6, 496–500

25. Hajishengallis, G., Nawar, H., Tapping, R. J., Russell, M. W., and Connell, T. D. (2004) \textit{Infect. Immun.} 72, 6351–6358

26. Pugin, J., Kravchenko, V. V., Lee, J. D., Kline, L., Ulevitch, R. J., and Tobias, P. S. (1998) \textit{Infect. Immun.} 66, 1174–1180

27. Moore, K. J., Andersson, I. P., Ingalls, R. R., Monks, B. G., Li, R., Arnaout, M. A., Golenbock, D. T., and Freeman, M. W. (2002) \textit{Infect. Immun.} 70, 4272–4280

28. Coxon, A., Rieu, P., Barkalow, F. J., Askari, S., Sharpe, A. H., von Andrian, U. H., Arnaout, M. A., and Mayadas, T. N. (1996) \textit{Immunol.} 6, 653–666

29. Wooten, R. M., Ma, Y., Yoder, R. A., Brown, J. P., Weis, J. H., Zachary, J. F., Kisching, C. J., and Weis, J. J. (2002) \textit{J. Immunol.} 169, 348–355

30. Hajishengallis, G., Tapping, R. J., Martin, M. H., Nawar, H., Lyle, E. A., Russell, M. W., and Connell, T. D. (2005) \textit{Infect. Immun.} 73, 1343–1349

31. Levy, S. M., Tabani, R., Kozel, T. R., MacGill, R. R., Ingalls, R. R., and Golenbock, D. T. (1997) \textit{Infect. Immun.} 65, 931–935

32. Stickel, J., Majdic, O., Pickl, W. F., Rosenkrantz, A., Prager, E., Gschwantler, E., and...
Mapping of Fimbrial Epitopes Interacting with Host Receptors

Knapp, W. (1995) J. Immunol. 154, 5452–5463
33. Hajishengallis, G., Martin, M., Schifferle, R. E., and Genco, R. J. (2002) Infect. Immun. 70, 6658–6664
34. Hajishengallis, G., and Genco, R. J. (2004) Infect. Immun. 72, 1188–1191
35. Diamond, M. S., and Springer, T. A. (1993) J. Cell Biol. 120, 545–556
36. Cunningham, M. D., Seachord, C., Ratcliffe, K., Bainbridge, B., Aruffo, A., and Darveau, R. P. (1996) Infect. Immun. 64, 3601–3608
37. Shimaoka, M., Takagi, J., and Springer, T. A. (2002) Annu. Rev. Biophys. Biomol. Struct. 31, 485–516
38. Diamond, M. S., Garcia-Aguilar, J., Bickford, J. K., Corbi, A. L., and Springer, T. A. (1993) J. Cell Biol. 120, 1031–1043
39. Seong, S. Y., and Matzinger, P. (2004) Nat. Rev. Immunol. 4, 469–478
40. Kim, J. I., Lee, C. J., Jin, M. S., Lee, C. H., Paik, S. G., Lee, H., and Lee, J. O. (2005) J. Biol. Chem. 280, 11347–11351
41. Watanabe, K., Yamaji, Y., and Umemoto, T. (1992) Oral Microbiol. Immunol. 7, 357–363
42. Naito, Y., Tohda, H., Okuda, K., and Takazoe, I. (1993) Oral Microbiol. Immunol. 8, 195–202
43. Medzhitov, R. (2001) Nat. Rev. Immunol. 1, 135–145
44. Dickinson, D. P., Kubiniec, M. A., Yoshimura, F., and Genco, R. J. (1988) J. Bacteriol. 170, 1658–1665
45. Yilmaz, O., Watanabe, K., and Lamont, R. J. (2002) Cell Microbiol. 4, 305–314
46. Lamont, R. J., Chen, A., Belton, C. M., Izutsu, K. T., Vasel, D., and Weinberg, A. (1995) Infect. Immun. 63, 3878–3885
47. Ernst, J. D. (1998) Infect. Immun. 66, 1277–1281
48. Trinchieri, G. (2003) Nat. Rev. Immunol. 3, 133–146