Fimbrial protein fimbrillin (FimA), a major structural subunit of Porphyromonas gingivalis, has been suggested as a vaccine candidate to control P. gingivalis-induced periodontal disease. Previously, cDNAs encoding IgG monoclonal antibodies (MAbs) against purified FimA from P. gingivalis 2561 have been cloned, and the MAbs have been produced in rice cell suspension. Here we examined the biological activities of the plant-produced MAb specific for FimA (anti-FimA plantibody) of P. gingivalis in vitro and in vivo. The anti-FimA plantibody recognized oligomeric/polymeric forms of native FimA in immunoblot analysis and showed high affinity for native FimA (K_D = 0.11 nM). Binding of P. gingivalis (10^8 cells) to 2 mg of saliva-coated hydroxyapatite beads was reduced by 53.8% in the presence of 1 μg/ml plantibody. Anti-FimA plantibody (10 μg/ml) reduced invasion of periodontal ligament cells by P. gingivalis (multiplicity of infection, 100) by 68.3%. Intracellular killing of P. gingivalis opsonized with the anti-FimA plantibody by mouse macrophages was significantly increased (77.1%) compared to killing of bacterial cells with irrelevant IgG (36.7%). In a mouse subcutaneous chamber model, the number of recoverable P. gingivalis cells from the chamber fluid was significantly reduced when the numbers of bacterial cells opsonized with anti-FimA plantibody were compared with the numbers of bacterial cells with irrelevant IgG, 66.7% and 37.1%, respectively. These in vitro and in vivo effects of anti-FimA plantibody were comparable to those of the parental MAb. Further studies with P. gingivalis strains with different types of fimbriae are needed to investigate the usefulness of anti-FimA plantibody for passive immunization to control P. gingivalis-induced periodontal disease.

Porphyromonas gingivalis is a black-pigmented Gram-negative anaerobic rod that is strongly associated with periodontal disease in adults (1–4). Fimbrial protein fimbrillin (FimA), a major structural subunit of P. gingivalis fimbriae, is believed to mediate bacterial attachment to the host cell surface (5). Since FimA is one of the critical cell surface virulence factors of P. gingivalis, it is of particular interest to the development of vaccine strategies (6). Immunization of mice with purified FimA elicited salivary IgG and IgA antifimbrial antibodies at high concentrations (7) and reduced periodontal destruction in a periodontitis rat model (8). These results suggest that FimA may be used as a vaccine candidate to control P. gingivalis-induced periodontal disease.

Meanwhile, in vitro studies have shown that FimA-specific monoclonal antibodies (MAbs) can inhibit the adherence of P. gingivalis to buccal epithelial cells (9) and saliva-coated hydroxyapatite (sHA) beads (10). These observations raise the possibility that passive immunization with antibodies against FimA may also be used to prevent P. gingivalis-induced periodontal disease. Passive immunization provides an immediate onset of protection but is known to be short-lived and remains effective only as long as the delivered antibodies persist (11). Therefore, continuous passive immunization on a regular basis may be required to provide protection from infections, demanding larger amounts of antibodies. Since animals and humans are used as the source of passive antibodies, it is of great concern to avoid pathogenic infectious agents of animal and human origin. These drawbacks still hamper the use of passive immunization for controlling infections. If a strategy overcoming the limitations is available, passive immunization can be widely employed to provide immediate control of life-threatening infections and, even more interestingly, the dissemination of drug-resistant microorganisms, poorly immunogenic pathogens/microorganisms, and the emergence of new microorganisms (12–14). It can also be used as an alternative to the ineffective antimicrobial drugs in immunocompromised hosts (13).

Transgenic plants or transient production systems in plants have been used to generate MAbs that can specifically bind to antigens or pathogens and protect the host from infection via passive immunization (15, 16). The advantages of MAB manufacturing in transgenic plants (plantibody) include large-scale production, low cost, and reduction of health risks that arise from contamination with human pathogens or toxins (17). In the context of oral disease, Guy’s 13 MAbs produced in tobacco were assembled into IgA and IgG molecules that recognized the native I/II cell surface adhesion molecules (AgI/II) of Streptococcus mutans, which is the most important bacterial cause of dental caries (18, 19). In some human trials, passive immunization using these plantibodies afforded specific protection against oral streptococcal colonization for up to 2 years (20).

The fimA gene, encoding FimA, exists as a single copy in the...
chromosome of P. gingivalis (21). Strains of P. gingivalis have been classified into six fimA genotypes called types I to V and Ib, and the most predominant fimA genotype in periodontitis patients is type II, which is now commonly referred to as the periodontitis-associated fimA genotype of P. gingivalis (22–26). Meanwhile, an earlier study (27) reported that anti-native FimA of serotype I strain 2561 reacts strongly with FimA from strains of serotype I and cross-reacts with serotype II. P. gingivalis strains of the FimA serotypes I and II used in the study are now known to belong to fimA genotypes I and II, respectively. These results suggest that FimA of serotype I strain 2561 is antigenically and serologically related to serotype II FimA (22). Since P. gingivalis strains of genotypes I and II are distributed in 60 to 80% of periodontally healthy and diseased patients (22, 26), passive immunization with the FimA plasmodium may be expected to protect not all, but a large portion, of the patients. In a previous study, cDNAs encoding MAbs specific for the purified FimA proteins from P. gingivalis 2561 were cloned, and the MAbs were produced in rice cell suspension (28). The present study aimed to examine the biological activities of the FimA-specific MAbs produced in a rice suspension culture against P. gingivalis (anti-FimA plasmodium) in comparison with the parental IgG MAb clone 265 (MAb 265).

MATERIALS AND METHODS

Production of plantibody specific for FimA of P. gingivalis. Previously prepared recombinant plant expression vectors (pMYV582 and pMYV583) containing cDNAs encoding the heavy (GenBank no. GQ984292) or light (GenBank no. GQ984393) chain of mouse MAb 265, which recognizes the FimA protein of P. gingivalis 2561 (10, 28), were used for this study. Using the plant expression vectors, plasmodium was prepared as described in a previous study (28). Briefly, scutellum-derived calli from mature rice seeds (Oryza sativa L. cv. Dongjin) were transformed via bombardment using gold particles (0.6 μm) coated with 10 μg of each recombinant plasmid. After bombardment, the calli were cultured on N6 coculture medium supplemented with 2,4-dichlorophenoxyacetic acid (2 mg/liter), supplemented with the antibiotic hygromycin B (50 mg/liter) for the selection of transgenic calli. Plantibody 265 was obtained from the rice cell suspension culture of transgenic rice calli showing positive signals by PCR. The plantibody was purified by using a HiTrap Protein G HP column.

Immunoblot analysis. Sonic extracts (crude fimbriae) were obtained from P. gingivalis 2561 and treated at 80°C for 5 min without β-mercaptoethanol (β-ME), as described previously (29, 30). The proteins were subjected to SDS-12% polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with anti-FimA plasmodium and MAb 265 at 4°C overnight. Immune complexes were detected by using alkaline phosphatase-labeled goat anti-mouse IgG-Fc-specific secondary antibody and visualized using 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) alkaline phosphatase substrate (Sigma, St. Louis, MO, USA).

SRR analysis. Surface plasmon resonance (SRR) experiments were performed on an SR7300DC instrument (Reichert Inc., Depew, NY), where purified native FimA of P. gingivalis 2561 (29) was immobilized on a polyethylene glycol (PEG) sensor chip (Reichert Inc.) via amine coupling. Briefly, the carboxyl groups of a PEG sensor chip surface were activated for 7 min with a solution containing 50 mM N-hydroxysuccinimide and 200 mM N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride. The purified native FimA protein was diluted to 6 μg/ml in 20 mM sodium acetate (pH 4.0) and immobilized by injecting the purified FimA over the surface for 10 min at a 20-μl/min flow rate. The remaining active sites of the surface were quenched with 1 M ethanolamine buffer at pH 8.0. One channel was kept blank and used as a reference. To monitor association, various concentrations (1.25 to 20.0 nM) of anti-FimA plantibody MAb 265 or irrelevant mouse IgG (Irr-IgG) in phosphate-buffered saline (PBS; 10 mM, pH 7.2) were applied to the ligand and reference channels at a flow rate of 30 μl/min for 3 min, and the changes in the response units were recorded. A regeneration step was performed using 10 mM NaOH, after dissociation with PBS for 3 min at a flow rate of 30 μl/min. Binding affinities of the antibodies to the FimA were analyzed using Scrubber2 software by selecting reference-subtracted curves.

Inhibition assay of P. gingivalis attachment to sHA beads. Antibody-mediated inhibition of bacterial attachment was measured with saliva-coated hydroxyapatite beads as described previously (31, 32). Briefly, 108 P. gingivalis 2561 cells were mixed with 2 mg sHA beads. The final volume of the mixture was adjusted to 400 μl with buffered KCl, and then either 100 ng, 200 ng, or 400 ng of antibodies was added to the mix. After a 1-h incubation at room temperature, the reaction mixture was layered onto 1 ml of 100% Percoll (Sigma) in new siliconized borosilicate tubes to remove unbound, free P. gingivalis. The number of the bacterial cells bound to sHA beads was determined by quantitative real-time PCR with genomic DNA extracted from sHA bead-bound bacteria, as described previously (32).

PDLC culture. Peridontal ligament (PDLC) cells were isolated and cultured as described previously (33). Briefly, healthy human premolars were extracted from adults (15 to 30 years of age) for orthodontic purposes under approved guidelines by the Review Board of Kyung Hee University (KHD IRB-1106-02). PDLC tissues were separated from the root surface using a scalpel and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY, USA) containing 110 mg/liter sodium pyruvate, 2 mM l-glutamine, 1,000 mg/liter l-glucose, 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco) and incubated at 37°C in 5% CO2. At confluence, the cells were treated with a trypsine-EDTA (0.05%) trypsin, 0.02% EDTA (Gibco) solution and resededed at 4 × 105 cells in 24-well culture dishes that did not contain antibiotics. Cells at passages 4 to 8 (P4 to P8) were used for invasion assay. Cultures were grown to confluence and then washed in DMEM without antibiotics before being infected with bacteria.

Bacterial invasion assay. Bacterial invasion of PDLC cells was quantified by the standard antibiotic protection assay as modified for P. gingivalis (34, 35). Briefly, P. gingivalis 2561 cells grown to mid-log phase were pelleted, washed, and adjusted to 2 × 106 cells/ml in only DMEM. Subsequently, the bacterial suspension was incubated with anti-FimA plantibody, MAb 265, or Irr-IgG (2.5, 5, or 10 μg/ml) at 37°C for 30 min. The bacterial cell suspension pretreated with the antibodies (200 μl) was inoculated onto a confluent PDLC cell monolayer at a multiplicity of infection (MOI) of 100 bacteria to 1 cell (4 × 106 CFU/4 × 105 cells) and then incubated at 37°C in 5% CO2 for 90 min. External nonadherent bacteria were removed by washing in PBS, and external adherent bacteria were then killed by incubation for 1 h in DMEM containing 100 μg/ml metronidazole. As reported in previous studies using different P. gingivalis strains (36, 37), this concentration of metronidazole was sufficient to completely kill 106 CFU/ml of P. gingivalis 2561 in 1 h (data not shown). The cells were washed in PBS and lysed with sterile distilled water for 30 min to release internal bacterial cells. Dilutions of the cell lysate were plated on brain heart infusion (BHI; Becton, Dickinson and Company, Sparks, MD, USA) agar supplemented with 5% sheep blood, 5 μg/ml of hemin, and 1 μg/ml of vitamin K1 (BHI-HK blood agar) and then incubated at 37°C anaerobically for 4 days in order to enumerate the viable bacterial cells in the lysate.

Phagocytosis and bacterial killing assay. All animal procedures were performed under the guidance of the Institutional Animal Care and Use Committee (IACUC) at Kyung Hee Medical Center using an approved protocol (KHMC IACUC 13-015). Peritoneal macrophages were obtained from male C57BL/6 (6 to 8 weeks old) by peritoneal lavage 4 days after an intraperitoneal injection of 3% Brewer thiglycollate medium.
isoflurane anesthesia (40). Mice were examined daily for signs of infection in subcutaneous tissue of the dorsolumbar region of each mouse under anesthesia. The skin incisions were prepared from 0.5-mm stainless steel wire and surgically implanted into four groups (107 cells) was opsonized with 10^10 to 10^11 bacteria/ml in DMEM in a 5% CO2 incubator at 37°C for 2 h. At specified times (0, 30, 60, and 120 min), the tube containing the phagocytic mixture was centrifuged at 150 × g for 10 min. The supernatant fraction was used to determine the number of extracellular bacteria, and the macrophage pellet was disrupted by incubation in sterile distilled water for 10 min. Triplicate preparations of the supernatant and the disrupted macrophage pellet were appropriately diluted in PBS and plated onto BHI-HK blood agar. After incubation at 37°C anaerobically for 4 days, the colonies were counted. Phagocytosis and killing indexes were expressed according to the following formulas: phagocytic index = [(N_cont - N_extra)/(N_cont)] × 100; killing index = [(N_cont - N_extra - N_intera)/(N_cont)] × 100, where N_extra and N_intera are the numbers of viable extracellular and intracellular bacteria, respectively, and N_cont is the number of viable bacteria without macrophage (39).

Chamber fluid analysis. Female BALB/c mice (8 weeks old) were separated into four groups (n = 5 mice/group). The coil-shaped chambers were prepared from 0.5-mm stainless steel wire and surgically implanted in subcutaneous tissue of the dorsolumbar region of each mouse under isoflurane anesthesia (40). Mice were examined daily for signs of infection, including hunched bodies, weakness, and weight loss. Following a 10-day healing period, the animals were infection challenged by transcutaneous injection of P. gingivalis group 1 mice received 100 μl P. gingivalis (10^7 cells) into the chamber; group 2, 3, and 4 mice received P. gingivalis anti-FimA plantibody, P. gingivalis MAb 265, and P. gingivalis Irr-IgG (100 μg), respectively. Chamber exudates were harvested from mice at 24 h postinfection by using a syringe with a 25-gauge needle (each chamber was sampled only once). The fluid samples were subjected to 10-fold serial dilutions in BHI broth, plated onto BHI-HK blood agar, and incubated at 37°C for 7 days under anaerobic conditions as described above.

Statistical analyses. The data were analyzed by use of either unpaired Student’s t test or one-way analysis of variance (ANOVA), which was followed by Tukey’s honestly significant difference multiple-comparison post hoc test. Statistical analysis was performed with IBM SPSS version 22 statistical software (IBM SPSS, Chicago, IL, USA). P values of <0.05 were considered statistically significant.

RESULTS

Immunoreactivity and binding kinetics of anti-FimA plantibody with P. gingivalis native FimA. Previously, native fimbriae purified from P. gingivalis 2561 showed a ladder-like pattern when treated at 80°C for 5 min under nonreducing conditions and were dissociated into monomeric forms of 43 kDa after heating at 100°C under reducing conditions (29, 41). In the immunoblot analysis, both anti-FimA plantibody and its parental MAB 265 reacted with oligomeric/polymeric FimA, as evidenced by the ladder-like pattern of bands (Fig. 1A). The SPR sensorgram was used to determine the kinetic binding parameters, the association rate constant (k_a) and the dissociation rate constant (k_d) of anti-FimA plantibody and purified native FimA. The equilibrium dissociation constant (K_D) was determined as k_d/k_a. Anti-FimA plantibody bound with nanomolar affinity to purified native FimA (K_D = 0.11 nM) (Fig. 1B) and showed a 264-fold-higher affinity than MAB 265 (K_D = 29 nM).

Inhibition of P. gingivalis binding to sHA beads by anti-FimA plantibody. In our previous study, the binding of P. gingivalis to sHA beads was significantly reduced by either of the plant-produced anti-FimA antibody (28) and its parental monoclonal antibody at a concentration as low as 25 ng/ml (10). In the present

FIG 1 Immunoreactivity and affinity of the anti-FimA plantibody with native FimA of P. gingivalis 2561. (A) The sonic extract from P. gingivalis 2561 was partially dissociated by treatment at 80°C for 5 min in the absence of β-ME. Immunoblot analysis was performed using anti-FimA plantibody produced in transgenic rice callus suspension culture (lane 1) and its parental mouse MAB 265 produced in hybridoma cell (lane 2). Note that the immunoblot shows a ladder-like pattern of bands as evidence for recognition of native fimbriae by the antibodies, as observed in our previous publication (21). (B) A representative SPR sensorgram of 20 nM anti-FimA plantibody, MAb 265, and Irr-IgG against purified FimA protein was obtained by injecting the protein over a PEG sensor chip at a 20-μl/min flow rate in an SR7500DC instrument.
study, we further investigated the maximal inhibitory properties of MAb 265 and anti-FimA plantibody against P. gingivalis-sHA bead interactions. As shown in Table 1, MAB 265 and anti-FimA plantibody ranging from 0.25 to 1 μg/ml inhibited the binding of P. gingivalis by 28.3 to 51.7% and 32.6 to 53.8%, respectively. Both the antibodies showed concentration-dependent inhibition of P. gingivalis binding to sHA beads and had a maximal inhibitory effect at 1 μg/ml. On the other hand, Irr-IgG showed no significant binding inhibition at all concentrations tested (Table 1).

**Inhibition of P. gingivalis invasion of PDL cells by anti-FimA plantibody.** Several recent studies have demonstrated that P. gingivalis is able to invade and activate different cell types in the tissue surrounding teeth (endothelial, gingival epithelial, and PDL cells) (42–45). We previously observed that MAb 265 and anti-FimA plantibody (20 to 100 μg/ml) inhibited the invasion of KB cells, a human oral epithelial cell line, by the bacterium (MOI = 100) (28). P. gingivalis FimA proteins have been proposed to play an important role in adherence to and invasion of PDL cells (46). Here, we investigated the inhibitory effects of anti-FimA plantibody against P. gingivalis invasion of primary PDL cell cultures, which are much better suited to mimic the in vivo situation than KB cells. As shown in Fig. 2, the invasion of PDL cells by P. gingivalis cells (MOI = 100) preincubated with MAB 265 at 2.5, 5, and 10 μg/ml was reduced to 42.3, 24, and 6.2%, respectively. At the same concentrations, anti-FimA plantibody reduced the bacterial invasion to 68.3, 59.6, and 5.0%, respectively.

**Intracellular killing of anti-FimA plantibody-opsonized P. gingivalis by macrophages.** We investigated phagocytosis and killing of the bacterial cells by mouse macrophages during 120 min. During the period of incubation, 69.3 to 81.3% of P. gingivalis 2561 cells were phagocytosed by macrophages (Fig. 3A), and the highest phagocytosis was measured at 120 min. Preincubation of P. gingivalis cells with either anti-FimA plantibody (10 μg) or MAB 265 (10 μg) for 30 min did not alter the levels of the phagocytosis that were determined with the bacterial cells preincubated with Irr-IgG as controls. On the other hand, the killing of P. gingivalis by macrophages was significantly increased when the bacterial cells were opsonized with anti-FimA plantibody or MAB 265 (Fig. 3B). The highest killing index was measured at 120 min, and there was no significant difference between the killing index of anti-FimA plantibody (77.1%) and that of MAB 265 (76.1%), while that of Irr-IgG was 36.7%.

**Enhanced in vivo phagocytosis of P. gingivalis by anti-FimA plantibody.** Chamber fluid was collected from each animal group and cultured to correlate bacterial survival and growth within the chambers in mice. As shown in Fig. 4, 10 μl of each chamber fluid sample was serially diluted in BH broth and plated on BHI-HK blood agar, and CFU were counted. The number of recoverable bacteria was significantly reduced when P. gingivalis cells opsonized with plantibody (7.8 × 104) or MAB 265 (5.6 × 104) were injected into the chamber compared to the number obtained with only P. gingivalis (2.1 × 104) or P. gingivalis opsonized with Irr-IgG (1.6 × 104).

**DISCUSSION**

Passive immunization with antibodies is thought to be safer than active immunization because it works for a limited time and has no effect on the systemic immune system (10, 12). Transgenic plants or transient production systems in plants have been used to generate MABs that can specifically bind to antigens or pathogens and protect the host from infection via passive immunization (15, 16). The advantages of MAB manufacturing in transgenic plants include large-scale production, low cost, and reduction of health risks that arise from contamination with human pathogens or toxins (17). As described above, Guy’s 13 MABs produced in tobacco were assembled into IgA and IgG molecules that recognized the native AgI/II of S. mutans, which is the most important bacterial cause of dental caries (18, 19). In some human trials, passive immunization using the plantibodies exhibited long-term effects on S. mutans recolonization, lasting up to 2 years (20). Recently, Robinette et al. (47) proposed that the long-term effect of passively administered Guy’s 13 plantibody may be due to the immunomodulatory property of the antibody. In the study, immune complexes of Guy’s 13 plantibody bound to S. mutans whole cells induced change in specificity, isotype, and functionality of the elicited anti-AgI/II antibodies. It was suggested that the passively applied Guy’s 13 plantibody does not act merely by blocking a

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**TABLE 1** Inhibitory effect of anti-FimA plantibody on the binding of P. gingivalis to sHA beads, an *in vitro* model for the pellicle-coated tooth surface

| Antibody concn (μg/ml) | Mean % inhibition of binding ± SD |
|------------------------|----------------------------------|
|                        | Anti-FimA plantibody | MAB 265 | Irr-IgG |
| 0.25                   | 32.56 ± 1.84b          | 28.28 ± 2.33b | −15.20 ± 6.78 |
| 0.5                    | 38.81 ± 3.63b          | 37.16 ± 3.06b | −7.40 ± 7.88 |
| 1.0                    | 53.82 ± 2.54b          | 51.67 ± 5.28b | 6.55 ± 8.65 |

*P. gingivalis* 2561 cells (10⁷) were mixed with 2 mg sHA beads in 400 μl with buffered KCl in the presence or absence of antibodies. The bacterial cells bound to sHA beads were enumerated by quantitative real-time PCR with genomic DNA extracted from sHA bead-bound bacteria. The number of the bacterial cells bound to beads in the absence of antibody was used as a control.

b *P* < 0.05, indicating a significant difference from the control. Student’s *t* test was used to compare the bacterial cell numbers.

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**FIG 2** Inhibitory effect of the anti-FimA plantibody on P. gingivalis invasion of PDL cells. Anti-FimA plantibody, MAB 265, and Irr-IgG were incubated with P. gingivalis 2561, and the antibody-opsonized P. gingivalis cells were added to PDL cells at an MOI of 100 bacteria to 1 PDL cell (4 × 10⁵ CFU/4 × 10⁶ cells). Invasion was calculated from CFU recovered intracellularly as a percentage of the total number of a bacterial inoculum with PDL cells. Values are means ± standard deviations from assays done in triplicate. Student’s *t* test was used to compare the cell numbers. *, *P* < 0.05; **, *P* < 0.001.
simple monovalent adhesive interaction but may act by a higher-order structural mechanism through which immune complexes of the plantibody bound to S. mutans whole cells increase the immunogenicity of relevant target epitopes. In fact, long-term effects of passively administered antibody are not uncommon and may stem from the deliberate or inadvertent formation of immune complexes in situ (47, 48).

For passive immunization to prevent P. gingivalis-induced periodontal disease, genes encoding monoclonal antibodies against native FimA proteins of P. gingivalis were cloned (10) and expressed in Nicotiana benthamiana using the agroinfiltration method (15). In the present study, anti-FimA plantibody was produced in the transgenic rice cell suspension culture under control of the RAmy3D expression system for the purpose of high yields as well as easy purification (28, 49). Native fimbriae of P. gingivalis are a polymer of FimA, which is a major structural subunit of the fimbriae. It has been demonstrated that antibodies generated against native fimbriae recognize only oligomeric and polymeric fimbrial antigen forms, while these antibodies generally do not react with completely denatured, monomeric FimA (27, 41, 50). Purified fimbrial proteins from P. gingivalis were assembled into native oligomeric and polymeric forms and recognized by anti-fimbria antibodies, revealing a series of reactive protein bands (29, 41, 51, 52). Like MAb 265, anti-FimA plantibody produced in the present study reacted with native oligomeric and polymeric forms of FimA, as observed in our previous study (28) (Fig. 1A). It indicates that the transgenic plants are reproducible and can exert their ability to produce the MAb that can recognize native fimbriae as the previous one. The association rate constants (k_o) obtained for MAB 265 and anti-FimA plantibody to purified FimA were 1.3 × 10^4 M^-1 s^-1 and 2.0 × 10^4 M^-1 s^-1, respectively (Fig. 1B), indicating that MAB 265 with FimA has a typical high k_o and the plantibody k_o is rather higher than that of MAB 265. The K_o of the plantibody was in the nanomolar range (K_o = 0.11 nM), suggesting that anti-FimA plantibody produced from a transgenic rice cell suspension culture recognizes the conformational epitope of native fimbriae with a high affinity. The present study also demonstrated that the biological activities of anti-FimA plantibody were comparable to those of MAB 265. The plantibody (1 μg/ml) showed approximately 54% inhibition of the binding of P. gingivalis cells to sHA beads (Table 1) and also inhibited ~95% of invasion of PD1 cells by P. gingivalis when 10 μg/ml plantibody was used (Fig. 2). Previously, we observed that MAB 265 and anti-FimA plantibody at concentrations as low as 25 ng/ml significantly inhibited the binding of P. gingivalis to sHA beads (10, 28). Furthermore, anti-FimA plantibody (20 μg/ml) inhibited approximately 91% of the invasion of KB cells, a human oral epithelial cell line, by the bacterium (28). Taken together, these results suggest that FimA-specific plantibody is reactive with native fimbriae present on P. gingivalis; therefore, the plantibody can block...
interactions of *P. gingivalis* with sHA beads, KB cells, and PDL cells.

The role of neutrophils or polymorphonuclear cells (PMNs) has generally been accepted as a double-edged sword in periodontitis (53). PMNs play an essential role in host defense against bacteria, while an excessive release of toxic products such as lysosomal enzymes and oxygen radicals during the microbial killing process has the potential to cause tissue destruction (53, 54). The major protective function of the PMN, phagocytosis and killing of bacteria, is often dependent on serum opsonization (35). Meanwhile, macrophages are an essential part of the innate immune response to intracellular infection, produce proinflammatory cytokines that enhance phagocytosis, and present antigens to T cells (56). Phagocytosis of pathogens by macrophages initiates innate immunity and creates a bridge between the innate and acquired immune responses (57). Antibody-Fc receptor interaction is important in phagocytosis and intracellular killing of pathogens by macrophages (58). In our previous study (28), *P. gingivalis* cells were preincubated with anti-FimA plantibody at various concentrations (10 to 250 μg) for 30 min, and then mouse PMNs were incubated with either *P. gingivalis* cells or mixtures of *P. gingivalis* cells and anti-FimA plantibody for 15 min. The levels of phagocytosis at 15 min were similar among the tested groups, while killing of *P. gingivalis* by PMNs was significantly increased when the bacterial cells were opsonized with anti-FimA plantibody. In the present study, the *in vitro* intracellular killing rate of *P. gingivalis* by macrophage was significantly increased when the bacterial cells were preincubated with the plantibody: 77.1% (Fig. 3). The result indicates that anti-FimA plantibody, which interacts with *P. gingivalis* FimA, can be recognized by IgG Fc receptor of macrophage, acting as an opsonin. The present study placed more emphasis on the *in vivo* efficacy of the plantibody, and as shown in Fig. 4, opsonization of *P. gingivalis* with the plantibody and MAb 265 resulted in a reduced number of the bacterial cells in chamber model. This result strongly implies that plantibody may be of value for use in treating periodontal disease.

Collectively, our results indicate that anti-FimA plantibody produced in the rice cell suspension culture inhibits the biological activities of *P. gingivalis* through interactions with native fimbiae on the bacterial cells. Guy’s 13 plantibody is intended for regular topical preventive administration by both dental hygienists and patients following a thorough cleaning and intervention for any existing decay. Similarly, anti-FimA plantibody may be used as a mouthwash or topically applied into the subgingival area of teeth after periodontal scaling and root planing and intervention for periodontal disease. Before applying such a strategy, further study is needed to determine whether anti-FimA plantibody has any modulatory effect on the host immune response to *P. gingivalis in vivo* and to define an optimal antigen/antibody ratio for eliciting the beneficial immunomodulatory effect, as observed in Guy’s 13 plantibody (47). Further studies employing *P. gingivalis* strains with different types of fimbiae are also required to investigate the inhibitory effects of anti-FimA plantibody and its usefulness for passive immunization to control *P. gingivalis*-induced periodontal disease.

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