Antibody Induced by *Porphyromonas Gingivalis* FimA-PVXCP DNA Vaccine Inhibit Host Cell Invasion and Enhance Phagocytosis

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Abstract

Porphyromonas gingivalis (P. gingivalis) is strongly correlated with periodontitis and systemic conditions such as aspiration pneumonia and cardiovascular disease. Vaccination against *P. gingivalis* will provide help to limit its pathogenicity. In order to investigate the function of antibody induced by DNA vaccine encoding *P. gingivalis* FimA fused with the immuno-enhancing molecule potato virus X coat protein (PVXCP), abilities of the induced antibody to inhibit host cell invasion and enhance phagocytosis were determined. Mice were vaccinated with the FimA-PVXCP fusion vaccine (pcDNA3.FimA-PVXCP) or FimA vaccine (pcDNA3.FimA). Antibody levels to FimA were measured by ELISA on day 14 after boosting. Both DNA vaccines induced anti-FimA antibody production at the comparable levels. Number of *P. gingivalis* invading into Caco-2 cells was significantly lowered when the bacteria were pre-incubated with serum from mice vaccinated with pcDNA3.FimA-PVXCP, comparing to the group that bacteria was not pre-incubated with serum or pre-incubated with normal mouse serum. In addition, when *P. gingivalis* was pre-incubated with serum from pcDNA3.FimA-PVXCP vaccinated mice, phagocytic index was significantly higher than the group that bacteria was not pre-incubated with serum or pre-incubated with normal mouse serum or serum from pcDNA3.FimA vaccinated mice.

Therefore, anti-FimA antibody induced by FimA-PVXCP DNA vaccine could inhibit *P. gingivalis* cell invasion and also enhance phagocytosis.

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Introduction

P. gingivalis is a non-motile asaccharolytic gram-negative rod anaerobic bacterium residing in human oral cavity. It is one of the major pathogens causing periodontal diseases. *P. gingivalis* is strongly correlated with periodontitis[1,2], a multi-microbial infection-driven inflammatory disease involves in the destruction of tooth-supporting tissues[3], which is the major cause of adult tooth loss. *P. gingivalis* could be detected in up to 85% of the periodontitis sites[4]. It is detected rarely at healthy periodontal sites. The presence of *P. gingivalis* in a periodontal pocket may indicate the disease progression[5].

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Apart of its role in periodontal diseases, *P. gingivalis* is also associated with other diseases and systemic conditions such as aspiration pneumonia and cardiovascular disease [6-8].

P. gingivalis express many virulent factors, such as cysteine proteinases (gingipains), lipopolysaccharide (LPS), and fimbriae, for escaping from host protective immunity. *P. gingivalis* uses fimbriae to adhere to salivary proteins, extracellular matrix, host cells and other bacteria. Thus it can attach to the early colonizing bacteria in oral biofilm. *P. gingivalis* fimbriae present in 2 forms, major and minor fimbriae[9]. Major fimbriae compose of 41 kDa fimbrillin subunits, encoded by fimA gene. Minor frimbriae compose of 67 kDa subunits which are encoded by mfa1 gene[9]. It's been suggested that minor fimbriae together with major fimbriae act coordinately in the development of mature *P. gingivalis* biofilm and both of them are required for the pathogenicity[9,10].

In attempt to control *P. gingivalis* pathogenicity, researchers have been developed many vaccines targeting the virulent factors such as gingipain and fimbriae. They also tried many strategies to obtain vaccine with ability to induce strong immunity against *P. gingivalis*. The vaccines provide protective immunity against *P. gingivalis* infection and alveolar bone loss induction[11-14].

In our study, we selected FimA as a candidate immunogen and linked FimA to PVXCP to enhance immunogenicity of the vaccine. PVXCP was chosen to be a fusion partner because of the lack of pre-existing immunity in human individuals and its great immunogenicity. The DNA fusion vaccine encoding scFv from the mouse A31 lymphoma or 5T33 myeloma induced antibodies that specifically recognized both the tumor antigen and PVXCP. In addition, the vaccine provided protection against the tumors and the protection appeared to involve CD4+ T cells [15]. A fusion of PVXCP provided T-cell help has been proven to be a crucial factor for both induction and maintenance of antibody responses [16]. An additional property of PVXCP fusion proteins is the ability to form aggregates [15], a feature which could further enhance immunogenicity.

In FimA vaccine studies, researchers showed that the vaccines could induce specific salivary IgA and serum IgG in the immunized mice [14, 17] and also protected mice from alveolar bone loss after challenging with *P. gingivalis* [14]. In this study, we showed another aspect of induced protective immunogenicity. We determined ability of the vaccine-induced antibody to enhance phagocytosis and to prevent host cell invasion by *P. gingivalis*.

Materials and methods

DNA vaccine construction

FimA type I gene was amplified from genomic DNA of *P.gingivalis* FimA genotype I (ATCC33277) by. For FimA-PVXCP vaccine construction, PCR primers contained ApaLI site at the 5' end of the forward primer and BspEI site at the 5'end of the reverse primer. FimA PCR product was ligated to leader sequence amplified from pcDNA3.BCL1scfv-PVXCP (pcDNA3 containing BCL1 scFv fusion with PVXCP) provided by Dr. Natalia Savelyeva, Faculty of Medicine, University of Southampton, UK. Forward primer for leader sequence PCR contained HindIII site at the 5' end and the reverse primer contained ApaLI site at the 5' end. The leader-FimA ligated product was amplified and subsequently ligated into HindIII/BspEI pre-digested pcDNA3.BCL1scfv-PVXCP. The resulting plasmid was pcDNA3 containing fusion FimA-PVXCP with leader sequence at the 5' end of the fusion sequence. For FimA vaccine construction, leader-FimA sequence was amplified with the forward primer containing HindIII site and the reverse primer containinig XhoI site at the 5' end. The leader-FimA sequence was then ligated into HindIII/XhoI pre-digested pcDNA3.BCL1scfv-PVXCP. The resulting plasmid was pcDNA3 containing FimA (pcDNA3.FimA) with leader sequence at the 5' end.

The ligation products was transformed into JM109 competent cells and the transformed cells was screened by colony PCR and DNA sequencing to check the correct sequence of the fusion vaccines. All enzymes and kits were purchased from Thermo Scientific, MA, USA.

Mice immunization

The plasmids for immunization were purified by GeneJet Plasmid Maxiprep Kit (Thermo Scientific, MA, USA). Mice experiments were approved by Naresuan University Institutional Animal Care and Use Committee (project no. NU-AE600814). Two groups of five 6-week-old female BALB/c mice (Nomura Siam International Co., Ltd., Bangkok, Thailand) were immunized intramuscularly with 50 microgram of pcDNA3.FimA or pcDNA3.FimA-PVXCP in sterile saline. A group of 3 mice was added as a control of normal (non-treated) mice. The boosting was done on day 14 and blood samples were collected from tail tip 2 weeks after boosting.

Antibody analysis

Anti-FimA antibody level in blood sample was detected by ELISA. One microgram per millilitre of in house purified FimA protein was coated on 96-well plate overnight. After incubation with serially diluted serum, the amount of bound IgG was detected by peroxidase-conjugated anti-mouse IgG (Seracare, MA, USA), followed by the addition of O-phenylenediamine substrate (Thermo Scientific, MA, USA). The OD490 was recorded using xMarkTM Microplate Spectrophotometer (Bio-Rad, California, USA). A mix of Anti-FimA antiserum was used to create

standard curve. The level of anti-FimA antibody of each sample was interpolated from standard curve and reported into units/ml value.

Caco-2 invasion assay

The human cell culture experiments were approved by the Naresuan University Research Ethics Committee (IRB No. 590/59). Caco-2 cells were provided by Asst.Prof.Dr. Kornchanok Wayakanon, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand. Caco-2 cells were seeded into 24-well plates at 5 x 10^4 cells per well in MEM (Gibco®, Thermo Fisher Scientific, MA, USA) containing 10% (vol/vol) heat-inactivated FBS (Hyclone™, GE Healthcare, Illinois, USA) and 100 IU/ml penicillin-streptomycin (Gibco®, Thermo Fisher Scientific, MA, USA), incubated for 24 h. The adherent cells were washed three times with PBS before addition of 2% (w/v) BSA in MEM and incubation for 1 h at 37°C to block the non-specific binding sites for bacteria. *P. gingivalis* was pre-incubated for 30 min at 37°C with mouse serum diluted at 1:100 in MEM. The number of bacteria for each well was 5 x 10^6 cells. The pre-incubated bacterial suspension was added to the cells and incubated for 4 h at 37°C, in 5% CO2. Bacterial suspension was removed and 1 ml of MEM containing 200 microgram/ml of metronidazole was added and incubated for a further hour at 37°C, in 5% CO2 to kill the adherent bacteria. Cells were washed twice with PBS and lysed by addition of 1 ml of distilled water and incubated for 30 min at room temperature. Cells were vortexed at maximum speed for 20 min as an extra step to disrupt the cells and release the bacteria. The lysates was then serially diluted and inoculated on ATCC medium 2722 supplemented Tryptic Soy agar plate for 5-7 days before colony counting.

Phagocytosis assay

THP-1 cells were cultured in RPMI-1640 containing 25 mM L-glutamine (Gibco®, Thermo Fisher Scientific, MA, USA), 10% (vol/vol) heat-inactivated FBS, 100 IU/ml penicillin-streptomycin, and 0.05 mM 2-mercaptoethanol. Amount of 3 x 10^5 cells were suspended in 200 microlitre suspensions of *P. gingivalis* pre-incubated with serum. The mixture of cells and bacteria were incubated for 1 h at 37°C. After incubation, the mixture was centrifuged at 200 x g for 5 minutes and the bacterial suspension containing bacteria outside the THP-1 cells was collected for bacteria enumeration. The collected suspensions were serially diluted and inoculated on ATCC medium 2722 supplemented Tryptic Soy agar plate for 5-7 days before colony counting. Phagocytic index = [(Ncont-Nextra)/(Ncont)] x 100. Ncont is a number of bacteria that did not incubate with the cells; Nextra is a number of bacteria outside the cells after incubation.

Statistical analysis

Differences of level of antibody, number of cell invading *P. gingivalis* and phagocytic index between groups were analyzed by Kruskal-Wallis test and Mann-Whitney test using GraphPad Prism version 8.1.2 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com

Results

DNA vaccines induced anti-FimA antibody production

The DNA vaccine was constructed in a fusion form of *P. gingivalis* FimA typeI and PVXCP (FimA-PVXCP) DNA vaccine, in which the mammalian expression plasmid pcDNA3 will be used as a backbone plasmid. A DNA vaccine containing only FimA sequence was also constructed to be used as a control vaccine for investigation of PVXCP immune enhancing ability in this model. Mice were injected with pcDNA3.FimA-PVXCP or pcDNA3.FimA and boosted at day 14 after priming. Blood was collected and the level of FimA antibody was measured by ELISA on day 14 after boosting. Both DNA vaccines induced anti-FimA antibody production at the comparable levels. Serum from non-vaccinated mice showed no detectable level of anti-FimA antibody.

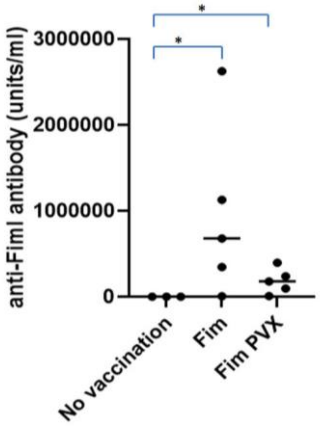

Figure 1. Anti-FimA antibody responses.
Mice were injected intramuscularly with pcDNA3.FimA-PVXCP

(FimPVX) or pcDNA3.FimA (Fim) and boosted at day 14 after priming. Blood was collected on day 14 after boosting and the serum antibody levels to FimA protein were measured. Serum from non-vaccinated mice (no vaccination) was also tested. *: P = 0.04. Each dot represents an individual mouse. Horizontal bars are median values.

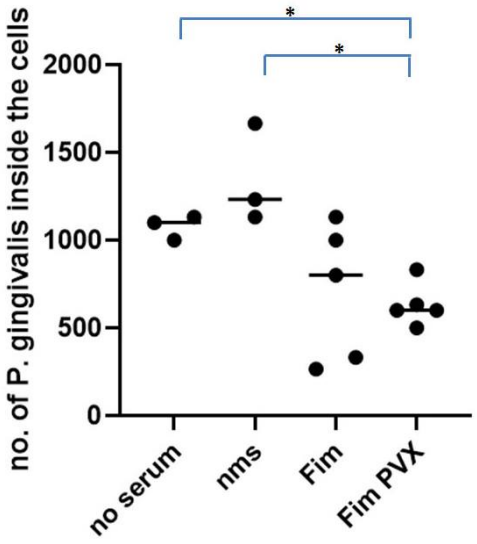

Figure 2. Inhibition of *P. gingivalis* invasion.
P. gingivalis was pre-incubated with serum from pcDNA3.FimA-PVXCP (FimPVX) vaccinated mouse or pcDNA3.FimA (Fim) vaccinated mouse or normal mouse (nms) before the bacteria was incubated with Caco-2 cells. *P. gingivalis* that was not pre-incubated with serum (no serum) was incubated with Caco-2 cells as a baseline of *P. gingivalis* invasion. The number of bacteria inside Caco-2 cells was counted from agar plate. *: P = 0.04. Each dot represents an individual mouse. Horizontal bars are median values.

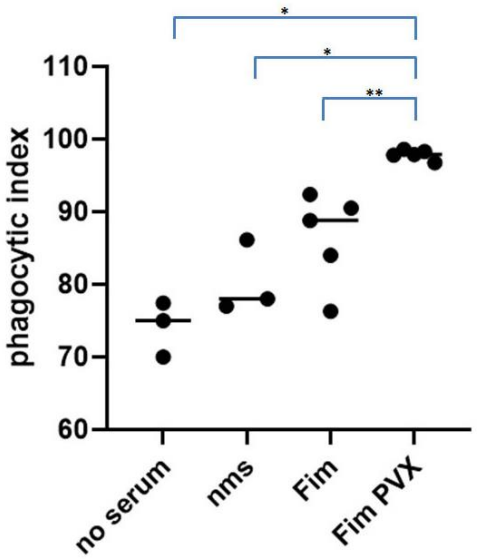

Figure 3. Enhancement of phagocytic activity.
P. gingivalis was pre-incubated with serum from pcDNA3.FimA-PVXCP (FimPVX) vaccinated mouse or pcDNA3.FimA (Fim) vaccinated mouse or normal mouse (nms) before the bacteria was incubated with THP-1 cells. *P. gingivalis* that was not pre-incubated with serum (no serum) was incubated with THP-1 cells as a baseline of THP-1 phagocytic activity. Phagocytic index represents the amount of bacteria disappeared from bacterial suspension outside THP-1 cells. *: P = 0.04; **: P = 0.008. Each dot represents an individual mouse. Horizontal bars are median values.

Serum from pcDNA3.FimA-PVXCP vaccination inhibit Caco-2 invasion

Comparing to the group that bacteria was not pre-incubated with serum or pre-incubated with normal mouse serum, number of *P. gingivalis* invading into Caco-2 was significantly lower when the bacteria were pre-incubated with serum from mice vaccinated with pcDNA3.FimA-PVXCP. Serum from pcDNA3.FimA vaccination seems to lower the number of invading bacteria but no significant was shown.

Serum from pcDNA3.FimA-PVXCP vaccination enhance phagocytic activity

Phagocytic activity of THP-1, a monocyte cell line, was determined after incubation of THP-1 cells with *P. gingivalis* pre-incubated with mouse serum. *P. gingivalis* with no pre-incubation was also used as a baseline of THP-1 phagocytosis. Phagocytic index, calculated with formula showed in the method section, represents amount of bacteria disappeared from bacterial suspension outside THP-1 cells. When *P. gingivalis* was pre-incubated with serum from pcDNA3.FimA-PVXCP vaccinated mice, phagocytic index was significantly higher than the group that bacteria was not pre-incubated with serum or pre-incubated with normal mouse serum or serum from pcDNA3.FimA vaccinated mice. Serum from pcDNA3.FimA vaccination seems to enhance phagocytic activity of THP-1 but no significant was shown.

Discussion

A fusion of PVXCP provided T-cell help which has been proven to be a crucial factor for both induction and maintenance of antibody responses to tumour antigens [16]. Therefore, the incorporation of PVXCP in FimA DNA vaccine was predicted to provide higher antibody level than the DNA vaccine without PVXCP sequence. Howerever, the result showed that both pcDNA3.FimA-PVXCP and pcDNA3.FimA could induce vaccinated mice to produce anti-FimA antibody and the antibody levels were not significantly different.

Even though the antibody levels are comparable, only serum from pcDNA3.FimA-PVXCP vaccination showed ability to inhibit *P. gingivalis* invasion into Caco-2 cells and enhance

phagocytic activity of THP-1 cells. This might due to different IgG subclasses induced by the vaccines. Different linking of antigen to immune-enhancing molecules could lead to different profile of IgG subclasses production [18]. In mice, 5 subclasses of IgG (IgG1, IgG2a, IgG2b, IgG2c and IgG3) were identified [19, 20]. Ability of each IgG subclasses to activate immune cell function depends on the ratio of binding to activating and inhibitory receptors expressed on the cell surface of immune cells (Fc ɣ Rs). According to the ratio, the most immune activating mouse IgG subclass would be IgG2a, following with IgG2b, IgG1 and IgG3, subsequently[20, 21]. IgG2c bind to Fc ɣ RIV (an activating receptor) better than IgG2b [19], but measurement of affinity to inhibitory receptor is required to address IgG2c ability to activate immune cells.

IgG from pcDNA3.FimA-PVXCP vaccination might be the isotypes that more helpful in inhibition of bacterial invasion and phagocytosis enhancement. Opsonization and phagocytosis, the features of Th1 response, are associated with IgG2a and IgG2b as phagocytes express Fc ɣ RIV which bind strongly to both IgG2a and IgG2b. Neutralization of microbes, mediated by Th2 response, is associated with mouse IgG1 and IgG3 [22, 23].

From this experiment, it was shown that for *P. gingivalis* outside the host cells, the FimA-PVXCP vaccine induced antibody was able to control host cell invasion and enhance phagocytic activity of monocytes. For intracellular *P. gingivalis*, weather the vaccine could induce immunization to eradicate infected cells should be further investigated.

Conclusions

FimA-PVXCP and FimA DNA vaccines both could induce production of anti-FimA antibody. However, only the antibody induced by FimA-PVXCP vaccine could inhibit *P. gingivalis* cell invasion and enhance phagocytosis.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Amano A, Nakagawa I, Kataoka K, Morisaki I, Hamada S. Distribution of Porphyromonas gingivalis strains with FimA genotypes in periodontitis patients. Journal of clinical microbiology 2000;37(5):1426-30.
2. Missailidis C, Umeda J, Ota-Tsuzuki C, Anzai D, Mayer M. Distribution of fimA genotypes of Porphyromonas gingivalis in subjects with various periodontal conditions. Oral Microbiology Immunology 2004;19:224-29.
3. Kinane D, Bartold P. Clinical relevance of the host responses of periodontitis. Periodontol 2000 2007;43:278-93.
4. Yang H, Huang Y, Chou M. Occurrence of Porphyromonas gingivalis and Tannerella forsythensis in periodontally diseased and healthy subjects. J Periodontol 2004;75:1077-83.
5. Winkelhoff Av, Loos B, Reijden W, Velden Uvd. Porphyromonas gingivalis, Bacteroides forsythus and other putative periodontal pathogens in subjects with and without periodontal destruction. J Clin Periodontol 2002;29:1023-28.
6. Haraszthy V, Zambon J, Trevisan M, Zeid M, Genco R. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71:1554-60.
7. Meurman J, Sanz M, Janket S. Oral health, atherosclerosis, and cardiovascular disease. Crit Rev Oral Biol Med 2004;15:403-13.
8. Seymour G, Ford P, Cullinan M, Leishman S, Yamazaki K. Relationship between periodontal infections and systemic disease. Clin Microbiol Infect 2007;13(suppl 4):3-10.
9. Amano A, Nakagawa I, Okahashi N, Hamada N. Variations of Porphyromonas gingivalis fimbriae in relation to microbial pathogenesis. J Periodont Res 2004;39:136-42.
10. Kuboniwa M, Amano A, Hashino E, et al. Distinct roles of long/short fimbriae and gingipains in homotypic biofilm development by Porphyromonas gingivalis. BMC Microbiology 2009;9:105.
11. Puth S, Hong SH, Na HS, et al. A built-in adjuvant-engineered mucosal vaccine against dysbiotic periodontal diseases. Mucosal Immunol 2019;12(2):565-79.
12. Puth S, Hong SH, Park MJ, et al. Mucosal immunization with a flagellin-adjuvanted Hgp44 vaccine enhances protective immune responses in a murine Porphyromonas gingivalis infection model. Hum Vaccin Immunother 2017;13(12):2794-803.
13. Huang N, Shimomura E, Yin G, et al. Immunization with cell-free-generated vaccine protects from Porphyromonas gingivalis-induced alveolar bone loss. J Clin Periodontol 2019;46(2):197-205.
14. Yu F, Xu Q, Chen W. A targeted fimA DNA vaccine prevents alveolar bone loss in mice after intra-nasal administration. J Clin Periodontol 2011;38:334-40.
15. Savelyeva N, Munday R, Spellerberg M, Lomonossoff G, Stevenson F. Plant viral genes in DNA idiotypic vaccines activate linked CD4+ T-cell mediated immunity against B-cell malignancies. Nat Biotechnol 2001;19(8):760-64.
16. Savelyeva N, King C, Vitetta E, Stevenson F. Inhibition of a vaccine-induced anti-tumor B cell response by soluble protein antigen in the absence of continuing T cell help. Proc Natl Acad Sci U S A 2005;102(31):10987-92.
17. Kawabata S, Terao Y, Fujiwara T, Nakagawa I, Hamada S. Targeted salivary gland immunization with plasmid DNA elicits specific salivary immunoglobulin A and G antibodies and serum immunoglobulin G antibodies in mice. Infection and Immunity 1999;67(11):5863-68.
18. Jobsri J, Allen A, Rajagopal D, et al. Plant virus particles carrying tumour antigen activate TLR7 and Induce high levels of protective antibody. PLoS One 2015;10(2):e0118096.
19. Falconer DJ, Barb AW. Mouse IgG2c Fc loop residues promote greater receptor-binding affinity than mouse IgG2b or human IgG1. PLoS One 2018;13(2):e0192123.
20. Bruhns P, Jonsson F. Mouse and human FcR effector functions. Immunol Rev 2015;268(1):25-51.

21. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 2005;310(5753):1510-2.
22. Fornefett J, Krause J, Klose K, et al. Comparative analysis of humoral immune responses and pathologies of BALB/c and C57BL/6 wildtype mice experimentally infected with a highly virulent Rodentibacter pneumotropicus (Pasteurella pneumotropica) strain. BMC Microbiol 2018;18(1):45.
23. Yamanaka A, Pitaksajjakul P, Ramasoota P, Konishi E. Expression of enhancing-activity-free neutralizing antibody against dengue type 1 virus in plasmid-inoculated mice. Vaccine 2015;33(45):6070-7.