J Clin Periodontol 2011; 38: 334–340 doi: 10.1111/j.1600-051X.2010.01700.x

Journal of Clinical Periodontology

A targeted *fimA* DNA vaccine prevents alveolar bone loss in mice after intra-nasal administration

Fei Yu[1]*, Qing-An Xu[2]* and Weimin Chen[1]

[1]Center of Stomatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; [2]The Key Laboratory for Oral Biomedical Engineering of Ministry of Education, School and Hospital of Stomatology, Wuhan University, Wuhan, Hubei, China

Yu F, Xu Q-A, Chen W. A targeted fimA DNA vaccine prevents alveolar bone loss in mice after intra-nasal administration. J Clin Periodontol 2011; 38: 334–340. doi: 10.1111/j.1600-051X.2010.01700.x.

Abstract

Aim: To construct a dendritic cell (DC)-targeted DNA vaccine against FimA of *Porphyromonas gingivalis* and evaluate the immunogenicity and protection in mice.

Materials and Methods: A targeted DNA plasmid pCTLA4-FimA, which encodes the signal peptide and extracellular regions of mouse cytotoxic T lymphocyte-associated antigen 4 (CTLA4), the hinge and Fc regions of human Igγ1 and FimA of *P. gingivalis*, was constructed. Mice were immunized with pCTLA4-FimA, the non-targeted DNA plasmid pFimA, which contains only *fimA* gene, or pCI vector intra-nasally. Serum and saliva antibody responses were detected by enzyme-linked immunosorbent assay. The protection against *P. gingivalis*-induced periodontitis was evaluated by measuring alveolar bone loss in mice.

Results: Mice immunized with pCTLA4-FimA showed elevated levels of specific serum IgG and salivary IgA antibody responses compared with mice immunized with pFimA ($p < 0.01$). Both pFimA and pCTLA4-FimA immunized groups showed significantly lower alveolar bone loss, with the magnitude protection greater in the latter ($p < 0.01$), compared with the pCI immunized group.

Conclusions: The DC-targeted DNA construct pCTLA4-FimA enhanced both systemic and mucosal immunity following intra-nasal immunization. A DNA-based immunization strategy may be an effective way to attenuate periodontitis induced by *P. gingivalis*.

Key words: cytotoxic T-lymphocyte antigen 4; DNA; fimbrillin; vaccines

Accepted for publication 19 December 2010

Porphyromonas gingivalis, a gram-negative anaerobic bacterium, is considered to be one of the major pathogens of chronic periodontitis, a condition that results in destruction of the tooth-supporting tissues and eventually teeth loss. The process of destruction is irreversible. Hence, once the damage occurs, it is permanent. Prevention of this disease can be achieved through plaque control. One of the ways to control the plaque is to interrupt the colonization of the periodontal pathogens such as *P. gingivalis* to the tooth surfaces or the oral tissues (Takahashi et al. 2007). Among a number of potential virulence factors of *P. gingivalis*, fimbriae, with fimbrillin (FimA) the major subunit protein, are major adherence-mediating determinants. They mediate bacterial adherence to a variety of oral substrates and molecules, such as salivary proline-rich proteins, salivary proline-rich glycoproteins, statherin, oral epithelial cells, antecedent bacteria such as oral streptococci and *Actinomyces naeslundii*, fibrinogen, fibronectin and lactoferrin (Ogawa et al. 1994, Ogawa & Uchida 1995, Lamont & Jenkinson 1998). Dysfunction in the fimbriae leads to a loss of adhesive properties in this bacterium with reduced invasion and subsequently

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest in this study.
This study was supported by grants from the National Natural Science Foundation of China (No.30700756) and the Doctoral Fund of Ministry of Education of China (No. 200804861011).

*Contributed equally.

decreased periodontal bone decay in experimental animals (Evans et al. 1992b, Njoroge et al. 1997, Weinberg et al. 1997). Besides their role in colonization, fimbriae have been conclusively implicated in immune evasion by *P. gingivalis* through interactions with CXCR4 and CR3 (Hajishengallis et al. 2007, 2008). Fimbriae are also potent inducers of many pro-inflammatory cytokines involved in tissue destruction and alveolar bone loss (Hajishengallis et al. 2002). The concept of vaccination for periodontitis is based on the aim of disturbing the bacterial infection through induction of specific serum IgG or secretory IgA responses. Studies showed that nasal vaccination of fimbriae with cholera toxin as a mucosal adjuvant induced antigen-specific Th1 and Th2 cell-driven IgA immune responses that possessed the ability to inhibit bacterial attachment to epithelial cells and subsequent inflammatory cytokine production (Yanagita et al. 1999). Immunization with FimA protein reduced *P. gingivalis*-induced alveolar bone loss in rats and protection against subsequent lethal infection with *P. gingivalis* in mice (Evans et al. 1992a, Deslauriers et al. 1996). The results of these studies suggest that fimbriae are effective candidate antigens for vaccine development and prevention of *P. gingivalis*-induced periodontitis by vaccination with fimbriae of *P. gingivalis* would become feasible if effective immune responses could be induced.

Dendritic cells (DCs) are considered to be the most potent antigen-presenting cells that are pivotal for the initiation and regulation of immune responses. DCs are strategically located at potential sites of pathogen entry, such as peripheral epithelial and mucosal inductive sites. Upon encountering pathogens, DCs capture and process antigens, undergo maturation as defined by up-regulation of MHC Class II (MHCII) molecules and co-stimulatory molecules such as CD40, CD80 and CD86 and migrate to lymph nodes, where they present antigens primarily as MHC–peptide complexes to naive T lymphocytes and initiate T cell-mediated immune responses (Lakey et al. 2009). Because of the antigen-presenting functions of DCs, they have been made logical targets for vaccine designs. Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is a membrane-bound molecule mainly located on activated T cells. Its extracellular V-domain is considered to be involved in mediating the binding to the B7 molecule on DCs (Parsons et al. 1996). By utilizing the interaction between CTLA4 and B7, specific antigens can be targeted to DCs by fusion to CTLA4. Accelerated and increased antibody responses were induced by immunization with DNA vaccines containing fused CTLA4 and antigen genes (Deliyannis et al. 2000, Jia et al. 2006). To enhance the immunological potency of vaccines against periodontitis, in the study reported here, we constructed a targeted DNA plasmid pCTLA4-FimA, which encoded the signal peptide and extracellular regions of mouse CTLA4, the hinge and Fc regions of human Igγ1 and FimA of *P. gingivalis* and evaluated the immunogenicity and protection in mice.

Materials and Methods

Construction of DNA plasmids and expressions in cultured cells

For targeted *fimA* DNA plasmid construction, the signal peptide and extracellular regions of mouse *Ctla4* gene (Genbank number: BC042741.1), and the hinge and Fc regions of human *Igγ1* gene (Genbank number: AF487336) were amplified from plasmid pGJGLU/GFP (Xu et al. 2009) with primers: forward primer 5′-ATTACGCGTATGGCTTGTCTTGGACTCC-3′, incorporated a MluI restriction site immediately upstream of the transcription initiation codon of mouse *Ctla4* gene, and reverse primer 5′-ATTGTCGACCTTTACCCGGAGACAGGGA 3′, incorporated a SalI restriction site immediately downstream of the last codon of human *Igγ1* gene. The *fimA* gene (Genbank number: M19405.1) was amplified from plasmid pcDNA3/fimA (Kawabata et al. 1999) with primers: forward primer 5′-ACTGGTCGACGTGGTATTGAAGAC-3′, incorporated a SalI restriction site immediately upstream of the transcription initiation codon of *fimA* gene, and reverse primer 5′-ATATTGCGGCCGCTTACCAAGTAGC-3′, incorporated a NotI restriction site immediately downstream of the last codon of *fimA* gene. The targeted plasmid pCTLA4-FimA was constructed by cloning these two fragments into pCI expression vector (Promega Corporation, Madison, WI, USA).

For non-targeted DNA plasmid construction, the *fimA* gene was amplified from plasmid pcDNA3/fimA with primers: forward primer 5′-ACTGGTCGACGTGGTATTGAAGAC-3′, incorporated a SalI restriction site immediately upstream of the transcription initiation codon of *fimA* gene, and reverse primer 5′-ATATTGCGGCCGCTTACCAAGTAGC-3′, incorporated a NotI restriction site immediately downstream of the last codon of *fimA* gene. The non-targeted DNA plasmid pFimA was constructed by cloning the amplified fragment into pCI vector.

Recombinant protein expressions in cultured cells

To examine protein expressions, CHO cells were transfected by pCTLA4-FimA, pFimA or pCI with Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). The slides were incubated with anti-FimA serum (1:500) at 37°C for 30 min. and kept at 4°C overnight. Then, they were incubated with FITC-conjugated goat anti-rabbit IgG (1:160, Sigma-Aldrich Corp., St. Louis, MO, USA) and viewed under a fluorescence microscope.

Preparation of DNA vaccines for immunization

Plasmids for vaccination were isolated and purified with EZ Spin Column Plasmid Maxi-Preps Kit (Sangon, Shanghai, China). For animal immunization, bupivacaine:DNA complexes were prepared by adding bupivacaine hydrochloride to the aqueous DNA solutions using a fast mixing method (Pachuk et al. 2000). The final bupivacaine and DNA concentrations were 0.25% and 1 μg/μl, respectively.

Immunization of mice

Approval to conduct this study was obtained from the review board of Hubei Medical Laboratory Animal Center. Three groups of 4-week-old female BALB/c mice, 10 per group, were immunized with pCTLA4-FimA, pFimA or pCI intra-nasally. Fifty micrograms of bupivacaine:DNA complex was deposited into each nostril with the aid of a micropipette. The immunizations were given on days 0 and 14. Blood and saliva samples were collected at 4 weeks after the last immunization. Serum samples were obtained after centrifugation of blood collected from the retro-orbital plexus. Saliva samples were obtained after intraperitoneal injection of 5 μg of pilocarpine (Sigma Chemical Co.) to stimulate salivation.

Antibody analysis

Anti-FimA and anti-*P. gingivalis* antibodies in serum and saliva were detected by ELISA as previously described (Nelles et al. 1985, Parsons et al. 1996). Individual wells of 96-well plates were coated with 1 μg rFimA in carbonate buffer or formalin-fixed *P. gingivalis* ATCC33277 suspensions, which were diluted in PBS to an optical density of 1.0 at 4°C overnight, and then blocked with 3% BSA in PBST for 2 h. Serially diluted sera or saliva were added to individual wells and the amount of bound antibodies were detected with peroxidase-conjugated goat anti-mouse IgG (1:2500, Vector Lab Inc., Burlingame, CA, USA) or peroxidase-conjugated goat anti-mouse IgA (1:1000, Sigma Inc.) diluted in the blocking buffer, followed by the addition of *O*-phenylenediamine substrate with H_2O_2. The reaction was then stopped with 0.2 M H_2SO_4 and OD_{490} was recorded. The antibody titre was defined as the reciprocal of the highest dilution giving an OD_{490} of 0.1 above the control (no sample added).

P. gingivalis challenge studies

To evaluate the protection of the vaccination against *P. gingivalis*, mice were infected with *P. gingivalis* as described previously (Baker et al. 1994). BALB/c mice were divided into five groups of 10 each: pCTLA4-FimA intra-nasal immunized and *P. gingivalis*-infected group, pFimA intra-nasal immunized and *P. gingivalis*-infected group, pCI intra-nasal immunized and *P. gingivalis*-infected group, non-immunized and *P. gingivalis*-infected group and non-immunized and non-infected group. The immunizations were given on days 0 and 14. Mice were then given 5 mg each of kanamycin and ampicillin by gavage, once a day for 4 days. This was followed by a 3-day antibiotic-free period. Mice were then orally infected with 10^9 CFU of *P. gingivalis* ATCC33277 in 100 μl of PBS containing 2% carboxymethylcellulose *via* a feeding needle for three times at 2-day intervals. Forty-two days after *P. gingivalis* challenge, mice were sacrificed and the mouse jaws were isolated as previously described (Gonzalez et al. 2003). The degree of horizontal alveolar bone loss [cementoenamel junction to alveolar bone crest (CEJ–ABC) distance] was assessed microscopically at 14 separate sites along the maxillary buccal surface of each jaw.

Statistical analysis

The differences in antibody levels and alveolar bone loss among the test and control groups were determined by ANOVA followed by multiple-mean comparisons using the Student–Newman–Keuls test. A value of $p < 0.05$ was considered significant.

Results

Expression of DNA plasmids in vitro

pCI vector is used for transient expression of *fimA* gene. It contains a human cytomegalovirus major immediate-early gene enhancer/promoter region, which drives strong and constitutive gene expression in many mammalian cell types and a late SV40 polyadenylation signal that causes the termination of transcription. Here we showed that green fluorescences were detected in the cytoplasm of pCTLA4-FimA or pFimA transfected CHO cells after incubating with anti-FimA antibody, whereas no fluorescence was detected in cells transfected with pCI vector (Fig. 1). These data indicate that pCTLA4-FimA and pFimA could express recombinant proteins in eukaryotic cells.

Antibody responses

Mice immunized with pCTLA4-FimA exhibited the highest levels of specific serum IgG and salivary IgA anti-*P. gingivalis* and anti-FimA responses, which were significantly higher than those of other groups ($p < 0.01$). Mice immunized with pFimA induced significantly higher levels of serum anti-*P. gingivalis* and anti-FimA IgG responses and salivary anti-*P. gingivalis* and anti-FimA IgA responses compared with the pCI group ($p < 0.01$) (Fig. 2).

Protection against challenge with *P. gingivalis*

The non-immunized and non-infected mice were used to determine the baseline value from CEJ to ABC in normal mice. The infected mice exhibited significantly greater maxillary alveolar bone loss than the non-infected mice ($p < 0.01$). Both pFimA and pCTLA4-FimA immunized groups showed significantly lower alveolar bone loss, with the magnitude protection greater in the latter ($p < 0.01$), compared with the pCI immunized group ($p < 0.01$). There was no significant difference between pCI immunized group and the non-immunized and *P. gingivalis* infected group ($p > 0.05$) (Fig. 3).

Discussion

DNA-based immunization is a process in which the immune responses are induced to a protein antigen expressed in vivo following the introduction of eukaryotic expression vector-carried DNA encoding the polypeptide sequence (Giri et al. 2004). The protein is expressed in transfected mammalian cells, including DCs, myocytes and keratinocytes, and enters into antigen-processing and presentation pathways to initiate immune responses (Coombes & Mahony 2001). DNA plasmids offer many potential advantages over protein-based vaccines, such as greater chemical stability, relatively easier purification, the expressed protein in its correct and native conformation and the possibility for creation of a polyvalent vaccine against several kinds of pathogens (Kowalczyk & Ertl 1999, Conry et al. 2002). The rates of integration-induced mutation with DNA plasmid in animal models were found to be much lower than the rates of spontaneous mutation for a mammalian genome (Nichols et al. 1995, Martin et al. 1999). Thus, DNA vaccines, in addition to being immunogenic, are much safe.

Studies showed that *P. gingivalis*-specific IgG antibodies were commonly observed in patients with periodontitis. But the avidity of IgG anti-*P. gingivalis* antibodies in these periodontitis patients was quite low (Lopatin et al. 1991). Hence, despite these potent antibody responses, the host is apparently unable to clear this organism. Certain *P. gingivalis* antigens which can provoke antibodies with high avidity may render a high degree of protection against the infection (Whitney et al. 1992, Mooney & Kinane 1994). Several studies reported active immunization with fimbriae resulted in antibody titre enhancement and potentially improved the outcomes of periodontitis (Evans et al. 1992a, Takahashi et al. 2007, Kim et al. 2009). Here, we found that both serum IgG and salivary IgA production were induced in mice intra-nasally immunized with pFimA or pCTLA4-FimA. Protective effects were observed in both of these two immunized groups after

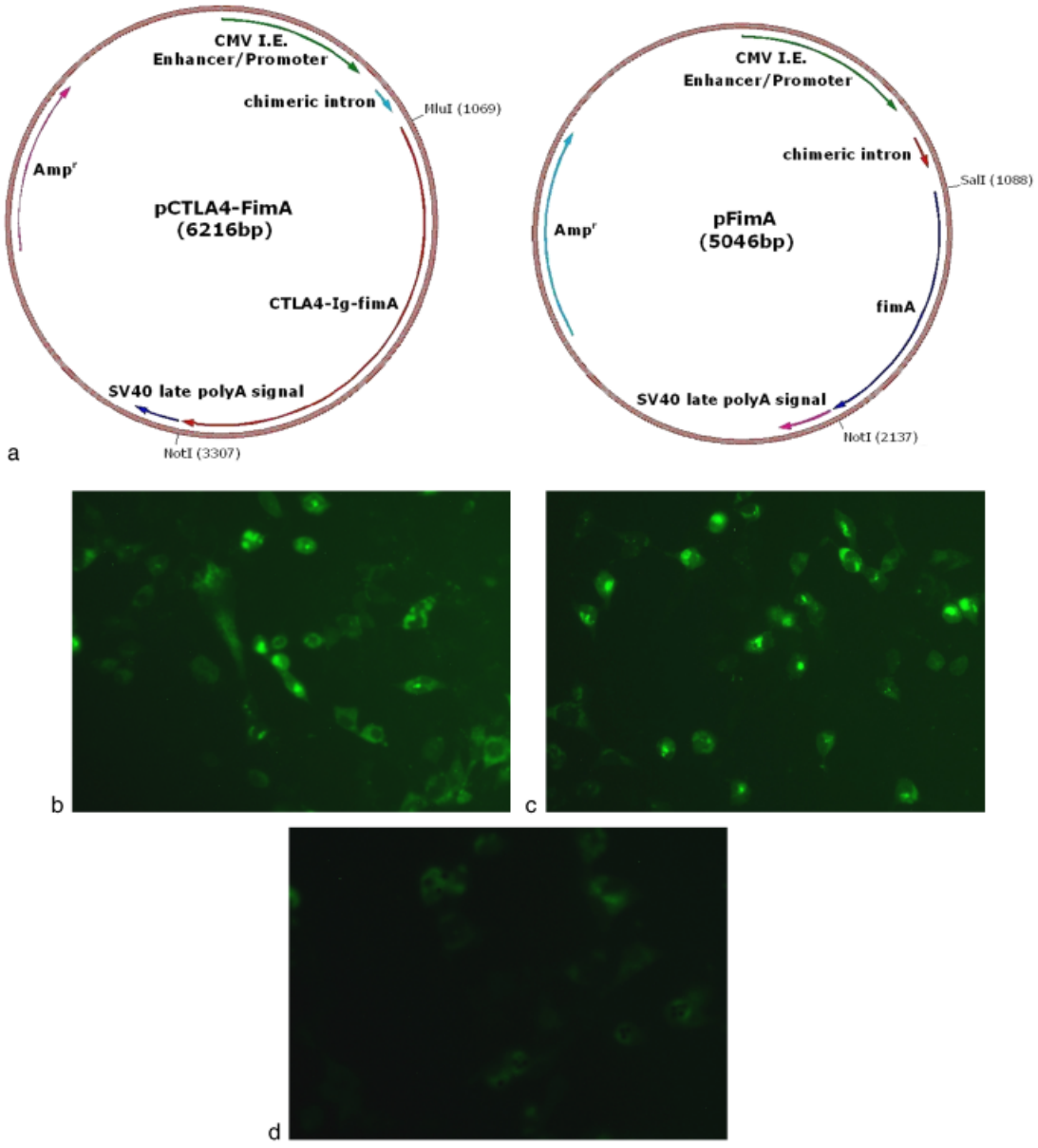

Fig. 1. Expression of FimA in eukaryotic cells. (a) Schematic maps of plasmids pCTLA4-FimA and pFimA. pCTLA4-FimA encodes the signal peptide and extracellular regions of mouse CTLA4, the hinge and Fc regions of human Igγ1, and FimA of *Porphyromonas gingivalis*. pFimA encodes FimA of *P. gingivalis*. (b) Recombinant CTLA4 fusion protein detected by anti-FimA serum in CHO cells transfected with pCTLA4-FimA. Green fluorescence could be found in the cytoplasm. (c) FimA protein detected by anti-FimA serum in CHO cells transfected with pFimA. Green fluorescence could be found in the cytoplasm. (d) CHO cells transfected with pCI vector, detected by anti-FimA serum. No specific signal was detected in the cells.

P. gingivalis infection. Miyachi et al. (2007) found that immunization with a *rpgA* DNA vaccine via the nasal cavity induced salivary IgA and serum IgG responses against *P. gingivalis* and prevented alveolar bone loss incurred by *P. gingivalis* infection in mice. These data suggest that DNA immunization offers an effective way to protect against experimentally induced periodontal destruction. Although several studies have demonstrated the effectiveness of FimA protein in eliciting protective responses in animal models, to date there is no available information comparing the effect of protein vaccines with DNA vaccines against *P. gingivalis*. Prime-boost vaccination regimens, which combine the strengths of DNA vaccines and protein vaccines, were used in other infectious diseases such as AIDS, hepatitis B and dental caries, and showed to be able to better activate the immune system (Rasmussen et al. 2006, Yang et al. 2008, Li et al. 2010). Therefore, further study is required to compare the capacity of *fimA* DNA vaccine with FimA protein to elicit the immune responses. *fimA* DNA vaccine and FimA protein might be used together to trigger a more effective protection against *P. gingivalis* infection.

While periodontitis is not considered life threatening, recent reports demonstrate a link between periodontitis and systemic sequelae of morbidity and mortality in cardiovascular and other systemic diseases, emphasizing the

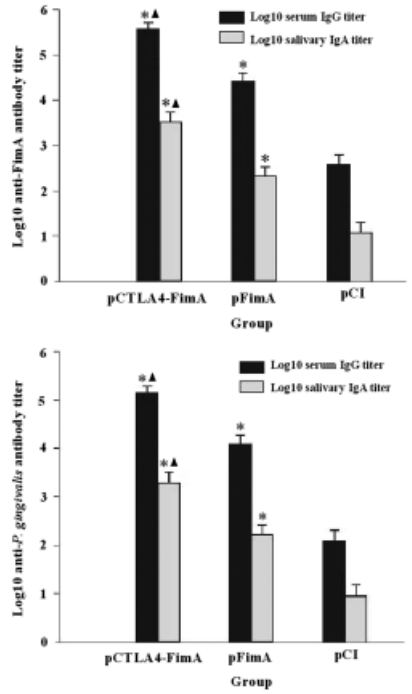

Fig. 2. Serum IgG and salivary IgA antibody responses to FimA and formalin-fixed *Porphyromonas gingivalis* following intra-nasal immunization in mice. BALB/c mice were immunized with pCTLA4-FimA, pFimA or pCI by the intra-nasal route on days 0 and 14. Blood and saliva samples were collected 4 weeks after the last immunization for ELISA. Data were presented as means plus standard deviations of the $\log_{10}$ antibody titre. One-way ANOVA was used for inter-group comparisons, followed by the Student–Newman–Keuls test for multiple-mean comparisons. *Significantly different from pCI control group ($p<0.01$). ▲pCTLA4-FimA-immunized group significantly different from pFimA-immunized group ($p<0.01$).

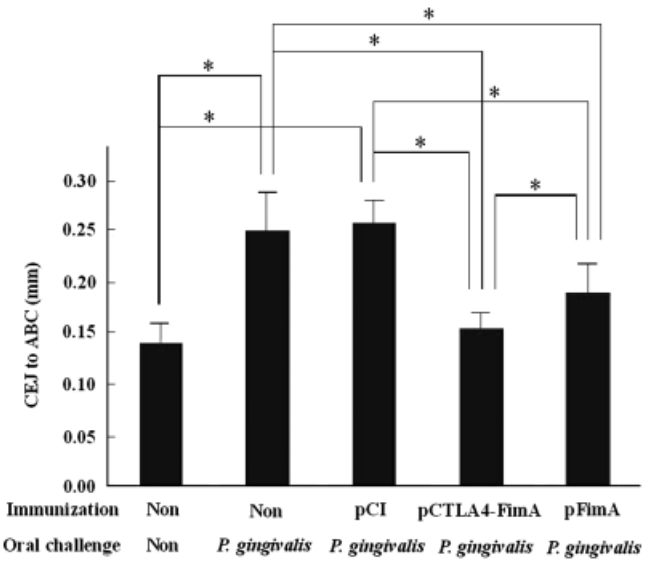

Fig. 3. Levels of alveolar bone loss elicited following *Porphyromonas gingivalis* challenge in mice. BALB/c mice were immunized with pCTLA4-FimA or pFimA intra-nasally. pCI immunized and non-immunized mice orally challenged with *P. gingivalis* were used as controls. The non-immunized and non-infected mice were used to determine the baseline value from cementoenamel junction to alveolar bone crest in normal mice. Forty-two days after *P. gingivalis* challenge, mice were sacrificed. Linear measurements ($n = 14$ sites) were obtained from the maxillary molars of each mouse, and the data presented are the means ± standard errors of the means of the pooled linear measurements (in micrometres) obtained from each group. One-way ANOVA was used for inter-group comparisons, followed by the Student–Newman–Keuls test for multiple-mean comparisons. *$p<0.01$.

potential broader importance of preventing this oral disease (Garcia et al. 2001). To limit the transmission and intra-oral dissemination of periodontal bacteria, it appears advantageous for an effective vaccine to induce immunity against periodontitis. DCs have always been logical targets for vaccine designs. For example, specific immunity could be induced and reduction in the size of established tumour was observed after tumour antigen-loaded DCs were re-infused as a vaccine (Eggert et al. 2002). Antibodies directed against the DC-specific, endocytic receptor DEC-205 allowed efficient uptake and presentation of antigens coupled to the antibodies by the targeted DCs (Nchinda et al. 2008). Researchers found that directing antigens directly to DCs by CTLA4 could enhance the immune responses. It was suggested that the enhanced antibody responses were due to the enhanced capture of antigens by DCs and subsequently more rapid antigen migration to lymph nodes (Drew et al. 2001). Here, we showed that increased serum IgG and salivary IgA responses were observed in targeted plasimd pCTLA4-FimA immunized mice compared with in non-targeted plasmid pFimA immunized mice, which proved the targeted DNA vaccine strategy could augment antibody responses against *P. gingivalis* antigen FimA.

The systemic immune and mucosal immune systems are two distinct compartments of the immune system. Antibodies associated with the systemic compartment are mainly of which function to neutralize pathogens in the circulatory system. In contrast, antibodies in the mucosa are primarily sIgA, which function to prevent entry of pathogens into the body via the mucosal surface (Lamm 1977). Systemic immunization can induce humoral immune responses in systemic (e.g. spleen, blood and draining lymph nodes) but not mucosal (e.g. salivary glands, aspiratory tract and genitourinary tracts) compartments. Hence, trials to induce mucosal immune responses have been performed by using some mucosal immunization routes, such as intra-vaginal or oral immune routes. These studies demonstrated that sIgA were induced not only at the immune sites but also at remote mucosal effector sites. Systemic IgG immune responses could also be induced after mucosal immunization (Park et al. 2003, Fayad et al. 2004). Thus, the development of an appropriate mucosal vaccine could lead to the induction of two layers of pathogen-specific immunity in both mucosal and systemic immune compartments. Our results here displayed that DNA vaccines against *P. gingivalis* could induce systemic IgG and salivary IgA responses after intra-nasal immunization. Compared with other mucosal delivery routes such as oral administration, nasal immunization is more effective for the induction of antigen-specific antibody responses in the mucosal compartment of the upper part of the body (Yanagita et al. 1999). Lower doses of antigens are needed because intra-nasal immunization does not expose antigens to low pH and a broad range of secreted degradative enzymes (Vajdy & O'Hagan 2001).

In conclusion, in this study, intranasal immunization with a DC-targeted *fimA* DNA vaccine induced enhanced systemic and mucosal immunity and offered strong potential in preventing alveolar bone loss incurred by *P. gingivalis* infection in mice. A DNA-based immunization strategy may be as an effective way to attenuate periodontitis induced by *P. gingivalis*. Although implantation of *P. gingivalis* in experimental animal models may not actually represent a monoinfection because *P. gingivalis* infection leads to the overgrowth of the indigenous flora that could contribute to the observed bone loss, animal models involving co-infection with periodontal bacteria, which more closely mimic the nature of this disease (Polak et al. 2010), would be used to evaluate the effect of FimA of *P. gingivalis* as an immunogen on the outcome of poly-microbial infections in our future studies. A polyvalent DNA vaccine containing different antigen epitopes from *P. gingivalis* and other periodontal bacteria may be needed to be constructed to better prevent the progression of experimental periodontitis induced by mixed bacterial infections.

References

Baker, P. J., Evans, R. T. & Roopenian, D. C. (1994) Oral infection with *Porphyromonas gingivalis* and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. *Archives of Oral Biology* **39**, 1035–1040.

Conry, R. M., Curiel, D. T., Strong, T. V., Moore, S. E., Allen, K. O., Barlow, D. L., Shaw, D. R. & LoBuglio, A. F. (2002) Safety and immunogenicity of a DNA vaccine encoding carcinoembryonic antigen and hepatitis B surface antigen in colorectal carcinoma patients. *Clinical Cancer Research* **8**, 2782–2787.

Coombes, B. K. & Mahony, J. B. (2001) Dendritic cell discoveries provide new insight into the cellular immunobiology of DNA vaccines. *Immunology Letters* **78**, 103–111.

Deliyannis, G., Boyle, J. S., Brady, J. L., Brown, L. E. & Lew, A. M. (2000) A fusion DNA vaccine that targets antigen-presenting cells increases protection from viral challenge. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 6676–6680.

Deslauriers, M., Haque, S. & Flood, P. M. (1996) Identification of murine protective epitopes on the *Porphyromonas gingivalis* fimbrillin molecule. *Infection and Immunity* **64**, 434–440.

Drew, D. R., Boyle, J. S., Lew, A. M., Lightowlers, M. W., Chaplin, P. J. & Strugnell, R. A. (2001) The comparative efficacy of CTLA-4 and L-selectin targeted DNA vaccines in mice and sheep. *Vaccine* **19**, 4417–4428.

Eggert, A. O., Becker, J. C., Ammon, M., McLellan, A. D., Renner, G., Merkel, A., Bröcker, E. B. & Kämpgen, E. (2002) Specific peptide-mediated immunity against established melanoma tumors with dendritic cells requires IL-2 and fetal calf serum-free cell culture. *European Journal of Immunology* **32**, 122–127.

Evans, R. T., Klausen, B. & Genco, R. J. (1992a) Immunization with fimbrial protein and peptide protects against *Porphyromonas gingivalis*-induced periodontal tissue destruction. *Advances in Experimental Medicine and Biology* **327**, 255–262.

Evans, R. T., Klausen, B., Sojar, H. T., Bedi, G. S., Sfintescu, C., Ramamurthy, N. S., Golub, L. M. & Genco, R. J. (1992b) Immunization with *Porphyromonas* (Bacteroides) *gingivalis* fimbriae protects against periodontal destruction. *Infection and Immunity* **60**, 2926–2935.

Fayad, R., Zhang, H., Quinn, D., Huang, Y. & Qiao, L. (2004) Oral administration with papillomavirus pseudovirus encoding IL-2 fully restores mucosal and systemic immune responses to vaccinations in aged mice. *The Journal of Immunology* **173**, 2692–2698.

Garcia, R. I., Henshaw, M. M. & Krall, E. A. (2001) Relationship between periodontal disease and systemic health. *Periodontology 2000* **25**, 21–36.

Giri, M., Ugen, K. E. & Weiner, D. B. (2004) DNA vaccines against human immunodeficiency virus type 1 in the past decade. *Journal of Clinical Microbiology* **17**, 370–389.

Gonzalez, D., Tzianabos, A. O., Genco, C. A. & Gibson, F. C. III (2003) Immunization with *Porphyromonas gingivalis* capsular polysaccharide prevents *P. gingivalis*-elicited oral bone loss in a murine model. *Infection and Immunity* **71**, 2283–2287.

Hajishengallis, G., Martin, M., Sojar, H. T., Sharma, A., Schifferle, R. E., DeNardin, E., Russell, M. W. & Genco, R. J. (2002) Dependence of bacterial protein adhesins on toll-like receptors for proinflammatory cytokine induction. *Clinical and Diagnostic Laboratory Immunology* **9**, 403–411.

Hajishengallis, G., Shakhatreh, M. A., Wang, M. & Liang, S. (2007) Complement receptor 3 blockade promotes IL-12-mediated clearance of *Porphyromonas gingivalis* and negates its virulence in vivo. *Journal of Immunology* **179**, 2359–2367.

Hajishengallis, G., Wang, M., Liang, S., Triantafilou, M. & Triantafilou, K. (2008) Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 13532–13537.

Jia, R., Guo, J. H., Fan, M. W., Bian, Z., Chen, Z., Fan, B., Yu, F. & Xu, Q. A. (2006) Immunogenicity of CTLA4 fusion anti-caries DNA vaccine in rabbits and monkeys. *Vaccine* **24**, 5192–5200.

Kawabata, S., Terao, Y., Fujiwara, T., Nakagawa, I. & Hamada, S. (1999) 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* **67**, 5863–5868.

Kim, T. G., Huy, N. X., Kim, M. Y., Jeong, D. K., Jang, Y. S., Yang, M. S., Langridge, W. H. & Lee, J. Y. (2009) Immunogenicity of a cholera toxin B subunit *Porphyromonas gingivalis* fimbrial antigen fusion protein expressed in E. coli. *Molecular Biotechnology* **41**, 157–164.

Kowalczyk, D. W. & Ertl, H. C. (1999) Immune responses to DNA vaccines. *Cellular and Molecular Life Sciences* **55**, 751–770.

Lakey, R. L., Morgan, T. G., Rowan, A. D., Isaacs, J. D., Cawston, T. E. & Hilkens, C. M. (2009) A novel paradigm for dendritic cells as effectors of cartilage destruction. *Rheumatology* **48**, 502–507.

Lamm, M. E. (1997) Interaction of antigens and antibodies at mucosal surfaces. *Annual Review of Microbiology* **51**, 311–340.

Lamont, R. J. & Jenkinson, H. F. (1998) Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiology and Molecular Biology Reviews* **62**, 1244–1263.

Li, Y. H., Huang, S., Du, M., Bian, Z., Chen, Z. & Fan, M. W. (2010) Immunogenic characterization and protection against *Streptococcus mutans* infection induced by intranasal DNA prime–protein boost immunization. *Vaccine* **28**, 5370–5376.

Lopatin, D. E., LaBelle, D. & Lee, S. W. (1991) Measurement of relative avidity of antibodies reactive with *Porphyromonas* (Bacteroides) *gingivalis* in the sera of subjects having adult periodontitis. *Journal of Periodontal Research* **26**, 167–175.

Martin, T., Parker, S. E., Hedstrom, R., Le, T., Hoffman, S. L., Norman, J., Hobart, P. & Lew, D. (1999) Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. *Human Gene Therapy* **10**, 759–768.

Miyachi, K., Ishihara, K., Kimizuka, R. & Okuda, K. (2007) Arg-gingipain A DNA vaccine prevents alveolar bone loss in mice. *Journal of Dental Research* **86**, 446–450.

Mooney, J. & Kinane, D. F. (1994) Humoral immune responses to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in adult periodontitis and rapidly progressive periodontitis. *Oral Microbiology and Immunology* **9**, 321–326.

Nchinda, G., Kuroiwa, J., Oks, M., Trumpfheller, C., Park, C. G., Huang, Y., Hannaman, D., Schlesinger, S. J., Mizenina, O., Nussenzweig, M. C., Uberla, K. & Steinman, R. M. (2008) The efficacy of DNA vaccination is enhanced in mice by targeting the encoded protein to dendritic cells. *The Journal of Clinical Investigation* **118**, 1427–1436.

Nelles, M. J., Niswander, C. A., Karakawa, W. W., Vann, W. F. & Arbeit, R. D. (1985) Reactivity of type-specific monoclonal antibodies with *Staphylococcus aureus* clinical isolates and purified capsular polysaccharide. *Infection and Immunity* **49**, 14–18.

Nichols, W. W., Ledwith, B. J., Manam, S. V. & Troilo, P. J. (1995) Potential DNA vaccine integration into host cell genome. *Annals of the New York Academy of Sciences* **772**, 30–39.

Njoroge, T., Genco, R. J., Sojar, H. T., Hamada, N. & Genco, C. A. (1997) A role for fimbriae in *Porphyromonas gingivalis* invasion of oral epithelial cells. *Infection and Immunity* **65**, 1980–1984.

Ogawa, T. & Uchida, H. (1995) A peptide, ALTTE, within the fimbrial subunit protein from *Porphyromonas gingivalis*, induces production of interleukin 6, gene expression and protein phosphorylation in human peripheral blood mononuclear cells. *FEMS Immunology and Medical Microbiology* **11**, 197–205.

Ogawa, T., Uchida, H. & Hamada, S. (1994) *Porphyromonas gingivalis* fimbriae and their synthetic peptides induce proinflammatory cytokines in human peripheral blood monocyte cultures. *FEMS Microbiology Letters* **116**, 237–242.

Pachuk, C. J., Ciccarelli, R. B., Samuel, M., Bayer, M. E., Troutman, R. D., Zurawski, D. V., Schauer, J. I., Higgins, T. J., Weiner, D. B., Sosnoski, D. M., Zurawski, V. R. & Satishchandran, C. (2000) Characterization of a new class of DNA delivery complexes formed by the local anesthetic bupivacaine. *Biochimica et Biophysica Acta* **1468**, 20–30.

Park, J. S., Oh, Y. K., Kang, M. J. & Kim, C. K. (2003) Enhanced mucosal and systemic immune responses following intravaginal immunization with human papillomavirus 16 L1 virus-like particle vaccine in thermosensitive mucoadhesive delivery systems. *Journal of Medical Virology* **70**, 633–641.

Parsons, K. R., Young, J. R., Collins, B. A. & Howard, C. J. (1996) Cattle CTLA-4, CD28 and chicken CD28 bind CD86: MYPPPY is not conserved in cattle CD28. *Immunogenetics* **43**, 388–391.

Polak, D., Wilensky, A., Shapira, L., Weiss, E. I. & Houri-Haddad, Y. (2010) Vaccination of mice with *Porphyromonas gingivalis* or *Fusobacterium nucle-*

atum modulates the inflammatory response, but fails to prevent experimental periodontitis. *Journal of Clinical Periodontology* **37**, 812–817.

Rasmussen, R. A., Ong, H., Kittel, C., Ruprecht, C. R., Ferrantelli, F., Hu, S. L., Polacino, P., McKenna, J., Moon, J., Travis, B. & Ruprecht, R. M. (2006) DNA prime/protein boost immunization against HIV clade C: safety and immunogenicity in mice. *Vaccine* **24**, 2324–2332.

Takahashi, Y., Kumada, H., Hamada, N., Haishima, Y., Ozono, S., Isaka, M., Yasuda, Y., Tochikubo, K. & Umemoto, T. (2007) Induction of immune responses and prevention of alveolar bone loss by intranasal administration of mice with *Porphyromonas gingivalis* fimbriae and recombinant cholera toxin B subunit. *Oral Microbiology and Immunology* **22**, 374–380.

Vajdy, M. & O'Hagan, D. T. (2001) Microparticles for intranasal immunization. *Advanced Drug Delivery Reviews* **51**, 127–141.

Weinberg, A., Belton, C. M., Park, Y. & Lamont, R. J. (1997) Role of fimbriae in *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infection and Immunity* **65**, 313–316.

Whitney, C., Ant, J., Moncla, B., Johnson, B., Page, R. C. & Engel, D. (1992) Serum immunoglobulin G antibody to *Porphyromonas gingivalis* in rapidly progressive periodontitis: titer, avidity, and subclass distribution. *Infection and Immunity* **60**, 2194–2200.

Xu, Q. A., Yu, F., Fan, M., Xing, Q., Liu, G. & Liu, C. (2009) Analysis of the molecular mechanisms of targeted anti-caries DNA plasmid enhancing antibody responses by gene arrays. *The Journal of Gene Medicine* **11**, 354–360.

Yanagita, M., Hiroi, T., Kitagaki, N., Hamada, S., Ito, H. O., Shimauchi, H., Murakami, S., Okada, H. & Kiyono, H. (1999) Nasopharyngeal-associated lymphoreticular tissue (NALT) immunity: fimbriae-specific Th1 and Th2 cell-regulated IgA responses for the inhibition of bacterial attachment to epithelial cells and subsequent inflammatory cytokine production. *The Journal of Immunology* **162**, 3559–3565.

Yang, K., Whalen, B. J., Tirabassi, R. S., Selin, L. K., Levchenko, T. S., Torchilin, V. P., Kislauskis, E. H. & Guberski, D. L. (2008) A DNA vaccine prime followed by a liposome-encapsulated protein boost confers enhanced mucosal immune responses and protection. *The Journal of Immunology* **180**, 6159–6167.

Address:
Fei Yu
Center of Stomatology
Tongji Hospital
Tongji Medical College
Huazhong University of Science and Technology
Jiefang Road 1095
Wuhan 430030
Hubei
China
E-mail: ymai016@yahoo.com.cn

Clinical Relevance

Scientific rationale for the study: Vaccines are considered to have immunoprophylactic potential against periodontitis. Improving the immunological potency of vaccines against periodontal pathogens can increase the protective efficacy of these vaccines. Targeting antigens to antigen-presenting cells such as DCs by fusion to CTLA4 has been shown to be a highly efficient way to enhance the efficacy of DNA vaccines.

Principal findings: Intra-nasal immunization with DC-targeted *fimA* DNA vaccine induced higher levels of serum IgG and salivary IgA antibodies compared with the non-targeted DNA vaccine. The targeted vaccine significantly reduced the alveolar bone loss incurred by *P. gingivalis* infection in mice.

Practical implications: FimA is a potential candidate antigen for the development of a DNA vaccine against periodontitis. A targeted DNA-based strategy may improve the efficacy of such a vaccination.