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Immunization of *Macaca fascicularis* against experimental periodontitis using a vaccine containing cysteine proteases purified from *Porphyromonas gingivalis*

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Introduction: Periodontitis is a common infectious disease to which *Porphyromonas gingivalis* has been closely linked, in which the attachment tissues of the teeth and their alveolar bone housing are destroyed. We conducted a study to determine if immunization using a purified antigen could alter the onset and progression of the disease.
Methods: Using the ligature-induced model of periodontitis in *Macaca fascicularis*, we immunized five animals with cysteine protease purified from *P. gingivalis* and used an additional five animals as controls. Alveolar bone loss was measured by digital subtraction radiography.
Results: Immunization induced high titers of specific immunoglobuin G serum antibodies that were opsonic. Total bacterial load, levels of *P. gingivalis* in subgingival plaque and levels of prostaglandin E_2 in gingival crevicular fluid were significantly reduced. Onset and progression of alveolar bone loss was inhibited by approximately 50%. No manifestations of toxicity were observed.
Conclusions: Immunization using a purified protein antigen from *P. gingivalis* inhibits alveolar bone destruction in a ligature-induced periodontitis model in *M. fascicularis.*

Key words: alveolar bone loss; cysteine protease; periodontitis; *Porphyromonas gingivalis*; serum antibodies; vaccine

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Periodontitis is an infectious disease caused by predominantly gram-negative, anaerobic bacteria, among which *Porphyromonas gingivalis* is the most strongly linked (18, 56). The disease causes destruction of the periodontal ligament and the alveolar bone housing the teeth and is a major cause of tooth loss (40). Prevalence of the disease is high in the USA and most other industrialized countries. Periodontitis affects about 35% of Americans aged 18–65 years, and disease of sufficient severity to endanger the dentition in the absence of treatment is seen in approximately 13% (1). The prevalence of the disease may have decreased in the 1990s (7). Although successful treatment is possible, it is costly, of limited availability, unsuccessful in many cases and may be painful.

Development of a vaccine for the prevention and control of periodontitis appears to be possible. Studies conducted in rodents have demonstrated that alveolar bone destruction can be inhibited by immunization (5, 17, 22, 23, 37, 38, 41, 47, 60). Using a vaccine containing intact killed *P. gingivalis* as antigen and Syntex Adjuvant Formulation-M (SAF-M adjuvant), Persson et al. (41) were able to

inhibit the progression of experimental periodontitis in the non-human primate *Macaca fascicularis*, as measured by radiographic assessment of alveolar bone loss. Thus, although periodontitis in humans and most other species appears to be a polymicrobial infection, alveolar bone loss, its predominant pathological feature, can be inhibited by vaccination using a single bacterial species, formalinized *P. gingivalis*, as antigen.

The next logical step in vaccine development was to identify and test a purified antigen from *P. gingivalis* that, when used in a vaccine, can induce protection. Of all of the *P. gingivalis* components studied to date, the cysteine proteases (porphypains, gingipains) have shown the most promise. The cysteine proteases are present in large quantities on the cell surface of *P. gingivalis* (28), and they account for much of the proteolytic, hemagglutinin and adhesin activities of this species (44–46). They can not only degrade proteinase inhibitors, immunoglobulins and iron transporting and sequestering proteins but also activate the complement, coagulation and kinin cascades and destroy bactericidal proteins and peptides (27, 44, 46, 48, 57). Cysteine proteases appear to be present in all clonal types and serotypes of *P. gingivalis* studied and no cysteine protease-negative clones have been reported except for laboratory-generated mutants. Furthermore, local application in humans of anti-*P. gingivalis* monoclonal antibodies specific for an epitope present in cysteine proteases prevented recolonization by *P. gingivalis* for up to 9 months (6).

The purposes of the present study were to determine whether a vaccine containing purified cysteine protease from *P. gingivalis* could induce protection against experimental peridontitis in *M. fascicularis*, to assess safety and to obtain sufficient data to design a larger and more definitive study.

Materials and methods

Animal screening and enrollment

The protocol was approved by the Animal Care Committee of the Health Sciences Center, University of Washington, Seattle, WA. Young adult male and female *M. fascicularis* monkeys were obtained through the National Primate Research Center of the University of Washington, and were housed in that facility. The animals had been released from quarantine, acclimated and tested by Primate Center staff. Only retrovirus D- and tuberculosis-negative animals that had passed a general examination by a veterinarian and had received no antibiotic treatment for the previous 3 months were considered for screening.

Table 1. Enrollment criteria

Estimated age 4–8 years; weight 3–6 kg
All molar and premolar teeth present
Early to moderate gingival inflammation
No probing depths ≥4 mm
No manifestations of oral or systemic disease
No radiographic periapical lesions
Presence of *Porphyromonas gingivalis* and at least three of the other five microorganisms being monitored
Serum immunoglobulin G titers of anti-*P. gingivalis* antibodies between 2 and 25 ELISA units
Pregnant or lactating animals excluded.

Animals selected for screening were sedated as described below, weighed and visually examined for injuries, coat quality and overall condition. An oral examination was performed, subgingival plaque and venous blood were sampled and analysed, and periapical radiographs were taken as described below. Enrollment criteria are described in Table 1. Qualifying animals were randomized into an experimental (to be immunized) group and a control group of five animals each. General health and well-being of the animals were monitored and maintained by staff veterinarians at the Center.

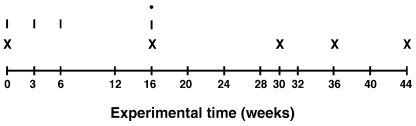

Fig. 1. Protocol outline with time-points in weeks. Samples of blood, subgingival plaque and saliva were harvested at all time-points. Additional blood samples were taken at weeks 1, 2 and 6. Control and immunized animals were vaccinated at baseline and at weeks 3, 6 and 16 as indicated (vertical bars). Ligatures were placed around mandibular and maxillary posterior teeth at week 16 (dot). Radiographs of the mandibular teeth were taken at baseline and at weeks 16, 30, 36 and 44 (X) and the ligated teeth were supra-infected with viable *Porphyromonas gingivalis* at weeks 36 and 40.

Clinical and radiographic examination and tooth ligation

The study protocol was the same as that described by Persson et al. (41) with minor modifications, except that cysteine protease (porphypain) purified from *P. gingivalis* instead of intact formalin-killed *P. gingivalis* was used as antigen (11) (Fig. 1). Throughout the study animals were sedated by administering 10 mg/kg ketamine, supplemented with the same drug as needed during the examination and data gathering procedures. At each examination animals were weighed and their overall health was assessed. Oral examination consisted of examination of the teeth, oral mucosa and tongue and recording missing teeth and the extent of gingival inflammation (30); probing pocket depth and attachment levels at four positions around each mandibular and maxillary posterior tooth were measured using a hand-held pressure-controlled digital probe (Florida probe; Florida Probe Corp., Gainesville, FL). Bleeding on probing was recorded as yes or no for each site.

At week l6, subgingival ligatures were placed on the mandibular molars and premolars using 000 braided silk suture material (Ethicon; Johnson and Johnson, Somerville, NJ) as described previously (41). At each examination, ligatures were checked and, if loose or missing, they were replaced. All investigators, except the person who prepared the vaccines, were blinded throughout the study as to which animals received complete vaccine and which did not.

At the times indicated in Fig. l, periapical radiographs of the maxillary and mandibular posterior teeth were taken using Kodak ultra-speed type D pediatric film and a Trophy ETX X-ray machine (Vincennes, France) at a distance of 60 mm at 70 kVp and 8 mA with a 0.25-s exposure. All films were developed using the same automatic processor (Dent-X, Elmsford, NY). Changes in alveolar bone height around the test teeth were determined by digital subtraction radiography as described by Jeffcoat et al. (21) and used as the primary outcome measure.

Sample collection

At each time-point noted in Fig. 1 plus days 1 and 2, 10-ml samples of venous blood were drawn and allowed to clot, and serum was prepared by centrifugation. Serum samples were separated into aliquots and stored at −70°C until assayed. Gingival crevicular fluid was sampled from test teeth before harvesting the subgingival flora or probing using filter paper strips as described previously (41).

Samples were stored −70°C. The subgingival flora were sampled from molars and premolars in each quadrant using paper points as described previously (41). Approximately 100 µl saliva was harvested using a microliter pipette and stored at −70°C.

Immunizations and super-infection

Porphypain was purified chromatographically from *P. gingivalis* strain W12 (11). The enzyme activity was irreversibly inactivated by precipitation with trichloroacetic acid, and the proteins were washed with ethanol and dissolved in 50 mM Tris buffer at pH 7.4. At the time the study was performed, porphypain-1 and porphypain-2 had not been successfully separated. The preparation we used contained both 150 kDa porphypain-1 and 120 kDa porphypain-2 at a protein concentration of 0.842 mg protein/ml. The preparation was shipped to our laboratory frozen. It was diluted to 42.85 µg/µl with 50 mM Tris buffer at pH 7.4, separated into portions needed for each immunization and stored at −20°C. A single batch of antigen was used for the entire experiment. The adjuvant used, SAF-M (2), was kindly provided by Dr A.C. Allison. It consisted of termutide powder (threonyl murymal dipeptide) and a liquid containing L-pluronic 121, squalene and Tween-80. These were stored at 4°C.

The vaccine was formulated as follows at the time of use and was not stored: 0.2 mg termutide was added to 0.325 ml of the liquid; this was combined with an aliquot of the antigen solution containing 75 µg porphypain in 0.175 ml. The suspension was well mixed and 0.25 ml was injected subcutaneously in the skin of the back near the scapula and 0.25 ml into the deltoid muscle of the arm. Animals were vaccinated at baseline with booster injections at weeks 3, 6 and 16. The control vaccine was formulated and administered in an identical manner except that buffer with no antigen was added to the preparation.

At weeks 32 and 36, the ligated teeth were super-infected by application of viable *P. gingivalis* (strain #5083 originally isolated from *M. fascicularis*) bacteria using a cotton swab. Bacteria to be used for super-infection were grown on blood agar plates as follows. A loop of *P. gingivalis* was applied to approximately three-quarters of each plate using a zig-zag motion; two isolation strips were placed on the remainder of the plate, and a loop full of a coagulase-positive *Staphylococcus* was touched to the plate surface at four locations sufficiently distant from *P. gingivalis* to ensure that cross-contamination did not occur. *Staphylococcus* serves as a growth enhancer for *P. gingivalis*. The plates were incubated for 4 days. Bacteria were removed from the plates using a cotton swab and applied directly to the teeth of the sedated animals using one plate for two teeth.

Laboratory studies

Subgingival plaque samples were analysed using specific DNA probes (12, 33) as described previously (41). A universal bacterial probe was used to measure total bacterial load, and species-specific probes were used to measure levels of *P. gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans*. Titers of serum immunoglobulin G antibodies and serum and salivary IgA antibodies reactive with antigens of *P. gingivalis* were determined by enzyme-linked immunosorbent assay using microtiter wells coated with sonicates of the monkey isolate of *P. gingivalis* strain 5083 as described previously (53). Specific antibody titers were calculated as described by Peterman and Butler (42). Western immunoblots were prepared as described by Nakagawa et al. (35) using immune and preimmune sera and arg-gingipain (HrgpA) and lys-gingipain (Kgp) purified from *P. gingivalis* strain HG66 (43) (kindly provided by Dr James Travis and Dr Jan Potempa, Department of Biochemistry and Molecular Biology, University of Georgia, Athens GA).

Levels of prostaglandin E_2 in gingival crevicular fluid were measured using competitive inhibition kits (Amersham Life Sciences, Little Chalfont, Buckinghamshire, UK) following the instructions of the manufacturer.

Chemiluminescence, a measure of opsonization of target bacteria by a specific immunoglobulin G antibody, was determined as described by Easmon et al. (13) and modified by Nakagawa et al. (35). Blood was drawn from a healthy, periodontally normal adult male, and polymorphonuclear leukocytes were isolated from the heparinized blood using Mono-Poly Resolving Medium (ICN Biomedical Corp., Costa Mesa, CA). Controls included tubes with polymorphonuclear leukocytes or bacteria only, polymorphonuclear leukocytes plus bacteria, and polymorphonuclear leukocytes plus bacteria plus preimmune sera. Immune sera from experimental weeks 6, 20, 24 and 44 were tested in triplicate for all control and immunized animals. Laboratory studies were repeated a minimum of three times and representative data are shown.

Data analysis

Given the small number of animals in the study, all statistical comparisons between immunized animals and controls were performed using the Wilcoxon rank sum test. A non-parametric test procedure based on ranked data, and exact methods were used to compute the statistical significance (SAS Version 9.1 software; SAS Institute, Inc., Cary, NC). Correlation analysis was used to assess the relationship between chemiluminescence and bone loss.

Toxicity evaluation

Vaccine injection sites were marked with a black ink dot and visually evaluated for swelling, ulceration and induration. Following inoculation and data collection at week 16, biopsy specimens were harvested from some injection sites at 24 h and from others at 72 h for histological evaluation. Serum samples harvested at week 44 were transported to the clinical laboratories at the University of Washington Medical Center where general serum chemistry, liver panel, renal panel, parathyroid calcium and phosphate and blood lipids were measured.

Results

The experimental and control animal groups each contained one male and four female animals. Mean animal weights at baseline were 5.0 (±1.1) and 4.5 (±1.3) kg for the control and immunized animal groups respectively and they did not differ significantly. At week 44, none of the animals had lost weight. There were no visual signs of swelling, acute inflammation, induration or ulceration at the injection sites, and histological evaluation of biopsies taken at days 1 and 3 post vaccination failed to reveal an inflammatory infiltrate (data not shown). Throughout the course of the study, there were no indications of malaise, change in coat quality or color, or loss of appetite in any of the animals. None of the laboratory values for general serum chemistry, liver panel, renal panel, parathyroid calcium and phosphate, and blood lipids for the two animal groups differed significantly ($P > 0.05$; data not shown).

Mean titers of serum immunoglobulin G antibody reactive with antigens of *P. gingivalis* 5083, a strain isolated originally from the oral cavity of *M. fascicularis*, are shown in Fig. 2. In sera from the immunized animals, mean titers increased by week 2, peaked at week 6, then began to decrease. Following the booster injection at week 16, values rose to a maximum at week 20 then decreased, but remained above control values through week 40. As indicated by the error bars, there was a great deal of variation among the animals in the magnitude and time to peak titers. An increase in specific antibody titers in the serum of control animals was seen following placement of the ligatures. Using immunoblots, we demonstrated that the induced serum antibodies bound to cysteine proteases isolated from *P. gingivalis*, to components in sonicates of *P. gingivalis* strain W12, the strain from which the antigen used was isolated, and to components in sonicates of *P. gingivalis* strain 5803, the monkey strain likely to be involved in causing periodontitis in *M. fascicularis* (data not shown).

Enhancement of specific IgA antibodies was observed in the sera of some, but not all, of the immunized animals but titers were very low and variable from one animal to another (data not shown). Enhancement of specific IgA antibodies could not be demonstrated in saliva from the immunized animals by the methods we used.

Immunization appeared to affect total subgingival bacterial load as well as values for *P. gingivalis* and some of the other species that were monitored. When calculated as change from baseline for weeks 3–16 and 20–32 total subgingival bacterial loads for immunized animals relative to controls were significantly reduced ($P = 0.008$) (Fig. 3).

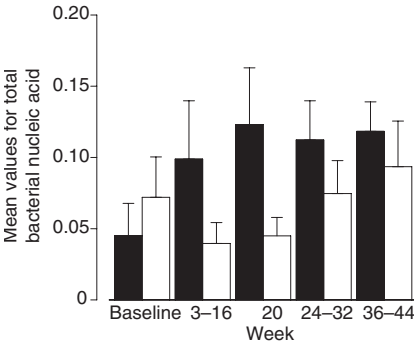

Fig. 3. Changes in total bacterial load for control (mean ± SE, black bars) and immunized animals (mean ± SE white bars) as determined using the universal DNA probe and reported as μg total bacterial nucleic acid.

Before the placement of ligatures (weeks 3–16) and 2 weeks after ligature placement (week 20), values for *P. gingivalis* were reduced significantly relative to controls ($P = 0.032$) (Fig. 4). For weeks 36–44, values were similar for immunized and non-immunized animals, most likely because that was the period of super-infection with living *P. gingivalis*. At week 20, *C. rectus* was also reduced significantly ($P = 0.008$) in samples from the immunized animals. There were no immunization effects on *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia* or *F. nucleatum* when values for samples from control and immunized animals were compared statistically (data not shown).

Results of the radiographic alveolar bone height measurement for ligated teeth in control and immunized animals are shown in Fig. 5. Mean and median loss in alveolar bone height from the time of tooth ligation at week 16 to weeks 36 and 44 were reduced more than 50% in the immunized animals relative to the controls ($P = 0.0079$), and the extent of reduction among animals was quite uniform. It is notable that during the period of super-infection from week 36 to 44, alveolar bone loss advanced considerably in the control animals but only slightly in the immunized animals. Before ligature placement at weeks 12–16, mean attachment loss was significantly less for vaccinated (−0.39 mm) than for control (− 0.72 mm) animals ($P = 0.05$) (data not shown).

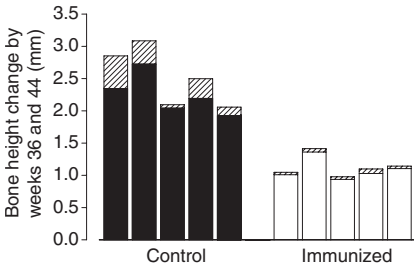

Fig. 5. Mean bone height change for each animal from week 16 to week 36 for controls (black bars) and immunized animals (white bars) and from week 36 to week 44 (stippled portions of all bars) as measured by digital subtraction radiography and reported in mm.

Prostaglandin E_2 levels in gingival crevicular fluid samples from ligated teeth in immunized animals were significantly reduced relative to values for control animals at weeks 16, 20 and 36 ($P < 0.05$), but not at week 44 (Fig. 6). There was a direct, site-specific association between prostaglandin E_2 levels in gingival crevicular fluid and the presence and extent of alveolar bone lost as measured by digital subtraction radiography; higher levels of prostaglandin E_2 related strongly to higher levels of bone loss (Fig. 7).

Chemiluminescence measurements were performed using *P. gingivalis* strain 5083 as the target organism and using preimmunization and week 6, 20, 24 and 44 sera from all control and immunized animals. Chemiluminescence is a measure of specific enhancement of phagocytosis by anti-*P. gingivalis* antibody and of killing of *P. gingivalis* by phagocytes. Chemiluminescence was enhanced by immune relative to control sera for all five animals, but the mean differences were statistically significant only for the 20-week samples ($P < 0.05$) (Fig. 8). In the 20-week samples, chemiluminescence was much lower for sera from control animals relative to immunized animals. For immunized

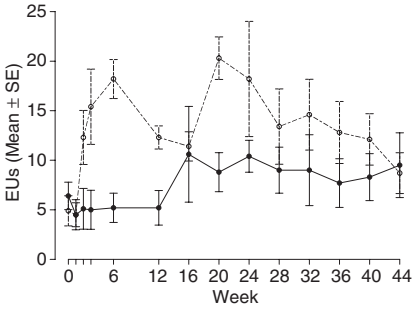

Fig. 2. Serum immunoglobulin G antibody titers (mean ± SE) reactive with antigens of sonicates of *Porphyromonas gingivalis* strain 5083 (monkey isolate) as determined using ELISA: samples from immunized animals (○) samples from controls (•).

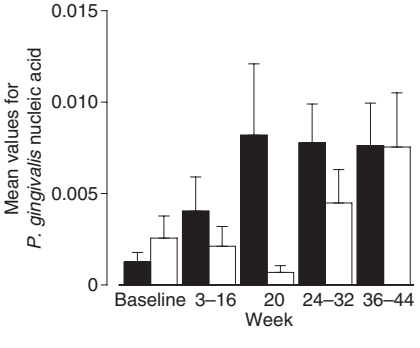

Fig. 4. Changes in amounts of *Porphyromonas gingivalis* in samples of the subgingival flora for control (mean ± SE, black bars) and immunized (mean ± SE, white bars) animals reported as μg DNA determined using the specific *P. gingivalis* DNA probe.

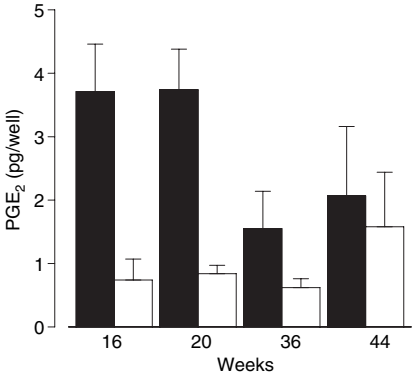

Fig. 6. Prostaglandin E_2 levels in gingival crevicular fluid for samples harvested at weeks 16, 20, 36 and 44 reported in pg/well. Black bars are mean values for samples from control animals (mean ± SE) and white bars are from immunized animals.

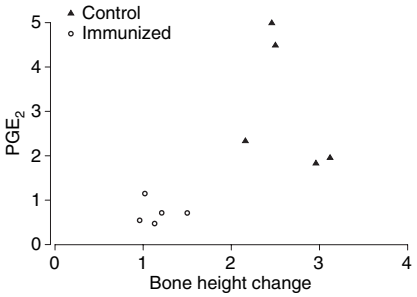

Fig. 7. Relationship between change in alveolar bone height (mm) reported on the horizontal vs. gingival crevicular fluid prostaglandin E_2 concentration (pg/well) reported on the vertical axis for control and immunized animals.

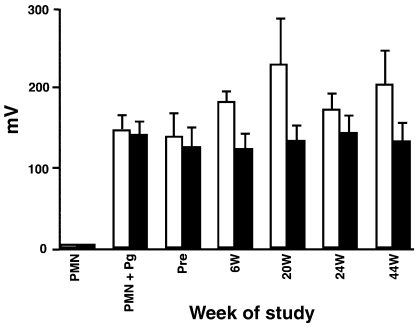

Fig. 8. Mean chemiluminescence reported as mean peak mV for control cultures containing polymorphonuclear leukocytes alone and polymorphonuclear leukocytes plus *Porphyromonas gingivalis* (Pg) and experimental cultures containing polymorphonuclear leukocytes, Pg and preimmune sera (Pre) or immune sera from weeks 6, 20, 24 and 44. Black bars represent sera from control animals and stippled bars represent sera from immunized animals.

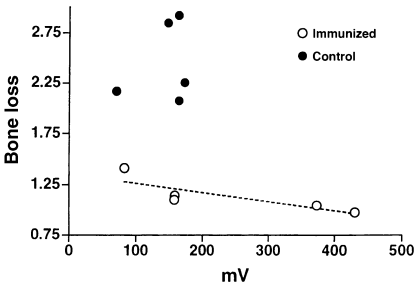

Fig. 9. The relationship between opsonic activity (mV) and alveolar bone loss (mm) for sera from control and immunized animals; $r = -0.9$ for immune sera ($P = 0.083$); $r = -0.1$ for control sera ($P = 0.95$).

animals, the extent of bone loss decreased linearly with increasing chemiluminescence ($r = -0.9$) (Fig. 9).

That was not the case for the 20-week samples from non-immunized animals ($P = 0.35$ for week 36; $P = 0.95$ for week 44). Enhancement of chemiluminescence by immune sera was not altered by prior inactivation of complement (data not shown).

Discussion

There are compelling reasons for the development of an anti-periodontitis vaccine. Periodontitis afflicts millions of people worldwide (1, 7, 8, 32). Not only is it a major cause of tooth loss, but also recent evidence indicates that periodontitis significantly enhances risk for several potentially disabling and fatal systemic diseases and conditions including heart disease, stroke, complications of diabetes and low-birthweight premature infants (3). In addition, existing treatments fail to control the progression of the disease in more than 10% of cases (19, 20, 31). A successful vaccine offers the most direct and cost-effective pathway to a reduction in the prevalence of periodontal disease in large populations and may provide an effective therapy for cases that do not respond to traditional therapies.

The infectious nature of periodontitis (18, 56), characteristics of the immune response in periodontitis patients (9, 37, 55) and vaccination studies conducted in rodents (17, 22, 23, 38, 47) and in non-human primates (34, 41) indicate that development of a successful vaccine for humans may be possible. The immunization experiment reported here was designed to determine whether a vaccine containing a purified *P. gingivalis* component as antigen could induce protection against periodontitis in a non-human primate model, to obtain preliminary data on the safety of the vaccine and to obtain sufficient data to design and conduct a larger and more definitive study. Our observations support the idea that a safe, effective anti-periodontitis vaccine can be developed.

We successfully demonstrated the principle that although periodontitis is considered to be a polymicrobial infection, a vaccine containing cysteine protease purified from *P. gingivalis* as antigen can significantly inhibit the induction and progression of the disease as measured by radiographic alveolar bone loss in experimental periodontitis in *M. fascicularis*. In addition, our evidence, although not definitive, indicates that the vaccine is non-toxic and safe. The pattern and magnitude of immunoglobulin G antibody response were very similar to results of a previous study using the same animal model and a vaccine containing intact formalin-killed *P. gingivalis* (41) but contrast with those in a report by Ebersole et al. (14).

Our study was similar to that conducted by Moritz et al. (33) in that we used the same antigen preparation and animal model but our study design and outcomes were somewhat different. Moritz et al. (34) used a split mouth design, fed a soft diet to induce gingivitis before placing ligatures and used Freund's incomplete adjuvant, while we used mandibular posterior teeth as test teeth without a split mouth, fed a standard monkey chow diet and used an adjuvant, SAF-M, that has been used in humans. The outcomes were similar with regard to the overall immune response and effect of immunization on suppressing alveolar bone loss. However, we observed a greater effect of the vaccine on suppressing alveolar bone loss and on reducing levels of plaque and *P. gingivalis*. In addition, we found evidence that a reduction in prostaglandin E_2 levels in gingival crevicular fluid and antibody-mediated enhancement of phagocytosis and killing of bacteria may underlie the immunization effects.

The potential of numerous purified components of *P. gingivalis* for use as antigen in an anti-periodontitis vaccine have been evaluated. These studies have included lipopolysaccharide (9, 50, 58), carbohydrate K surface antigens (10, 25, 26, 51, 54), various proteins including fimbriae and fimbrillin and the 53- and 67-kDa surface proteins (15, 16, 24, 29, 59, 61), and cysteine proteases (11, 28, 43). Of the *P. gingivalis* components studied, cysteine protease appears to be the only one that is expressed by all 50 or more genetically distinct *P. gingivalis* types.

The vaccine appeared to reduce alveolar bone loss by more than 50%. Although the number of animals studied was small, the magnitude and uniformity of the reduction in bone loss resulted in strong statistical significance ($P = 0.0079$). The extent of the reduction was greater and more uniform among animals than observed previously using the whole cell vaccine (41). The uniformity of the reduction in bone loss among the immunized animals was striking relative to the previous study (41) in which a great variation in inhibition of bone loss among the animals was observed.

Our data suggest two possible pathways by which specific antibodies may attenuate alveolar bone loss. Serum immunoglobulin G antibodies induced by the vaccine were opsonic and opsonic activity persisted throughout the study period. These antibodies were present in the gingival crevicular fluid (49), an exudate that originates from inflamed small blood vessels, traverses the tissue to enter periodontal pockets where it bathes the subgingival bacterial biofilms. Thus, one mechanism of immune protection may be a reduction in *P. gingivalis* and in total bacterial load by antibody-mediated enhancement of phagocytosis and killing of bacteria by polymorphonuclear leukocytes and other phagocytes. The observed reduction in *P. gingivalis* in subgingival plaque and in total plaque load supports this idea.

Reduction in levels of prostaglandin E_2 may provide a second mechanism whereby immunization may attenuate alveolar bone destruction. Prostaglandins are major mediators of bone resorption at sites of inflammation where they induce differentiation and maturation of osteoclasts and these cells resorb alveolar bone (52). Large amounts of prostaglandin E_2 are present in gingival crevicular fluid and inflamed periodontal tissue (39). Activated macrophages and fibroblasts are a major source of prostaglandin E_2 (36, 48). Levels of prostaglandin E_2 in the gingival crevicular fluid, and presumably in the periodontal tissue, were significantly reduced in the immunized relative to the control animals, especially through week 36, and the site-specific level of prostaglandin E_2 strongly related to the extent of bone loss at each site. Immune, but not control or preimmune, sera were able to inhibit prostaglandin E_2 production by human monocytes activated with *P. gingivalis* lipopolysaccharide (4). Inhibition was antigenically specific and did not occur when monocytes were activated with lipopolysaccharide from *E. coli*. Thus, elevated levels of antibody specific for *P. gingivalis* in the inflamed periodontal tissues may suppress alveolar bone resorption by inhibiting prostaglandin E_2 production.

In summary, although periodontitis is a chronic polymicrobial infection, previous studies in rodents and non-human primates have shown that immunization using a single microorganism, *P. gingivalis*, as antigen can provide protection. The present study shows that purified protein antigens from *P. gingivalis* can also provide protection as measured by reduction in alveolar bone destruction. Similar results have been reported by others (34). Possible mechanisms of immune protection appear to be specific antibody-mediated enhancement of phagocytosis of *P. gingivalis* and specific antibody-mediated inhibition of production of prostaglandins by resident fibroblasts and macrophages in the gingival tissue. Furthermore our data, although not definitive, suggest that the vaccine is safe.

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