

REVIEW ARTICLE

Immunization in Periodontics: A Systematic Review

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ABSTRACT

Background: Formulation of a vaccine against pathogenic microorganisms causing periodontal diseases has been under research for a decade. Many *in vitro* and *in vivo* studies have been made in this regard. The aim of this study is to review all preclinical (i.e. animal) and *in vivo* studies that presents supporting evidence for the feasibility of formulating a prophylactic human periodontal vaccine.

Materials and methods: A manual and electronic literature search was made for animal studies up to 2011 that presented clinical, morphologic (alveolar bone level), and immunologic data for the efficacy of a prophylactic periodontal vaccine. A total of 31 studies are included out of which nine are *in vivo* studies.

Results: *In vitro* studies revealed a definitive inhibitory effect of vaccines over periodontal disease causing organisms and their vulnerability toward such agents. Among the studies reviewed, *in vitro* outnumbered the *in vivo* studies and there is definitely a lack in quality and quantity of human trials in this regard. Most *in vitro* studies have shown results in favor of vaccines preventing the periodontal diseases by action against pathogenic organisms.

Conclusion: Because of the insufficient quality and quantity of human trials, no adequate evidence could be gathered to use the beneficial effects of these animal experiments to formulate a prophylactic human periodontal vaccine. Thus, good quality animal and human trials are needed in this field of vaccination against periodontal diseases.

Keywords: Periodontal vaccine, Animal studies, Periodontal diseases.

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INTRODUCTION

Periodontal diseases belong to a heterogeneous family of diseases, which demands a clear need for a better understanding of the etiology and pathogenesis behind formulation of a vaccine against the same. Both specific and nonspecific plaque hypothesis has its own merits and demerits.^{1,2} However, epidemiological evidence indicates that host factors are likely to be of over-riding importance for the most severe forms. Specific inhibitors of virulence factors provide a logical approach, but their clinical application still demands improvement. Improvement of general health and resistance to disease by proper nutrition, the avoidance of intercurrent disease, and elimination of smoking and stress-induced risk are encouraged. The genetic basis of susceptibility to periodontitis is increasingly

understood, and, while gene therapy is not likely to be a practicable approach to prevention, genetic markers of risk are emerging. The vaccine should also be investigated first in animal models like rodents, followed possibly by nonhuman primates, before being studied in human beings. Various bacterial strains being investigated are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* (previously *T. forsythensis*), *Bacteroides macacae*, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) and *Campylobacter rectus*. These strains are used for periodontal vaccine preparation. The immunogens being tested included whole cells, sonicated cell walls, fimbriae and few purified proteins.

Reviews so far conducted have stressed on the need for preventive therapy for periodontal disease because of its worldwide prevalence, systemic disease linkage, and the failure of traditional periodontal therapy to regenerate the lost periodontium or to eliminate the disease. Thus, the aim of this review is to discuss all the preclinical studies, which support the evidence for the feasibility of formulating a prophylactic human periodontal vaccine.

MATERIALS AND METHODS

The PubMed (MEDLINE) database of the US National Library of Medicine and the Cochrane Library of the Cochrane Collaboration (CENTRAL) were used as electronic databases, and a literature search was accomplished of articles published in English from 1950 to February 2010. Articles available online in electronic form before their publication in material form (Epub ahead of print or early online articles) were considered to be eligible for inclusion in the present article. The electronic search was carried out by applying the following terms and keywords: Vaccination, immune response, genomic vaccine, recombinant vaccine, subunit vaccine, adjuvant, animal model, animal study, preclinical study, monkey study, rat study, dog study, rabbit study, periodontal vaccine, human periodontal vaccine, vaccine for periodontal disease, prevention of periodontal disease by vaccine, vaccination against periodontal disease, vaccination against periodontal bacteria, animal trials for periodontal vaccine, animal studies for periodontal vaccine, periodontal vaccine using animal models, periodontal vaccine using nonhuman primates, periodontal vaccine using murine models, active immunization for periodontal

disease, passive immunization for periodontal disease, DNA vaccine for periodontal disease, periodontal vaccine against *Porphyromonas gingivalis*, periodontal vaccine against *Actinobacillus actinomycetemcomitans*, periodontal vaccine against *Fusobacterium nucleatum*, periodontal vaccine against *Prevotella intermedia*, periodontal vaccine against *Tannerella forsythia*, periodontal vaccine against *Treponema denticola*, and periodontal vaccine against Herpes virus.

In addition, a hand search (up to 2011) of the Journal of Periodontology, Journal of Clinical Periodontology, Journal of Periodontal Research, Oral Microbiology and Immunology, Critical Reviews in Oral Biology and Medicine, Advances in Dental Research, and Periodontology 2000 was performed.

DATA ANALYSIS

In vitro Studies

In the present review, *in vitro* and *in vivo* studies were analyzed based on whether the study concerned belonged to the active or passive type of immunization. Among the *in vitro* studies conducted from 1970 (Ivanyi et al) till 2011 (Gibson and Genco), analysis was done regarding different types of agents and adjuvants, different routes of administration of vaccines, different animals used and different parameters employed in each study to check the efficiency of vaccines.

Inducing immunity by injecting whole cell inactivated antigens has been the cornerstone of vaccination against any disease. Bone loss was found to be less in the group that received vaccine. Clark et al (1991) assessed the potential for vaccination with *P. intermedia* and Ebersole et al (1991) with *P. gingivalis* and *P. intermedia*. Both studies showed a substantial systemic immune response and a reduction of microorganisms. Various antigenic components in a microorganism possessed different degree of immune stimulation. Fimbrial protein of *P. gingivalis* was found to be more effective than the cell surface receptor of the same (Evans et al 1992). Similar studies by Persson et al (1994), Chen et al (1995) and Katz et al (1999) on immunization induced by *P. gingivalis* also proved the same results. Attempt of vaccine preparation employing molecular proteins of pathogenic periodontal organisms like fimbrial protein (Sharma et al 2001, Takahashi et al 2007), bacteroides secretory protein (Sharma et al 2002), outer membrane protein (Yoshiaki et al 2003), capsular protein (Gonzalez et al 2003), gingipain protease secreted by *P. gingivalis* which tend to reduce inflammation (Rajapakse et al 2002, Miyachi et al 2007), heat shock proteins (Lee et al 2006) and cysteine protease (Page et al 2007) have been

some important contributions to the present status of periodontal vaccination.

Chronic disease is not generally an indication for passive immunization by the repeated administration of an immunoglobulin. However, passive immunization against periodontal microorganisms has been attempted. Study by Yamashita et al (1991) on Rowett rats with vaccine consisting of cloned *A. actinomycetemcomitans*-specific T helper cells was the first ever major step in formulating a periodontal vaccine. Passive immunization against periodontal pathogens temporarily prevents colonization of *P. gingivalis*. Ellen VG Frensdén et al (1996), attempted characterization of IgA1, in different subspecies of Capnocytophaga. Bezerra et al study in 2000 comparing the effects of drugs like indomethacin and meloxicam on periodontal diseases have added an additional dimension of using drugs as vaccines.

In vivo Studies

In vivo are longitudinal studies before and no human trials are made till present. Aukhil et al (1988), Kohyama et al (1989) and Sjostrom et al (1994) in their longitudinal studies measured the reduction in serum IgG titers following initial cause related periodontal therapy (ICRT) to different periodontally pathogenic organisms like *P. gingivalis*, *T. denticola*, *A. actinomycetemcomitans* and *F. nucleatum*. The effectiveness of monoclonal antibodies against the pathogens has been further reinforced by the longitudinal study of Booth et al (1996). Among the other *in vivo* studies Takichi et al in 2000, investigated expression of mRNA, IL-2, IL-5, IFN- γ , to ascertain the nature of infiltrate associated with destruction and pocket formation. The study also revealed that under appropriate stimulation there was an upregulation of Th type 2 cells, emphasizing the potential of CD8 T lymphocytes to participate in periodontal disease pathology. In order to stress on the great influence of environment and socioeconomic variables, Craig et al (2002), measured the serum IgG antibody response to six periodontal pathogens among three entirely different urban population consisting of Asiatic, African-American and Hispanic subjects and found a positive correlation.

An important link in innate immunity in *Drosophila* is a receptor protein named toll, which binds fungal antigens and triggers activation of genes coding for antifungal proteins. An expanding list of toll-like receptors (TLRs) have now been identified in humans (Teng et al 2006), (Kinane et al 2007). The *in vivo* studies also concentrated on the role of RANK-L/osteoprotegerin system and its effect on bone resorption in conjunction to the lipopolysaccharide (LPS) stimulation. Thus, it is evident that keeping the

IN VITRO STUDIES

Study	Animals used	Vaccine administered	Vaccine dose	Outcomes measured	Results of outcomes measured
Clark et al ³ (1991)	12 squirrel monkeys 6 monkeys → immunized 6 monkeys → sham immunized	<i>P. intermedia</i> whole cells	6 monkeys 1 ml of <i>P. intermedia</i> (14,447) (10 ⁹), subcutaneously in chest, abdomen and 3 booster dose Sham immunization → 6 monkeys Freund's adjuvant without bacteria	IgG anti- <i>P. intermedia</i> antibody. 2 weeks: 1. <i>P. intermedia</i> detected in 5 of 6 sham immunized 2. <i>P. intermedia</i> detected in 3 of 6 immunized.	Immunization attached with a reduction in emergence of indigenous <i>P. intermedia</i> in gingival crevice.
Yamashita et al ⁴ (1991)	39 male Rowett rats	<i>A. actinomycetem-comitans</i> specific clone A3.	10 ⁶ cells A3 clone cells injected intravenous to first group (AaTh; n = 13); second group received oral <i>A. actinomycetem-comitans</i> cells and no T cells (AaNT; n = 15) and third group was neither infected nor received T cells (NAaNT; n = 11)	Serum IgG and IgM Ab II-2 level in gingival wash fluid bone loss	First group showed significantly elevated serum IgG and IgM Ab to <i>A. actinomycetemcomitans</i> when compared to both other groups. Significant difference in IL-2 levels between the two control groups (AaNT and NAaNT) and the group that received the cloned cells and was infected (AaTh). Bone loss was significantly lower in recipient of <i>A. actinomycetemcomitans</i> —specific cloned cells compared to an infected group and was approximately equal to the bone loss of an uninfected group.
Ebersole et al ⁵ (1991)	<i>M. fascicularis</i> female 10 experimental + 10 control	Experimental groups: Whole cell antigens of <i>P. gingivalis</i> and <i>P. intermedia</i> Control groups: Whole cell antigens of <i>B. fragilis</i> and placebo group (PBS in IFA). Both experimental and control animals were immunized three times bi-weekly up to week 14.	10 ⁹ total cells intramuscularly	2 weeks after the last immunization (at week 16) ligatures were placed, and microbiologic, immunologic, clinical and radiographic samples were taken during the subsequent 35 weeks (up to week 51).	The immunization elicited ~ 2-log increase in serum IgG, IgM and IgA isotype Ab that was highly specific for these immunogens. Postimmunization and postligation, there was a minimal change in the levels of specific Ab. <i>P. gingivalis</i> immunization significantly inhibited the emergence of this species during disease progression. In contrast, the induction of anti- <i>P. intermedia</i> Ab had a minimal effect on this species within the subgingival plaque. Plaque indices showed few changes that could be attributed to active immunization. Both bleeding on probing and loss of attachment were higher in ligated sites of immunized animals than in the placebo-treated group. A significant increase in bone-density loss was observed in the ligated teeth from immunized versus control animals.
Evane et al ⁶ (1992)	Male germ-free Sprague-Dawley rats	Heat killed whole cells; 43 kDa fimbrial protein from <i>P. gingivalis</i> ; 75 kDa cell surface component from <i>P. gingivalis</i> and cell surface protein fraction that included the 43 kDa protein, 75 kDa protein and other proteins extracted during the purification process.	10 ¹⁰ heat killed whole cells of <i>P. gingivalis</i> or 20 µg of soluble cell surface protein antigens in IFA (subcutaneous)	Ab response, gingival enzyme activity (collagenase, gelatinous, cathepsin B and L) and bone loss	Immunization with highly purified 43 kDa fimbrial protein protection against periodontal tissues destruction when tested in the <i>P. gingivalis</i> infected gnotobiotic rat model. A similarly highly purified 75 kDa cell surface component did not provide protection. Heat-killed whole-cell and sonicated cell-surface extracts that contained a 43 kDa protein as well as a 75 kDa component were also protective.

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Perisson et al ⁷ (1994)	28 <i>M. fascicularis</i> monkeys	Formalin killed <i>P. gingivalis</i> 5,083 in SAF adjuvant	0.5 ml of vaccine in upper arm and 0.5 ml subcutaneous in the back	Ab titers Bone loss Gingival inflammation, probing depths and attachment loss. Plaque sample analysis	Although vaccination elicited a relatively high mean Ab titer, high titers were not enduring. Although superinfection with <i>P. gingivalis</i> resulted in rapid bone loss in control animals, it had no measurable effect on CADIA scores for the 3 immunized animals. Effect of immunization on gingival inflammation, probing depths and attachment ion were not a clear cut as the effects on bone. Immunization did not clear <i>P. gingivalis</i> infection from the subgingival areas, even at nonligated teeth.
Katz et al ⁸ (1999)	Conventional Fischer 344 rats (8-10 weeks old, 6 rats/group)	Recombinant HagB + Freund's adjuvants (hemagglutinin B)	100 µg followed by exposure to <i>P. gingivalis</i> at days 13 and 14.	Serum IgA level, interferon, IL-2, 1, 4	Serum IgA levels increased in both immunized and infected only groups. High levels of interferon, IL-2, IL-1 and IL-4 in immunized and infected group suggesting a thin type of immune response. Immunization with rHagB inducer protective immunity against <i>P. gingivalis</i> infection.
Martuscelli et al ⁹ (2000)	20, 18-month-old female beagle dogs. 3 treatment group + 1 control group	Recombinant human IL-11	15, 30 or 80 µg/kg of rh-11 subcutaneously	Gingival inflammation, plaque, bleeding on probing, attachment loss, bone height	All three treatment groups last significantly less attachment then placebo Rh-11 subcutaneously injected slow progressive of attachment loss and radiographic bone loss in ligature-induced beagle dog model.
Mirna M Bizarre et al ¹⁰ (2000)	Wistar rats	IND (0.5, 1 or 2 mg/kg) MLX (10.75, 1.5 or 3 mg/kg)	IND (0.5, 1 or 2 mg/kg) MLX (10.75, 1.5 or 3 mg/kg)	Alveolar bone loss Histopathological analysis	Nontreated group: Severe alveolar bone loss neutrophilia and lymphomonocytosis at 6 hours and at 7 days Treated groups: Reduced alveolar bone loss. Neutrophilia and lymphomonocytosis MLX may provide a better risk/benefit ratio.
Sharma et al ¹¹ (2001)	Male Sprague-Dawley germ free rats	Oral immunization with two <i>Streptococcus gordonii</i> recombinants expressing N (residues 55-145) and C (residues 226-337) terminal episodes of <i>P. gingivalis</i> FimA	200 ml bacterial suspension (10 ⁹ colony-forming units)	Fim-A specific serum IgG and IgA and salivary IgA Ab responses. Alveolar bone loss	Induction of Fim-A specific serum IgG and IgA and salivary IgA Ab responses were protective against subsequent <i>P. gingivalis</i> -induced alveolar bone loss
Rajapakse et al ¹² (2002)	Male Sprague-Dawley rats	Arg-x and Lys-x specific cysteine proteinases and adhesion designated as RgpA-kgp complexes	Rats (randomly divided) were immunized subcutaneously with either formalin-killed whole <i>P. gingivalis</i> ATCC 33277 cells with IFA, RGP A-Kgp with IFA (100 mg/doss) or IFA bone loss	Bone loss Plaque sampling serum IgG2a response	Marked periodontal bone loss in animals immunized with IFA alone. This bone loss is significantly greater than that detected in animals immunized with formalin-killed whole cells or RgpA-kgp or in unchallenged animals. There is no significant difference in periodontal bone loss between animals immunized with formalin killed whole cells and those immunized with RgpA-kgp. The bone loss in these animals is also not significantly different from that in unchallenged

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					animals. 100% of animals immunized with IFA alone and challenged with <i>P. gingivalis</i> ATCC 33277 are positive for the bacterium. However <i>P. gingivalis</i> ATCC 33277 can not be detected in subgingival plaque samples from animals immunized with formalin-killed whole cells or with RgpA-kgp. Immunization with formalin-killed whole cells or rGpA-kgp induced a high titer serum IgG2a response.
Gonzalez et al ¹³ (2003)	6-week old, female BALB/C mice	CPs of <i>P. gingivalis</i>	0.1 ml of <i>P. gingivalis</i> CPs (1 mg/ml) in sterile pyrogen-free saline (subcutaneously)	Serum IgM and IgG Ab response bone loss	Animals immunized with <i>P. gingivalis</i> CPS developed elevated levels of IgM and IgG in serum that reacted with whole <i>P. gingivalis</i> organisms. Mice immunized with whole <i>P. gingivalis</i> CPS were protected from <i>P. gingivalis</i> elicited oral bone loss.
De carlo et al ¹⁴ (2003)	Conventional Fischer CD F (344) rats	rHA2 in PBS	50 µg of rHA2 in 0.5 ml of PBS (subcutaneously)	Specific IgG Ab response Bone loss	In animals inoculated with rHA2 in PBs, lower anti-rHA2 IgG1/IgG2b Ab ratios from both of the time points measured and lower IgG2a/IgG2b Ab ratios were significantly associated with less periodontal bone loss.
Roberts et al ¹⁵ (2004)	10 Macaca fascicularis monkeys	Formalin-killed <i>P. gingivalis</i> strain 5,083 in SAF adjuvant	Not mentioned	IL-1b, TNF-α, PGE2 and <i>P. gingivalis</i> specific IgG levels Bone loss	Only PGE2 levels were suppressed in immunized animals versus controls. There was a significant correlation between PGE2 levels and decreased bone-loss scores.
O' Brien-Simpson et al ¹⁶ (2005)	BALB/C mice	RgpA-kgp complex and synthetic ABM and proteinase active-side peptides conjugated to diphtheria toxoid administered subcutaneously	100 µl	IgG, Ab, IL-4 and IFN-γ response and periodontal bone loss	Most efficacious peptide and protein vaccines were found to induce a high titer IgG, Ab response. Mice protected in the lesion and periodontitis models had a predominant, <i>P. gingivalis</i> -specific IL-4 response, whereas mice with disease had a predominant IGn-γ response. Peptide specific Abs directed to the ABM2 (EGLATATTFEEDGVA) protected against periodontal bone loss and inhibited binding of the RgPA Kgp complex to fibrinogen, fibronectin and collagen type V. Peptide specific Abs directed to the ABM3 sequence protected against periodontal bone loss and inhibited binding to hemoglobin. Most protective Abs were those directed to the active sites of the RgpA and Kgp proteinases.
Lee et al ¹⁷ (2006)	12 Sprague-Dawley rats	Immunized with <i>P. gingivalis</i> rHSP60 IFA	5 µg	IgG levels Alveolar bone loss. Identification of periodontopathogenic bacteria in fecal samples	Very strong inverse relationship between post-immune anti- <i>P. gingivalis</i> HSP IgG levels and amount of alveolar bone loss induced by either <i>P. gingivalis</i> or multiple bacterial infection. Polymerase chain reaction data indicated that the vaccine successfully eradicated the multiple pathogenic species.

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Takahashi et al ¹⁸ (2007)	BALB/c mice	Fimbriae of <i>P. gingivalis</i> and rCTB, administered nasally	30 µl	IgG and IgA Ab, in sera and alveolar bone ion	rCTB significantly increased serum IgA levels when mice were administered a minimal amount (0.5 µg) of the fimbrial antigen. In contrast to systemic responses, a fimbria-specific secretory IgA response was strongly induced by coadministration of r-CTB and 0.5 µg fimbriae. Nasal administration of fimbrial vaccine significantly protected the mice from <i>P. gingivalis</i> -mediated alveolar bone loss.
Miyachi et al ¹⁹ (2007)	BALB/C mice	RgpA DNA vaccine with a gene gun and intranasally	Gene gun 2.5 µg DNA via abdominal skin. Intranasally: RgpA HVJE vector (20 µg/10 µl/mouse)	Ab response Bone loss	Immunization elicited IgG responses against <i>P. gingivalis</i> in both groups. Nasal immunization also induced specific sIgA response against <i>P. gingivalis</i> , gene gun immunization did not. Reduction of alveolar bone loss was more pronounced in the intranasal immunization group than in the gene gun group.
Page et al ²⁰ (2007)	<i>M. fascicularis</i> male and female monkeys. 5 control and 5 experimental	Cysteine protease of <i>P. gingivalis</i> (150 kDa porphyrin-1 and 120 kDa posphyain-2). Control group: Buffer with no antigen added to vaccine preparation. Control and immunized animals were vaccinated at baseline and at 3, 6 and 16 weeks.	0.5 ml subcutaneously	Samples of blood, subgingival plaque and saliva were harvested at all time points. Ligatures were placed around mandibular and maxillary posterior teeth at week 16. Radiographs of mandibular teeth were taken at baseline and at 16, 30, 36 and 44 weeks and the ligated teeth were supra-infected with viable <i>P. gingivalis</i> at 36 and 40 weeks.	Immunization induced high titers of specific IgG serum Ab that WEE opsonic. Total bacterial load, levels of <i>P. gingivalis</i> in subgingival plaque and levels of PGE2 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.
Momoi et al ²¹ (2008)	Female BALB/c Cr S/c (BALB/c) mice	40 kDa OMP of <i>P. gingivalis</i> normally administered with a nontoxic chimeric adjuvant that combines mCTA/LTB.	10 µl/nostril	IgG and IgA Ab response in sera and IgA in saliva, total IgE, 40 kDa OMP-specific IgE Abs and IL-4 levels. Alveolar bone loss.	Immunization induced high levels of 40 kDa OMP-specific IgG and IgA Abs in sera and elicited a significant IgA anti 40 kDa OMP Ab response in saliva. Levels of total IgE and 40 kDa OMP specific IgE Abs as well as IL-4 levels induced by the immunization with mCTA/LTB were lower than those induced by immunization with nCT. Mice given nasal 40 kDa OMP plus mCTA/LTB showed a significant reduction of alveolar bone loss caused by oral infection with <i>P. gingivalis</i> even 1 year after the immunization compared to the loss in unimmunized mice.
Zhang et al ²² (2009)	Female BALB/c mice	40 kDa OMP of <i>P. gingivalis</i> (40 koMP) sublingually with a DNA vector PFL.	8-10 µl	Serum IgG and IgA and salivary IgA Ab responses and alveolar bone loss.	Significant serum IgG and IgA and salivary IgA Ab responses that were comparable to those induced by 40 k OMP plus cholera toxin are adjuvant. Sublingual immunization with 40 k OMP plus PFL induced both IgG and IgG2a Ab responses. Sublingual 40 k OMP plus PFL administration showed a significant reduction of alveolar bone loss caused by oral infection with <i>P. gingivalis</i> .

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Liu et al ²³ (2009)	Mice	UV-inactivated <i>F. nucleatum</i>	25 µl inoculated intraorally for 9 weeks of a 3 weeks interval	Ability of VSC production and biofilm formation	Ability of VSC (volatile sulfur compounds) and biofilm production suppressed in <i>F. nucleatum</i> immunized mice whereas it was present in <i>S. mutans</i> immunized mice when exposed to <i>F. nucleatum</i> again.
Gibson and Genco ²⁴ (2011)	BALB/c mice	Immunized subcutaneously with Freund complete adjuvant or heat-killed <i>P. gingivalis</i> or adjuvant RgpA or RgpB	100 µg per injection	IgG Abs and maxillary bones loss	Mice immunized with heat-killed <i>P. gingivalis</i> or purified RgpA or RgpB possessed elevated levels of <i>P. gingivalis</i> -specific IgG. Only animals immunized with <i>P. gingivalis</i> whole cells or RgpA were protected from maxillary bone loss.

IN VIVO STUDIES

Authors	Study	Result	Outcomes
Ivanyi et al ²⁵ (1970)	Cellular immunity in periodontal diseases by the lymphocyte transformation test. C thymidine uptake.	In patients with severe periodontitis, lymphocyte transformation was significantly depressed when compared with mild or moderate periodontitis.	Cell-mediated immune response to some oral microorganisms may play a protective or aggressive part in the pathogenesis of periodontal disease.
Ellen V Frandsen et al ²⁶ (1987)	Characterized the IgA ₁ protease activity of <i>Bacteroides</i> and <i>Capnocytophaga</i> species	Immunoelectrophoretic and sodium dodecyl sulfate—polyacrylamide gel electrophoretic analyses suggested that all species cleave the α-chain at the same peptide bond, i.e. the prolyl-seryl bond between residues 223 and 224 in the hinge region.	IgA ₁ proteases from <i>Capnocytophaga ochracea</i> and <i>Capnocytophaga sputigena</i> strains were apparently identical, while <i>Capnocytophaga gingivalis</i> had a protease that differed from those of the other <i>Capnocytophaga</i> species.
Aukhil et al ²⁷ (1988)	Longitudinal case series of 23 subjects with CP over 12 months.	Serum IgG ELISA assay was used to estimate IgG levels.	Results showed a reduction in serum IgG titers to <i>P. gingivalis</i> , <i>A. viscorus</i> , <i>F. nucleatum</i> , <i>P. intermedia</i> , <i>T. vincentii</i> , <i>T. denticola</i> , but was unclear for <i>Capnocytophaga</i> spp.
Kohyama et al ²⁸ (1989)	A study consisting of longitudinal case series of subjects with chronic periodontitis (CP) (n = 52) before and after initial cause related periodontal therapy (ICRT).	Serum IgG ELISA assay was used for the investigation. Results showed that subjects with CP had higher serum IgG titers to <i>P. gingivalis</i> , <i>P. intermedia</i> , <i>A. actinomycetemcomitans</i> than healthy controls. It was also founded that titers increased after therapy whereas the presence of pathogens decreased.	It was concluded that subjects with CP experienced elevated serum IgG titers to pathogens associated with periodontitis. Potential passive immune response was present.
Sjostrom et al ²⁹ (1994)	Case intervention longitudinal study of 12 months consisting of 22 subjects with AgP and 20 healthy controls.	Serum IgG ELISA assay to <i>A. actinomycetemcomitans</i> and chemiluminescence (CL) assay for (PMN cell killing capacity) were conducted. Results consisting of zero conversion 1 year after ICRT showed elevated IgG serum antibodies in sero-negative subjects to the whole cell and LPS from <i>A. actinomycetemcomitans</i> antigens and an increased CL capacity.	SRP results in a humoral immune response in sero-negative subjects consistent with beneficial treatment effects.

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Horibe et al ³⁰ (1995)	Longitudinal case series with 20 AgP subjects used <i>P. gingivalis</i> (ATCC FDC 381) <i>P. intermedia</i> (ATCC 25611), <i>P. loeschii</i> (ATCC 15930), <i>F. nucleatum</i> (ATCC 25586), <i>A. actinomycetemcomitans</i> (ATCC 1/4), <i>E. corrodens</i> (FDC 1073), <i>C. ochracea</i> 9M1 antigens.	Serum IgG ELISA assay was used to measure the titers. Results showed a decrease in serum IgG titers to <i>P. gingivalis</i> , <i>P. intermedia</i> and decrease in serum IgG titers consistent with suppression of pathogens in subgingival plaque.	This study failed to demonstrate passive immunization effect. Study suggested that serum titers linked to presence of bacteria in plaque.
Mooney et al ³¹ (1995)	Longitudinal case-control intervention study of 18 subjects with CP and 23 healthy controls <i>P. gingivalis</i> (NCTC 11834) and <i>A. actinomycetemcomitans</i> (ATCC 29523) were used as antigens.	Serum IgG assay (ELISA) and avidity assay using ammonium thiocyanate. Results showed enhanced avidity and serum IgG titer to <i>P. gingivalis</i> and <i>A. actinomycetemcomitans</i> in sero-positive CP care. Elevated serum IgG ELISA titer in sero-negative CP subjects was found.	There was no difference in treatment effect explained by IgG titer or avidity changes. Thus unique humoral immune responses were found on previous exposure to pathogens of significance.
Booth et al ³² (1996)	Longitudinal case control study of subjects with periodontitis used radioimmune assay for serum IgG, IgA, IgM as antigens.	Results showed that there was no impact on difference in decrease of periodontal measures other than for <i>P. gingivalis</i> .	Passive immunization can selectively prevent colonization of <i>P. gingivalis</i> for up to 9 months.
Craig et al ³³ (2002)	Serum IgG antibody response to six periodontal pathogens and compared there data with microbiological, clinical and demographic parameters in three urban minority population consisting of 23 Asiatic, 48 African-American and 37 Hispanic subjects.	Pocket depth, attachment levels, gingival erythema, bleeding on probing, suppuration and supragingival plaque were clinical parameters, whereas checkerboard hybridization and ELISA were used for microbiological analysis. Results showed that mean serum IgG antibody to <i>P. gingivalis</i> was higher in African-American group. This group also showed increased mean probing depth, attachment loss, etc. Increasing pocket depth, attachment level, gingival erythema was positively correlated with serum IgG antibody to <i>P. gingivalis</i> species only.	Results suggest that elevated serum IgG antibody to <i>P. gingivalis</i> reflects destructive periodontal disease status and may be considered a risk factor for disease progression in these population. Environmental and socioeconomic variables may have a greater influence on serum IgG antibody levels in these population.

complex nature of periodontal diseases in mind, a multifactorial approach including host-derived factors (enzymes and other defense mechanisms), antibacterial, anti-inflammatory and specific virulence inhibitor components is required for the successful formulation of a periodontal vaccine (Johnson et al 1994).

Future of Periodontal Vaccines

DNA vaccines that were first described less than 5 years ago have already progressed to phase I clinical trials in healthy adult humans. They might induce immunity to numerous agents, including periodontopathic bacteria, following confirmation of their safety. Advantages of DNA vaccines are:

1. DNA vaccines can be manufactured more easily than vaccines consisting of an attenuated pathogen, an outer or internal protein or a recombinant protein.
2. DNA is stable by nature and resistant to extremes of temperature, storage, transport and distribution.
3. The immunogenicity of the modified protein may be directly assessed following an injection of DNA vaccine.
4. DNA plasmids encoding a gene required for antigen production are transferred by intramuscular needle injection without adjuvant. Alternatively, intradermal particle bombardment is also effective.

CONCLUSION

In this documentation various *in vitro* and *in vivo* studies are reviewed. The *in vitro* studies were conducted on experimental animal groups like *Macaca fascicularis*, BALB mice, Beagle dogs and Spring Dawley rats. These animals were inoculated with antigens consisting of either live or dead microorganisms. Various routes of vaccine administration namely intranasal, intravenous, intramuscular, subcutaneous, intradermal were tried and each route has proved its own significance, mucosal being the better among all. Among the outcomes measured are clinical features like alveolar bone loss, clinical attachment level, probing depth and also serological investigations like molecular levels of IL-1, TNF- α , PGE₂, IgG levels, IFN- γ . All the *in vitro* studies showed significant improvements in clinical and microbiological outcomes but the concrete evidence supporting the formulation of an analogs human periodontal vaccine is still missing.

The *in vivo* studies were conducted among different ethnic races that included Caucasoids, African-Americans and Asians. Most studies were conducted as longitudinal studies and serological investigations were given considerable importance. An emphasis on association of plaque control, socioeconomic status and role of genetics

with periodontal diseases is made. To end with, periodontal vaccine may be expensive in the near future but it may overcome the financial burden of periodontal treatment on patients.

Though many animal studies are conducted, there still is an insufficiency of data available on the transfer of the results obtained from animal trials on to humans. In conclusion long-term clinical trials are needed to formulate a beneficial prophylactic multispecies human periodontal vaccine.

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