Cumhuriyet Dental Journal

Volume 18 Issue 2

doi: 10.7126/cdj.58140.1008001432

available at http://dergipark.ulakbim.gov.tr/cumudj/

REVIEW

Vaccines for periodontal disease: A new hope?

Resmi Chanrahasan Nair, BDS, MDS[a], *Binu Neelikad Sugunan, BDS, MDS*[b]

[a]*Registrar, Department of Dentistry, Ministry of Defence, Kuwait*
[b]*Registrar, Department of Dentistry, Ministry of Health, Kuwait*

ARTICLE INFO

Article history:
Received: 13 March 2012
Accepted: 01 May 2012

Keywords:
Immunization,
periodontitis,
vaccines

ABSTRACT

Recent recognition of the importance of periodontal disease and its impact on the perpetuation and management of systemic diseases calls for a global effort to control periodontal disease. With the advent of advanced molecular diagnostic techniques, a better understanding of the role of specific pathogens and the contributory role of the host immune response in the initiation and progression of periodontal disease has been possible. However, successful vaccine development that fully utilizes the current level of understanding has not yet occurred for human use. This article reviews the various trials undertaken to develop a vaccine against periodontal disease, so as to construct a more sophisticated design which may be relevant in the future. Periodontal disease as a multifactorial and polymicrobial disease requires a vaccine design targeting multiple pathogenic species. As an innovative strategy, vaccine trials to stimulate antigen-specific T-cells polarized towards helper T-cells with a regulatory phenotype have been introduced. Conjugate and recombinant vaccines, immune regulation with coinfecting microorganisms within the biofilm and vaccine regimens like commonly shared antigens by selected periodontopathogenic species are new avenues that are being explored. The role of RecA protein and osteoprotegerin-ligand cannot be overlooked. Targeting not only a single pathogen, but polymicrobial organisms, and targeting not only periodontal disease, but also periodontal disease-triggered systemic disease could be a feasible goal.

Corresponding author at: Resmi Chanrahasan NAIR, Department of Dentistry, Jaber Al-Ahmed Armed Forces Hospital, Ministry of Defence, Kuwait. P.O Box NO.9657, Zip: 22097{salmiya}, Phone: 00965-94996105, Fax: 00965- 22552348. E-mail: docresbi@yahoo.co.in

INTRODUCTION

Vaccination is the induction of immunity by injecting a dead or attenuated form of a pathogen.[1] Ever since the introduction of the smallpox vaccine by Edward Jenner in 1798, antigens of pathogenic bacteria and viruses have been the targets for a variety of vaccines against a number of infectious diseases. Periodontal diseases are one such group of infectious bacterial diseases, against which vaccine research is still going on. The complexities in the etiopathogenesis of periodontal diseases have been the prime obstacle in the hunt for vaccine. Despite its poly-infectious nature, most immunization approaches, both active and passive, against periodontitis have been directed towards a very limited number of antigenic components of a single specific pathogen, either *Porphyromonas (P.) gingivalis* or *Aggregatibacter (A.) actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*).[2] The target antigens have evolved from the whole organism to specific virulence factors (structural components or secreted products) that could confer immunity against colonization or the virulent activity of putative periodontal pathogens.

The demanding primary role of any periodontal vaccine would be to eradicate the global periodontal disease burden with the ultimate purpose of lowering periodontal disease-associated morbidity in humans. The role of any vaccine, however, should also be seen within the context of changes in lifestyle. The vaccine should enhance the feasibility of maintaining oral health and maximize retention of the natural dentition, thus minimizing the need for prosthetic or implant restorations in the oral cavity. The so-called "healthy gum-healthy body" lifestyle could also lessen the economic burden incurred by restorative dental treatment. Moreover, recent novel findings linking periodontitis and systemic health concerns would suggest that prevention or treatment of periodontal diseases is fundamental to the effective management of atherosclerosis, uncontrolled diabetes and low-weight pre-term birth or preeclampsia.[3]

EMERGING CONCEPTS REGARDING PERIODONTAL VACCINE DEVELOPMENT

Three emerging concepts of periodontal disease may influence the development of a vaccine to eradicate or alleviate the disease burden. The first is that periodontal disease is a polymicrobial infection. The second is that it is a major cause of adult tooth loss worldwide. The third is that periodontal disease contributes to the perpetuation of systemic diseases of critical importance (atherosclerosis, diabetes mellitus, etc.).

DEVELOPMENT STRATEGY FOR A VACCINE AGAINST PERIODONTITIS AS A POLYMICROBIAL INFECTION

Traditional concepts of the etiology and initiation of periodontal disease stem from the observation that gingival inflammation ensues from the sequential and quantitative microbial load accumulating in the gingival sulcus as an organized biofilm known as bacterial plaque. The current concept emerges from extensive research findings on the polymicrobial nature of the associated biofilm. This has led to the notion that biofilm quality is the critical factor in the pathogenesis of periodontal disease. Indeed it is now thought that periodontal disease is a specifically combined infection of polymicrobial gram-negative anaerobic bacteria, including *Porphyromonas (P.) gingivalis*, *Treponema (T.) denticola*, *Tanerella (T.) forsythia* and *Aggregatibacter (A.) actinomycetemcomitans* all of which have been proposed as predominant pathogens, exclusively or synergistically

with other bacteria, including *Prevotella (P.) intermedia*, *Campylobacter (C.) rectus*, *Fusobacterium (F.) nucleatum* and herpes virus.[4] Although periodontal diseases are primarily initiated and perpetuated by the mixed biofilm (possibly also including viruses), other factors including host-associated factors, genetic predisposition, immune dysfunction and environmental factors can exacerbate the disease. Thus, a combined strategy, targeting both specific pathogenic species and the host immune response would have to be adopted for the effective management of the compromised subject.

DEVELOPMENT STRATEGY FOR A VACCINE AGAINST PERIODONTITIS AS A MAJOR CAUSE OF TOOTH LOSS

Epidemiological studies reveal that more than two-thirds of the world's population suffer from one of the chronic forms of periodontal disease. Thus periodontal disease is a major cause of adult tooth loss. Availability of a vaccine for preventing or modulating periodontal disease would be of great benefit in both developing and developed countries.

DEVELOPMENT STRATEGY FOR A VACCINE AGAINST PERIODONTITIS-TRIGGERED SYSTEMIC DISEASES

A number of different mechanisms have been postulated to explain the link between periodontal disease and atherosclerosis. In particular, increased systemic inflammation with elevated inflammatory biomarkers, in periodontal patients may contribute to the perpetuation of atherosclerotic cardiovascular disease.[5] Furthermore, it has been suggested that the microbial components responsible for periodontal infection may trigger the development of autoimmune disease. Most recently, heat shock protein (HSP) of *P. gingivalis* has been a molecule of considerable interest since it may be a candidate trigger molecule linking infectious disease (e.g. periodontitis) and systemic autoimmune diseases such as atherosclerosis, diabetes mellitus and rheumatoid arthritis.[6] Choi et al.[7] have mapped the immunodominant T- and B-cell epitopes of *P. gingivalis* HSP60 in periodontitis and atherosclerosis patients. Furthermore, they have cloned hybridomas producing anti-*P. gingivalis* HSP60 monoclonal antibodies with either mono-reactivity to homologous HSP60 or poly-reactivity to multiple bacterial HSP's and mammalian HSP60. The poly-reactive monoclonal antibody recognized peptide number 19 (TLVVNRLRGSLKICAVKAPG) of 37 synthetic peptides spanning the whole molecule of *P. gingivalis* HSP60. This novel finding could provide a clue to identify a possible candidate peptide epitope that could be further developed into a periodontal disease-systemic autoimmune disease vaccine. Interestingly, patients whose sera recognized both *P. gingivalis* HSP peptide number 19 and cross-reactive human HSP peptide number 19 have demonstrated a significantly higher level of alveolar bone, strongly suggesting an immune-modulating role for the cross-reactive peptide number 19 in periodontitis.[8] The fact that all atherosclerosis patients also exhibited antibodies to peptide number 19 suggests that it may be also involved in the pathogenesis of atherosclerosis.

EXPERIMENTAL MODELS FOR PERIODONTAL VACCINE DEVELOPMENT

Vaccine testing animal systems have ranged from mice and rats to dogs and nonhuman primates. Nonhuman primates and humans are similar in both periodontal structure and microflora composition. However, ligatures must be tied around the teeth to

elicit periodontitis in nonhuman primates because it is difficult to colonize the oral cavity with *P. gingivalis* and establish periodontal lesions. Animal models for vaccine trials may pose discrepancies with human models in major histocompatibility complex-restriction of antigens presented by antigen presenting cells, thus obscuring the immunodominant epitope(s). In order to evaluate vaccine efficacy in terms of the human immune system, a humanized mouse system has been introduced with the adoptive transfer of human peripheral blood lymphocytes (PBLs) into a severe combined immunodeficiency (SCID) mouse system as well as into a non-obese diabetes (NOD)-SCID mouse model. However there is frequent episodes of leakiness of these mouse systems. More recently, a genetically engineered mouse system with low leakiness such as the NOD.CB17-prkdc^{scid}/J mouse has been introduced for the study of infectious and autoimmune diseases in humans.[9] This model may also prove useful for the study of periodontal diseases and putative periodontal vaccines.

HISTORY OF PERIODONTAL VACCINE

In the early twentieth century, three types of periodontal vaccines were employed for the control of periodontal diseases.[10]

- Pure cultures of streptococcus and other organisms.
- Autogenous vaccines from dental plaque samples of patients with destructive periodontal diseases.
- Stock vaccines-Vancott's vaccine and Goldenberg's vaccine or Inava endocarp vaccine.

TYPES OF PERIODONTAL IMMUNIZATION

These include active, passive and genetic immunization.

ACTIVE IMMUNIZATION (FIGURE 1)

Active immunization has been carried out using whole bacterial cells, outer components or synthetic peptides as antigens.[11]

Whole cells

Here, the entire cell with its components is inoculated into a host to bring about active immunization. Evans et al.[12] reported that the levels of serum antibodies to whole cells from *P. gingivalis* were elevated in rats immunized with *P. gingivalis* cells and that the activities of collagenases and cysteine proteinases in gingival tissues as well as

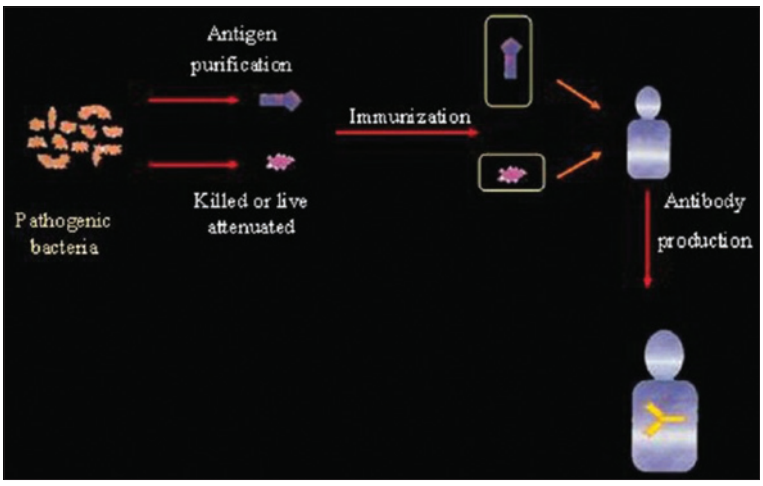

Figure 1. A schematic diagram of active immunization

periodontal tissue loss were decreased. Genco et al.[13] demonstrated that protection against invasion but not colonization by *P. gingivalis* was induced in the mouse chamber model by immunization with either killed heterologous invasive or heterologous non-invasive *P. gingivalis* strains. Mice vaccinated subcutaneously with an inactivated, whole cell vaccine preparation of *Porphyromonas denticanis*, *Porphyromonas gulae* and *Porphyromonas salivosa* displayed significantly reduced alveolar bone loss in response to heterologous and cross-species challenges as compared to sham vaccinated animals.[14]

Outer components

In this, a part of the bacterial cell is used for immunization. *P. gingivalis* has emerged as a major periodontopathogen in human periodontitis.[4] The virulence factors of *P. gingivalis* which have been used as subunits for the development of active immunization include gingipain, fimbriae, capsular polysaccharide and heat shock protein.[15]

Gingipain

These are cysteine proteases expressed on the outer membrane of *P. gingivalis*. Gingipains are classified into two groups based on substrate specificity. Gingipain R (Rgp) cleaves proteins at arginine residues and are encoded by two similar genes, RgpA and RgpB, while gingipain K (porphypain 2, Kgp) cleaves proteins at lysine residues. Both RgpA and Kgp (but not RgpB) have a hemagglutinin domain that is essential for the adherence to erythrocytes, while the catalytic domain (in RgpA, RgpB, and Kgp) plays an important role in the evasion of the host defense system by degrading immunoglobulins and complement proteins and by disturbing the functions of neutrophils. As a result, gingipains have drawn considerable interest as candidate antigens for periodontal vaccine development.[16]

Spurred by these findings, an active immunization program using purified *P. gingivalis* cysteine protease (porphypain-2) has been carried out, which resulted in a significantly elevated immunoglobulin G (IgG) antibody response that suppressed *P. gingivalis*-induced bone loss in *Macaca (M.) fascicularis*.[17] Gibson et al.[18] showed that immunization with RgpA stimulates the production of hemagglutinin domain specific antibodies which contribute to the prevention of *P. gingivalis* mediated oral bone loss. Clinical trials have reported that periodontal patients demonstrated high IgG titers to the hemagglutinin domain but not to the catalytic domain, because the catalytic domain is not exposed on the gingipain complex.[19] Furthermore, it is assumed that insufficient levels of human antibody to the catalytic subunits of RgpA and RgpB may be responsible for development of periodontitis, thus strengthening the need for inclusion of the catalytic subunit in the vaccine design. Studies performed in murine models incorporating peptides of the catalytic domains have demonstrated the protective function of the anti-catalytic domain antibodies against *P. gingivalis* infection.[20]

Fimbriae

These are cell surface structure components and serve as critical antigens. These are the most advanced immunogens. Functions of fimbriae are the following:[21]

- Adherence to host: Adherence is the first step regarding the virulence of microorganisms. Fimbriae bind to saliva-coated hydroxyapatite and mediate binding of *P. gingivalis* to the substrate.
- Invasion of oral epithelial cells and fibroblasts.

- Modulation of inflammation by release of interleukin-1α, interleukin-1β, tumor necrosis factor (TNF)-α.

Evans et al.[12] reported that immunization with highly purified *P. gingivalis* fimbrial preparations protected against periodontal destruction induced by *P. gingivalis* in gnotobiotic rats. They suggested that fimbrial protein might serve as a model of effective vaccines against periodontitis. However, it has been demonstrated that immunization with 43-kDa fimbrillin polymer of *P. gingivalis* did not show satisfactory levels of protection against all strains of *P. gingivalis* tested.[22] The feasibility of fimbrial protein of *P. gingivalis* as a vaccine candidate antigen may therefore be dependent on its effectiveness in protecting against all the *P. gingivalis* strains.

Capsular Polysaccharide (CPS)

Capsular polysaccharide, by virtue of its encapsulation and antigenic shift, constitutes a robust strategy for *P. gingivalis* survival against opsonophagocytic activity. A conjugate vaccine incorporating both fimbriae and *P. gingivalis* capsular polysaccharide has been introduced in a study by Choi et al.[23] A significantly high human IgG antibody response and in vivo protection against bacterial challenge was observed in the group immunized with the conjugate vaccine. It was concluded that capsular polysaccharide-fimbrial protein conjugate from *P. gingivalis* could potentially be developed as a vaccine against periodontal infection by *P. gingivalis*. More recently, *P. gingivalis* CPS alone has been used as an immunogen, and it has been reported to result in an elevated production of serum immunoglobulin G (IgG) and immunoglobulin M (IgM) that provided protection against *P. gingivalis*-induced bone loss.[24]

Heat shock protein 60 (HSP60 / GroEL)

The heat shock protein of *Porphyromonas gingivalis* is remarkably immunogenic, and both T-cell and antibody responses to HSP60 have been reported in various inflammatory conditions.[25] The GroEL homologs are also key molecules in auto-immune reactions because of the sequence similarity with human HSP60. Rats immunized with *P. gingivalis* HSP60 showed decrease in alveolar bone loss induced by infection with multiple periodontopathic bacteria. Polymerase chain reaction data indicated that the vaccine successfully eradicated the multiple pathogenic species. This study postulated that *P. gingivalis* HSP60 could potentially be developed as a vaccine to inhibit periodontal disease induced by multiple pathogenic bacteria.[26]

A. actinomycetemcomitans is considered another important pathogen in human periodontal disease, especially in the localized form of aggressive periodontitis.[4] An oligopeptide based on the amino acid sequence of *A. actinomycetemcomitans* fimbriae was synthesized and conjugated with branched lysine polymer resin beads by Honma et al.[27] Mice were immunized with the synthetic antigen together with one or more of Freund's incomplete adjuvant. A significantly high salivary immunoglobulin A (IgA) and serum IgG levels against the synthetic fimbrial antigen was observed by enzyme-linked immunosorbent assay. Also, subcutaneous and intranasal immunization of mice with capsular serotype b-specific polysaccharide antigen of *A. actinomycetemcomitans* resulted in a specific antibody that efficiently opsonized the organism.[28] Furthermore, when mice were immunized with anti surface-associated material from *A. actinomycetemcomitans*, it yielded a raised protective opsonic antibody response and rapid healing of the primary lesions following a challenge

with live *A. actinomycetemcomitans*.[29] However, relatively few studies have been conducted on developing vaccines against *A. actinomycetemcomitans*.

Synthetic Peptides

Mapping the adhesion, T-cell and B-cell epitopes is essential for investigating synthetic peptide vaccines.[30] T and B-cell epitopes are recognized by T cells and B cells respectively. Adhesion epitopes mediate adherence between bacteria and host tissue through a ligand-receptor interaction. It is important to design a synthetic peptide vaccine in which antigenicity does not imply immunogenicity. Since IgG and secretary immunoglobulin A (IgA) may play a role in preventing bacterial adhesion to salivary glycoproteins or mucosal receptors, adhesion epitopes are also indispensable to the immune response elicited by synthetic peptide vaccines. Small antigenic peptides are normally poorly immunogenic, and it is therefore necessary for small peptides to be added as carrier molecule for inducing an immune response. Lee et al.[31] found that synthetic peptides based on the protein structure of fimbrillin inhibit the adhesion of *Porphyromonas gingivalis* to saliva-coated hydroxyapetite crystals in vitro. Brant et al.[32] investigated the linear immunogenic and antigenic structure of *P. gingivalis* fimbrillin and identified the antigenic determinant on native fimbrillin protein as amino acid residues 99-110. The results indicate that amino acid residues 99-110 on the native fimbrillin protein are accessible to antibody binding. Ishikawa et al.[11] suggested that peptide immunogens would be effective as vaccines since they could adopt a more native conformation to produce effective antibodies.

PASSIVE IMMUNIZATION (FIGURE 2)

In essence, this approach employs preformed antibodies administered to "at risk" individuals or to individuals during "at risk" intervals to interfere with microbial pathogenic processes. Passive immunization studies have been carried out using monoclonal antibodies and plantibodies.

Monoclonal antibody (mAb)

In terms of an anti-infective scheme, monoclonal antibodies (mAbs) targeting the antigenic molecules of *P. gingivalis* could potentially be adopted as a sophisticated mode of immunotherapy. The antigenic factors of *P. gingivalis* which have been studied for the development of passive immunization are outer membrane protein and hemagglutinin.

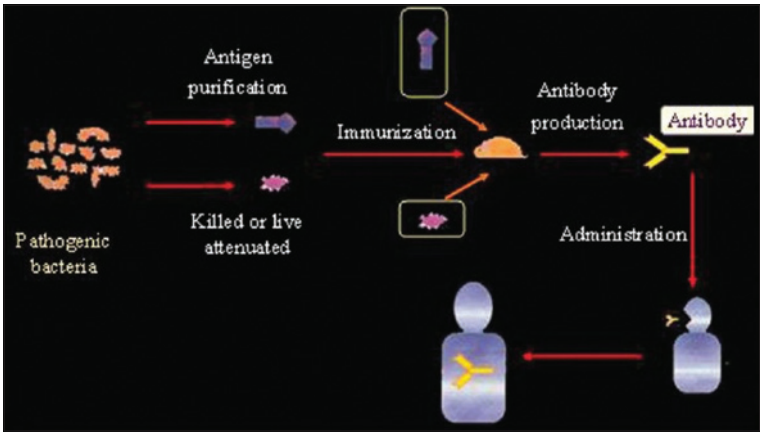

Figure 2. A schematic diagram of passive immunization

Outer membrane protein (OMP)

They are important coaggregation factors and as such are major colonization factors of *P. gingivalis*.[33] Since IgG specific for the 40 kDa-OMP inhibited coaggregation of *P. gingivalis* vesicles with *Streptococcus gordonii*, it could conceivably be used to prevent *P. gingivalis* infection.[34] In support of this, a panel of mouse mAbs against purified r40-kDa OMP specifically inhibited the coaggregation of *Actinomyces (A.) naeslundii* with several strains of *P. gingivalis*.[35] Furthermore, an IgG2 human mAb (HAB-OMP1) has been shown to significantly inhibit the coaggregation activity of *P. gingivalis* vesicles with *A. naeslundii*.[36] Takiguchi et al.[37] developed a panel of monoclonal antibodies by immunizing mice with purified 40 kDa of outer membrane protein. The objective of their study was to determine the bactericidal activity on *P. gingivalis* by the immunoglobulin G1 monoclonal antibody *P. gingivalis*-outer membrane protein A2 (Pg-OMP A2). The results showed that in the presence of complement, Pg-OMP A2 was lethal to *P. gingivalis* 381 as well as to the more virulent *P. gingivalis* strains. It was concluded that Pg-OMP A2 may contribute to the development of a local immunotherapy that can be applied in the gingival crevice of a patient with *P. gingivalis* related periodontitis, or be a vaccine candidate.

Hemagglutinin

Erythrocyte-derived protoheme is known to be one of the absolute requirements for the persistent growth of *P. gingivalis*.[38] It is the hemagglutinins of *P. gingivalis* that facilitate its attachment to the erythrocyte cell surface, allowing it to access protoheme. Hence, application of a monoclonal antibody against the hemagglutinin could be seen as a potential passive immunization strategy against the persistence of *P. gingivalis* in the subgingival niche. Based on this concept, a mAb using *P. gingivalis* vesicles as the immunogen (mAb-Pgvc) was raised and it was shown to inhibit the vesicle-associated hemagglutinating activity when incubated with rabbit erythrocytes.[39] As an advanced step in this approach, human lymphocytes, isolated from a donor with a high antibody titer against a recombinant 130 kDa hemagglutinin domain (r130k HMGD), were immortalized with Epstein-Barr virus, and specific antibody-producing B cells were established resulting in a human mAb, HMGD1. HMGD1 significantly inhibited the hemagglutinating activity of *P. gingivalis* vesicles in a dose-dependent manner and may prove to be a useful tool for passive immunization against periodontal disease.[40] Interestingly, in a novel introduction of XenoMouse technology, Shibata et al.[41] constructed an IgG2 Xeno-monoclonal antibody against the recombinant 130-kDa hemagglutinin domain of *P. gingivalis* and demonstrated a significant inhibition of hemagglutination by *P. gingivalis* and its vesicles.

These results support the hypothesis that a mAb specific to a bacterial antigen could prove to be an effective mode of passive immunization against *P. gingivalis* and possibly other periodontopathic bacteria.

PLANTIBODIES

A vaccination concept was developed using molecular biologic techniques to enable plants to synthesize and assemble antibody molecules, including antigen-binding domains, complete antibodies and multimeric antibodies. Ma et al.[42] characterized a secretory IgG antibody produced in transgenic plants. This antibody was more stable and exhibited a higher functional affinity than the native antibody, and provided protection against *Streptococcus mutans* in humans.

GENETIC IMMUNIZATION (FIGURE 3)

By the early 1990s, scientists had begun to study new approaches to the production of vaccines that differ in structure from traditional ones. The strategy involves genetic engineering or recombinant DNA technology.[43] DNA vaccines can be administered intranasally, intramuscularly or delivered by gene gun, an instrument that propels tiny DNA-coated gold beads into the body's cells. These recombinant vaccines activate the immune systems eliciting both antibody-type and killer cell-type immunity.

Kawabata et al.[44] demonstrated that salivary gland of mouse, when immunized using plasmid DNA encoding the *P. gingivalis* fimbrial gene, produced fimbrial protein locally in the salivary gland tissue, which resulted in the subsequent production of specific salivary IgA and IgG antibodies and serum IgG antibodies. This secreted IgA neutralized *P. gingivalis* and limited its ability to participate in plaque formation. Sharma et al.[45] studied the efficacy of immunization with genetically engineered *Streptococcus gordonii* vectors expressing *P. gingivalis* fimbrial antigen as vaccine against *P. gingivalis* associated periodontitis in rats. The results supported the potential usefulness of *Streptococcus gordonii* vectors expressing *P. gingivalis* fimbrillin as a mucosal vaccine against adult periodontitis. Most recently, a multi-centered genomic analysis of *P. gingivalis* has reported that recombinant OMP antigens PG32 and PG33, both known to play an important role in bacterial growth, coaggregation with other bacteria and transcription, are potential vaccine candidates.[46]

Suyama et al.[47] constructed a subclone, designated pMD160, encoding a fusion protein of 80-kDa HagA. The novel clone produced relatively large amounts of recombinant protein. The recombinant protein was purified to homogeneity and rabbit antiserum was raised. The antibody was capable of inhibiting the hemagglutinating activity of *P. gingivalis*. These findings suggest that HagA recombinant proteins can be produced and these may be useful in developing immunotherapy against periodontitis infected by *P. gingivalis*.

VACCINE DESIGN VIA FINE TUNING OF ANTIGEN SPECIFIC T CELLS

Polarization of helper T-cells depend, in part, on the nature of the antigens, source of adjuvants, duration of antigenic challenge, presence of co-stimulatory molecules and the type of antigen-presenting cell.[48] The helper T-cells produce enormous amounts of two types of cytokines, Th1 and Th2. Periodontal disease severity is counterbalanced by the fine-tuning of the Th1/Th2 lymphocyte axis and the array of cytokine profiles

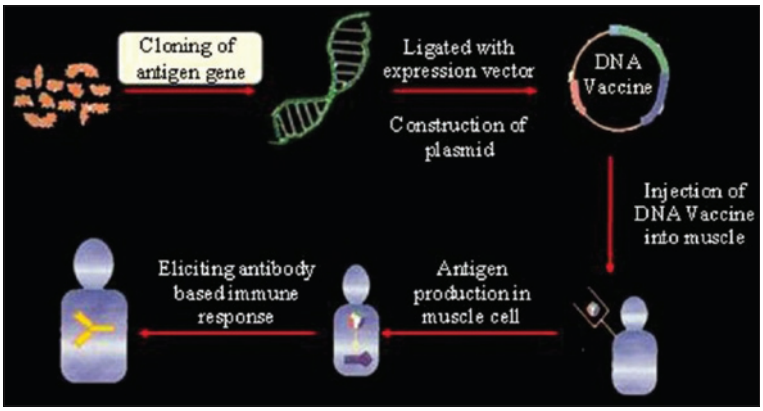

Figure 3. A schematic diagram of genetic immunization

contingent on T-cell polarization and immunoglobulin profiles secreted by B-lymphocytes. Interleukin-10 (IL-10) secreted by a number of different cell types is thought to exert a protective role against the progression of periodontal disease. This is supported by the observation that IL-10 knock-out mice demonstrate significantly lower bone levels and higher susceptibility to periodontal infection.[49] Care however must be taken in interpreting these results, as high levels of IL-10 may also stimulate IL-1 production by B cells and it has been suggested that the response curve to IL-10 follows a U shape such that both low levels as well as high levels of IL-10 are associated with disease progression.[50] Gemmell and Seymour[51] have shown in humans that Th1-dominated lesions are associated with stability, while Th2-dominated lesions are associated with progressive disease, such that downregulation of Th2 responses with a concomitant increase in Th1 responses selectively against the bacteria may have therapeutic effects. However, the polarization of *P. gingivalis*-specific T-cell lines or clones in periodontal lesions is still controversial.

Further experiments on immune modulation by pathogen-specific T-cell clones may lead to a greater understanding of the specific role of antigen-specific T-lymphocytes in the pathogenesis of periodontal disease at the species level. At this stage, however, it would appear that it is the balance between Th1 and Th2 cytokines that play an important role in maintaining alveolar bone homeostasis. [51] As an innovative strategy, vaccines designed to stimulate antigen-specific regulatory T-cells (Tregs, CD4+, CD25+, FoxP3+), secreting IL-10 and tumor necrosis factor beta (TGF-β), may provide new clues to periodontal disease prevention, through the induction of either immune tolerance or effector function. [52]

VACCINE DESIGN VIA IMMUNE MODULATION IN THE POLYMICROBIAL BIOFILM

The modulation of immune response by coinfecting microorganisms within the polymicrobial biofilm has been demonstrated in several studies. Two different independent research groups have evaluated immune modulation by immunizing *F. nucleatum* prior to subsequent immunization of *P. gingivalis*. When mice were immunized with *F. nucleatum* prior to *P. gingivalis*, a significantly decreased antibody response to *P. gingivalis* was observed.[53] At the same time, Choi et al[54] demonstrated that *P. gingivalis*-specific helper T cell clones derived from mice immunized with *P. gingivalis* alone had a Th1 profile while those derived from mice immunized with *F. nucleatum* prior to *P. gingivalis* had a Th2 profile. The latter research group also reported that anti-*F. nucleatum* antibody elicited by immunization of *F. nucleatum* prior to *P. gingivalis* down modulated the opsonophagocytic function of anti-*P. gingivalis* immune serum.

VACCINE DESIGN VIA COMMONLY SHARED ANTIGENS BY SELECTED PERIODONTOPATHOGENIC SPECIES

Most periodontal immunization studies have targeted a single pathogenic species. However, a number of the potential antigenic determinants may share a sequence homology with other periodontopathic bacteria. These antigens include phosphorylcholine,[55] capsular polysaccharide (CPS),[56] and heat-shock protein (HSP).[57] Phosphorylcholine, however, would not be a suitable candidate antigen as it has not been identified in *P. gingivalis*. In addition, CPS is not a potent inducer of T-cell-mediated immunity and would require protein conjugation in any vaccine design. Therefore HSP antigen, which has been identified in most putative

periodontal pathogenic bacteria with a high level of sequence homology, may be a suitable candidate molecule for the development of periodontal vaccines targeting the mixed microbial component.[26]

RecA PROTEIN

RecA protein is a multifunctional DNA-binding protein that plays an integral role in both homologous recombination and postreplicative DNA repair mechanisms. An ability to overcome oxidative stress is vital for colonization and survival of bacteria in an inflammatory environment such as the periodontal pocket. DNA damage is one of the lethal effects of oxidative stress. The recA protein plays a key role in DNA repair and homologous recombination.[58] Thus, it is possible that recA gene may play an important role in the virulence of periodontal pathogens. It has been shown that in the periodontal pathogen *Porphyromonas gingivalis*, the recA locus which carries the bacterioferritin co-migratory protein (bcp) gene plays a role in virulence regulation and oxidative stress resistance.[59] It is however unclear whether the recA gene in other periodontal pathogens may play a similar role. Researches are on the way to investigate the genetic architecture of the recA locus in other periodontal pathogens and to develop a vaccine against recA protein which might provide protection from developing periodontal disease.

OSTEOPROTEGERIN-LIGAND [OPG-L] / RECEPTOR ACTIVATOR OF NF-KB LIGAND [RANK-L]/ TRANCE

OPG-L (CD4+ T Cell mediated osteoclastogenic factor RANK-L) belongs to the tumour necrosis factor (TNF) family. RANK-L is expressed by bone marrow stromal cells, osteoblasts and chondrocytes on cell surfaces. RANK-L and its receptor RANK have been shown to be key regulators of bone remodeling and are directly involved in the differentiation, activation and survival of osteoclasts and osteoclasts precursors.[60] Inhibition of RANK-L thus has therapeutic value to prevent alveolar bone and / or tooth loss in human periodontitis.[61]

HURDLES IN PERIODONTAL VACCINE DEVELOPMENT

Though success has been achieved in the case of animal models, there are several reasons which still have to be overcome to make the dream of periodontal vaccine for humans a reality. Some of them are enlisted below:

- The multifactorial nature of periodontal disease. Hence, elimination of certain bacteria may not prevent the onset and progression of the disease.
- The difficulty to accurately differentiate between primary colonizers and secondary invaders.
- The relative difficulty to grow and identify many of the disease-associated microorganisms and the variability of the plaque composition from one individual to the other and between sites in the same individual.
- The fact that presumptive periodontal pathogenic microorganisms are members of the normal subgingival bacterial flora in humans and are not indigenous to the normal flora of the rodents.
- The variations in disease state and chronicity of the disease.
- The difficulty in clinically detecting and quantitating active periodontal disease.
- The location of gingival sulcus at the interface between the systemic immunity and the local immune responsive tissues, and the oral cavity bathed by the secretory immune system.
- The nonfatal nature of the disease.

WHAT DOES THE FUTURE HOLD?

The current treatment of periodontitis is nonspecific and is centered on the removal of subgingival plaque by mechanical debridement. This ongoing therapy is costly, painful and has variable prognosis, in part due to poor compliance of the patients. The significant reduction in periodontal disease progression in nonhuman primates and rodents by immunization with either killed whole *P. gingivalis* cells or *P. gingivalis* antigens suggests that vaccination may be an important adjunctive therapy to mechanical debridement in humans to prevent colonization of periodontal pathogens. As yet, there are no periodontal vaccine trials that have been successful in satisfying all requirements; to prevent the colonization of multiple pathogenic biofilms in the subgingival area, to elicit a high level of effector molecules such as immunoglobulins sufficient to opsonize and phagocytose the invading organisms, to suppress alveolar bone loss and to stimulate helper T-cell polarization that exerts cytokine functions optimal for protection against bacteria and tissue destruction.

Periodontal disease as a multifactorial and polymicrobial disease requires a sophisticated vaccine design targeting multiple pathogenic species. To accomplish this end, conjugate vaccines (i.e. protein-CPS conjugate) and subunit DNA vaccines may be helpful. As an innovative strategy, vaccines using cross-reactive immunodominant epitopes as antigenic molecules in an attempt to stimulate antigen-specific regulatory T-cells secreting IL-10 and TGF-β, may provide new clues for periodontal disease prevention. The immune regulating phenomenon observed with coinfecting microorganisms within the subgingival biofilm and vaccine regimens including the commonly shared antigens by selected periodontopathogenic species should also be taken into consideration when researchers design any periodontal vaccine.

Thus, it is important to use a combined proteomic, genomic and immunologic strategy to identify bacterial antigens of periodontopathogens and to evaluate their potential as vaccine candidates for the development of a multispecies vaccine for periodontitis as an adjunct to current periodontal therapies. In light of the increasing evidence that periodontitis significantly increases the risk for potentially fatal diseases such as coronary heart disease, stroke and complications from diabetes mellitus, a successful vaccine for periodontitis could have health benefits far exceeding the prevention of periodontitis.

REFERENCES

1. Roderich N. Immunology. In: Brooks GF, Butel JS, Morse SA (eds). Jawetz, Melnick and Adelberg's Medical Microbiology. New York: McGraw-Hill, 2004; 121.
2. Choi JI, Seymour GJ. Vaccines against periodontitis: a forward-looking review. J Periodontal Implant Sci 2010; 40(4): 153–163.
3. Raul GI, Michelle HM, Elizabeth KA. Relationship between periodontal disease and systemic health. Periodontology 2000 2001; 25: 21-36.
4. Guthmiller JM, Novak KF. Periodontal diseases. In: Brogden KA, Guthmiller JM (eds). Polymicrobial Diseases. Washington (DC): ASM Press, 2002; 138-139.
5. Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, et al. The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. *J Periodontol* 2009; 80:1021–1032.

6. Rajaiah R, Moudgil KD. Heat-shock proteins can promote as well as regulate autoimmunity. *Autoimmun Rev* 2009; 8: 388–393.

7. Choi JI, Chung SW, Kang HS, Rhim BY, Park YM, Kim US, et al. Epitope mapping of *Porphyromonas gingivalis* heat-shock protein and human heat-shock protein in human atherosclerosis. *J Dent Res* 2004; 83: 936–940.

8. Park CS, Lee JY, Kim SJ, Choi JI. Identification of immunological parameters associated with the alveolar bone level in periodontal patients. *J Periodontal Implant Sci* 2010; 40: 61–68.

9. Macchiarini F, Manz MG, Palucka AK, Shultz LD. Humanized mice are we there yet? JEM 2005; 202(10):1307-1311.

10. Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective. Periodontology 2000 1994; 5(1): 7-25.

11. Ishikawa I, Nakashima K, Koseki T, Nagasawa T, Watanabe H, Arakawa S, et al. Induction of immune response to periodontopathic bacteria and its role in pathogenesis of periodontitis. Periodontology 2000 1997; 14: 79-111.

12. Evans RT, Klausen B, Ramamurhty NS, Golub LM, Sfintescu C, Lee lY, et al. Periodontal bone level and gingival proteinase activity in gnotobiotic rats immunized with *Bacteroides gingivalis*. Oral Microbiol Immunol 1991; 6: 193-201.

13. Genco CA, Kapczynski DR, Cutler CW, Arko RJ, Arnold RF. Influence of immunization on *Porphyromonas gingivalis* colonization and invasion in the mouse chamber model. Infect Immun 1992**;** 60: 1447-1454.

14. Hardham J, Sfintescu C, Evans RT. Evaluation of cross-protection by immunization with an experimental trivalent companion animal periodontitis vaccine in the mouse periodontitis model. J Vet Dent 2008; 25(1): 23-27.

15. Nail BS, Paul VD, Staurt DG. Antigens of bacteria associated with periodontitis. Periodontology 2000 2004; 35: 101-34.

16. Taneaki N, Atsushi S, Yasuo H, Kazuyuki I. Gingipains as candidate antigens for *Porphyromonas gingivalis* vaccine. Keio J Med 2003; 52 (3): 158–162.

17. Moritz AJ, Cappelli D, Lantz MS, Holt SC, Ebersole JL. Immunization with *Porphyromonas gingivalis* cysteine protease: effects on experimental gingivitis and ligature-induced periodontitis in *Macaca fascicularis*. *J Periodontol* 1998; 69: 686–697.

18. Gibson FC, Genco CA. Prevention of *Porphyromonas gingivalis*-induced oral bone loss following immunization with gingipain R1. Infect Immun 2001; 69: 7959–7963.

19. Inagaki S, Ishihara K, Yasaki Y, Yamada S, Okuda K. Antibody responses of periodontitis patients to gingipains of *Porphyromonas gingivalis*. *J Periodontol* 2003; 74:1432–1439.

20. Genco CA, Odusanya BM, Potempa J, Mikolajczyk-Pawlinska J, Travis J. A peptide domain on gingipain R which confers immunity against *Porphyromonas gingivalis* infection in mice. Infect Immun 1998; 66: 4108–4114.

21. Amano A. Molecular interaction of *Porphyromonas gingivalis* with host cells: implication for the microbial pathogenesis of periodontal disease. J Periodontol 2003; 74(1): 90-96.

22. Fan Q, Sims T, Sojar H, Genco R, Page RC. Fimbriae of *Porphyromonas gingivalis* induce opsonic antibodies that significantly enhance phagocytosis and killing by human polymorphonuclear leukocytes. *Oral*

Microbiol Immunol 2001; 16: 144–152.

23. Choi JI, Schifferle RE, Yoshimura F, Kim BW. Capsular polysaccharide-fimbrial protein conjugate vaccine protects against *Porphyromonas gingivalis* infection in SCID mice reconstituted with human peripheral blood lymphocytes. Infect Immun 1998; 66(1): 391–393.

24. Gonzalez D, Tzianabos AO, Genco CA, Gibson FC. Immunization with *Porphyromonas gingivalis* capsular polysaccharide prevents *P. gingivalis*-elicited oral bone loss in a murine model. *Infect Immun* 2003; 71: 2283–2287.

25. Yamazaki K, Ohsawa Y, Tabeta K, Ito H, Ueki K, Oda T, Yoshie H, Seymour GJ. Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. Infect Immun 2002; 70(5): 2492-2501.

26. Lee JY, Yi NN, Kim US, Choi JS, Kim SJ, Choi JI. *Porphyromonas gingivalis* heat shock protein vaccine reduces the alveolar bone loss induced by multiple periodontopathogenic bacteria. J Periodontol Res 2006; 41 (1): 4-10.

27. Honma K, Kato T, Okuda K. Salivary immunoglobulin A production against a synthetic oligopeptide antigen of *Actinobacillus actinomycetemcomitans* fimbriae. Oral Microbiol Immunol 1999; 14 (5): 288-292.

28. Takamatsu-Matsushita N, Yamaguchi N, Kawasaki M, Yamashita Y, Takehara T, Koga T. Immunogenicity of *Actinobacillus actinomycetemcomitans* serotype b-specific polysaccharide-protein conjugate. *Oral Microbiol Immunol* 1996; 11: 220–225.

29. Herminajeng E, Asmara W, Yuswanto A, Barid I, Sosroseno W. Protective humoral immunity induced by surface-associated material from *Actinobacillus actinomycetemcomitans* in mice. *Microbes Infect* 2001; 3:997–1003.

30. Lehner T, Ma JKC, Walker P, Childerstone A, Todryk S, Kendal H, et al. T-cell and B-cell epitope mapping and construction of peptide vaccines. In: Genco RJ, Hamada S, Lehner T, McGhee J, Mergenhagen S (eds). Molecular Pathogenesis of Periodontal Disease. Washington (DC): ASM Press, 1994; 279-292.

31. Lee JY, Sojar HT, Bedi GS, Genco RJ. Synthetic peptides analogous to the fimbrillin sequence inhibit adherence of *Porphyromonas gingivalis*. Infect Immun 1992; 60:1662-1670.

32. Brant EE, Sojar HT, Sharma A, Bedi GS, Genco RJ, De Nardin E. Identification of linear antigenic sites on the *Porphyromonas gingivalis* 43-kDa fimbrillin subunit. Oral Microbiol Immunol 1995; 10(3): 146-150.

33. Mouton C, Bouchard D, Deslauriers M, Lamonde L. Immunochemical identification and preliminary characterization of a nonfimbrial hemagglutinating adhesin of *Bacteroides gingivalis*. *Infect Immun* 1989; 57:566–573.

34. Maeba S, Otake S, Namikoshi J, Shibata Y, Hayakawa M, Abiko Y, et al. Transcutaneous immunization with a 40-kDa outer membrane protein of *Porphyromonas gingivalis* induces specific antibodies which inhibit coaggregation by *P. gingivalis*. *Vaccine* 2005; 23: 2513–2521.

35. Saito S, Hiratsuka K, Hayakawa M, Takiguchi H, Abiko Y. Inhibition of a *Porphyromonas gingivalis* colonizing factor between *Actinomyces viscosus* ATCC 19246 by monoclonal antibodies against recombinant 40-kDa outer-membrane protein. *Gen Pharmacol* 1997; 28: 675–680.

36. Abiko Y, Ogura N, Matsuda U, Yanagi K, Takiguchi H. A human

monoclonal antibody which inhibits the coaggregation activity of *Porphyromonas gingivalis*. *Infect Immun* 1997; 65: 3966–3969.

37. Takiguchi H, Katoh M, Saito S, Abiko Y. Bactericidal activity of a monoclonal antibody against recombinant 40-kDa outer membrane protein of *Porphyromonas gingivalis*. J Periodontol 2000; 71: 368-375.

38. Chu L, Bramanti TE, Ebersole JL, Holt SC. Hemolytic activity in the periodontopathogen *Porphyromonas gingivalis*: kinetics of enzyme release and localization. *Infect Immun*1991; 59: 1932–1940.

39. Hosogi Y, Hayakawa M, Abiko Y. Monoclonal antibody against *Porphyromonas gingivalis* hemagglutinin inhibits hemolytic activity. *Eur J Oral Sci* 2001; 109:109–113.

40. Kaizuka K, Hosogi Y, Hayakawa M, Shibata Y, Abiko Y. Human monoclonal antibody inhibits *Porphyromonas gingivalis* hemagglutinin activity. *J Periodontol* 2003; 74: 38–43.

41. Shibata Y, Hosogi Y, Hayakawa M, Hori N, Kamada M, Abiko Y. Construction of novel human monoclonal antibodies neutralizing *Porphyromonas gingivalis* hemagglutination activity using transgenic mice expressing human Ig loci. *Vaccine* 2005; 23: 3850–3856.

42. Ma JK, Hikmat BY, Wycoff K, Vine ND, Charqeleque D, Yu L, et al. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med 1998; 4(5): 601-606.

43. Barry MA, Johnston SA. Biological features of genetic immunization. Vaccine 1997; 15: 788-791.

44. Kawabata S, Terao Y, Fujiwara T, Nakagawa I, Hamada S. Targeted salivary gland immunization with plasmid DNA elicits specific salivary immunoglobulin A and G antibodies and serum immunoglobulin G antibodies in mice. Infect Immun 1999; 67(11): 5863-5868.

45. Sharma A, Honma K, Evans RT, Hruby DE, Genco RJ. Oral immunization with recombinant *Streptococcus gordonii* expressing *Porphyromonas gingivalis* FimA domains. Infect Immun 2001; 69(5): 2928-2934.

46. Ross BC, Czajkowski L, Hocking D, Margetts M, Webb E, Rothel L, et al. Identification of vaccine candidate antigens from a genomic analysis of *Porphyromonas gingivalis*. *Vaccine* 2001; 19: 4135–4142.

47. Suyama T, Hayakawa M, Abiko Y. Subcloning of the 200-kDa *Porphyromonas gingivalis* antigen gene and inhibition of hemagglutination by an antibody against the recombinant protein. J Oral Sci 2004; 46(3): 163-169.

48. Seymour GJ, Taylor JJ. Shouts and whispers: an introduction to immunoregulation in periodontal disease. *Periodontol 2000* 2004; 35: 9–13.

49. Sasaki H, Okamatsu Y, Kawai T, Kent R, Taubman M, Stashenko P. The interleukin-10 knockout mouse is highly susceptible to *Porphyromonas gingivalis*-induced alveolar bone loss. *J Periodontal Res* 2004; 39: 432–441.

50. Cullinan MP, Westerman B, Hamlet SM, Palmer JE, Faddy MJ, Seymour GJ, et al. Progression of periodontal disease and interleukin-10 gene polymorphism. *J Periodontal Res* 2008; 43: 328–333.

51. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontol 2000* 2004; 35: 21–41.

52. Belkaid Y. Regulatory T cells and infection: a dangerous necessity. *Nat Rev Immunol* 2007; 7: 875–888.

53. Gemmell E, Bird PS, Ford PJ, Ashman RB, Gosling P, Hu Y, et al. Modulation of the antibody response by *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in a mouse model. *Oral Microbiol Immunol* 2004; 19: 247–251.

54. Choi JI, Borrello MA, Smith ES, Zauderer M. Polarization of *Porphyromonas gingivalis*-specific helper T-cell subsets by prior immunization with *Fusobacterium nucleatum*. *Oral Microbiol Immuno* 2000; 15: 181–187.

55. Gmur R, Thurnheer T, Guggenheim B. Dominant cross-reactive antibodies generated during the response to a variety of oral bacterial species detect phosphorylcholine. *J Dent Res* 1999; 78: 77–85.

56. Laine ML, Appelmelk BJ, van Winkelhoff AJ. Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. *J Dent Res* 1997; 76: 1840–1844.

57. Hinode D, Nakamura R, Grenier D, Mayrand D. Cross-reactivity of specific antibodies directed to heat shock proteins from periodontopathogenic bacteria and of human origin. *Oral Microbiol Immunol* 1998; 13: 55–58.

58. Chapple ILC. Role of free radicals and antioxidants in the pathogenesis of inflammatory periodontal diseases. J Clin Pathol Clin Mol Pathol 1996; 49: 247-255.

59. Johnson NA, McKenzie RM, Fletcher HM. The bcp gene in the bcp-recA-vimA-vimE-vimF operon is important in oxidative stress resistance in *Porphyromonas gingivalis* W83. Mol Oral Microbiol 2011; 26(1): 62-77.

60. Lancey DL, Timms E, Tan HL, Kelly MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation. Cell 1998; 93: 165-176.

61. Teng YT, Nquyen H, Gaox. Functional human T cell immunity and osteoprotegerin-ligand control alveolar bone destruction in periodontal infection. J Clin Invest 2000; 106 (6): 59-67.

How to cite this article: Resmi Chanrahasan Nair, Binu Neelikad Sugunan. Vaccines for periodontal disease: A new hope?. Cumhuriyet Dent J 2015;18(2):198-213.