Review

JPIS Journal of Periodontal & Implant Science

J Periodontal Implant Sci 2010;40:153-163 • doi: 10.5051/jpis.2010.40.4.153

Vaccines against periodontitis: a forward-looking review

Jeom-Il Choi[1]*, Gregory J. Seymour[2]

[1]Department of Periodontology, Pusan National University School of Dentistry, Yangsan, Korea
[2]Faculty of Dentistry, University of Otago, Dunedin, New Zealand

Periodontal disease, as a polymicrobial disease, is globally endemic as well as being a global epidemic. It is the leading cause for tooth loss in the adult population and has been positively related to life-threatening systemic diseases such as atherosclerosis and diabetes. As a result, it is clear that more sophisticated therapeutic modalities need to be developed, which may include vaccines. Up to now, however, no periodontal vaccine trial has been successful in satisfying all the requirements; to prevent the colonization of a multiple pathogenic biofilm in the subgingival area, to elicit a high level of effector molecules such as immunoglobulin sufficient to opsonize and phagocytose the invading organisms, to suppress the induced alveolar bone loss, or to stimulate helper T-cell polarization that exerts cytokine functions optimal for protection against bacteria and tissue destruction. This article reviews all the vaccine trials so as to construct a more sophisticated strategy which may be relevant in the future. As an innovative strategy to circumvent these barriers, vaccine trials to stimulate antigen-specific T-cells polarized toward helper T-cells with a regulatory phenotype (Tregs, CD4+, CD25+, FoxP3+) have also been introduced. 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.

Keywords: Immunization; Periodontitis; Vaccines.

PERIODONTITIS AS A DISEASE ENTITY

Periodontal disease refers to the processes of destruction of the peri-tooth structures that support the teeth. These are composed of the gingiva, the periodontal ligament, the cementum, and the alveolar bone. The chronic destruction of these supporting tissues leads to the eventual loss of teeth and hence partial or full edentulism. Epidemiological studies reveal that more than two-thirds of the world's population suffers from one of the chronic forms of periodontal disease. 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.

Two forms of periodontitis have been proposed: One is chronic periodontitis (previously termed "adult periodontitis"), which affects primarily the adult population who are >35 years of age. This type of periodontitis is frequently associated with an elevated number and frequency of *Porphyromonas (P.) gingivalis, Treponema (T.) denticola,* and *Tanerella (T.) forsythia* detected in the subgingival microbial community. Contributing local factors consisting of conspicuous dental plaque, calculus, root surface accretions, and overhanging restorations are closely associated quantitatively or qualitatively with disease expression. The other form, aggressive periodontitis (previously referred to as "early-onset periodontitis"), is associated with young adults (<35 years of age) and is characterized by rapid destruction with minimal signs of gingival inflammation. *Aggregatibacter (A.) actinomycetemcomitans*

Received: May. 1, 2010; **Accepted:** Jul. 2, 2010
***Correspondence:** Jeomil Choi
Department of Periodontology, Pusan National University School of Dentistry, Beomeo-ri, Mulgeum-eup, Yangsan 626-870, Korea
E-mail: jrapa@pusan.ac.kr, Tel: +82-55-360-5200, Fax: +82-55-360-5194

pISSN 2093-2278
eISSN 2093-2286

JPIS Journal of Periodontal & Implant Science

(formerly *Actinobacillus actinomycetemcomitans*) is defined as the predominant cultivable organism in localized afflicted sites, whereas *P. gingivalis, Prevotella (P.) intermedia* and *Capnocytophaga (C.) sputigena* are frequently isolated in the generalized form. The aggressive forms of periodontitis suggest a genetic predisposition with a minimal number of noticeable local factors. The American Academy of Periodontology (1999) proposed disregarding the association of age with either form of the disease, since both can affect young and old populations regardless of age. Other periodontal diseases include gingival diseases, necrotizing periodontal diseases, abscesses, developmental and acquired forms of periodontal diseases, and combined endodontic-periodontal lesions.

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 infectionof polymicrobial Gram-negative anaerobic bacteria, including *P. gingivalis, T. denticola* and *T. forsythia,* and *A. actinomycetemcomitans,* all of which have been proposed as predominant pathogens, exclusively or synergistically with other bacteria, including *P. intermedia, Campylobacter (C.) rectus, Fusobacterium (F.) nucleatum,* and herpes virus.

Although periodontal diseases are primarily initiated and perpetuated by 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 sophisticated management of the compromised subject.

EMERGING CONCEPTS REGARDING VACCINE DEVELOPMENT

Three emerging concepts of periodontal disease may influence the development of a sophisticated 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.).

Ever since the introduction of the smallpox vaccine by Jenner in 1798, antigens of infectious pathogenic bacteria and viruses have been the targets for a variety of vaccines against a number of infectious diseases. Within this context, vaccine strategy has been based on prevention of disease and less so on their treatment. Thus, most vaccines target one or multiple antigenic components of mono-infecting bacteria or viruses. At the same time, most experiments on immunization of periodontitis, despite its poly-infectious nature, have been directed toward a very limited number of antigenic components of a single specific pathogen, either *P. gingivalis* or *A. actinomycetemcomitans.*

THE SIGNIFICANCE OF PERIODONTAL VACCINE DEVELOPMENT

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 effect should be seen to enhance the feasibility of maintaining oral health and to 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 (atherosclerosis, diabetes mellitus, pre-term low-weight birth, rheumatoid arthritis, etc.) 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.

BASIC TENETS IN DEVELOPING STRATEGIES FOR PERIODONTITIS VACCINES

Despite the considerable numbers of cultivable microorganisms identifiable in the subgingival niche, researchers have narrowed the number of putative periodontal pathogens down to six or seven, *P. gingivalis, T. denticola* and *T. forsythia, A. actinomycetemcomitans, P. intermedia, C. rectus,* and *F. nucleatum,* which are predominantly cultivated in sites demonstrating disease activity. Socransky et al. [1] proposed the Red complex, namely *P. gingivalis, T. denticola* and *T. forsythus,* as the predominant disease-associated organisms. Later *P. inter-*

media, Dialister pneumosintes, Eubacterium nodatum, C. rectus, and *F. nucleatum*, were also added as putative periodontal pathogens [2]. Viruses (herpes virus) and their interaction with periodontal pathogenic bacteria have also been suggested to be periodontal pathogens with the recent advent of molecular diagnostic techniques [3-6]. In addition, aggressive periodontitis patients may not only harbor the distinct bacterial species associated with disease activity, but may also harbor *A. actinomycetemcomitans*, especially in a localized form.

Most immunization approaches, both active and passive, against periodontitis have been focused on *P. gingivalis* and *A. actinomycetemcomitans*. 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.

Vaccine testing animal systems have ranged from mice and rats to dogs and nonhuman primates. In order to evaluate vaccine efficacy in terms of the human immune system, a sophisticated 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, due to frequent episodes of leakiness of these mouse systems, a more stringent model with low leakiness has now been developed: NOD.CB17-prkdc^{scid}/J.

IMMUNIZATION AGAINST *PORPHYROMONAS GINGIVALIS*

As noted above, *P. gingivalis* has been implicated as a major periodontopathogen in human periodontitis [7]. In this context, it has developed a variety of survival strategies enabling it to evade host defense mechanisms. Virulence components of the bacterial cell include cysteine proteases, fimbriae, capsular polysaccharide (CPS), lipopolysaccharide, and outer membrane vesicles [8].

Both heat- and formalin-killed *P. gingivalis* whole cell vaccines, either alone or conjugated with syntax adjuvant, have been reported to inhibit the progression of periodontal disease and to elevate serum immunoglobulin G (IgG) and IgA titers that demonstrated opsonophagocytic capability in nonhuman primates [9-11]. Further, a recent study in mice immunized with heat-killed *P. gingivalis* reported the induction of *P. gingivalis*-specific IgG with optimal levels of opsonization of the microorganism and the prevention of alveolar bone loss [12].

These studies, however, lack evidence of long-term immune memory and cell-mediated immune responses that would allow them to be adopted as a feasible vaccine strategy.

Gingipain, the term adopted for *P. gingivalis* specific cysteine proteases, represents one of the major pathogenic virulence factors for this organism. It consists of two components: gingipain R (RgpA and RgpB) that cleaves proteins at arginine residues, and gingipain K (porphypain 2, Kgp) that cleaves proteins at lysine residues. As a result, it has drawn considerable interest as a candidate target antigen for periodontal vaccine development [13].

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 [14,15]. 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 specific IgG antibody response that suppressed *P. gingivalis*-induced bone loss in *Macaca (M.) fascicularis* [16].

However, with repeated immunization, the authors realized that only animals immunized with RgpA produced hemagglutinin domain-specific antibodies that contributed to the prevention of *P. gingivalis*-mediated periodontal disease [17]. Furthermore, immunization with the RgpA-Kgp proteinase-adhesin complexes of *P. gingivalis* protected against periodontal bone loss by eliciting a high titer of serum IgG_{2a} response in the rat. This approach seems to open a new venue for further trials to pursue.

As *P. gingivalis* requires the hemagglutinin 2 (HA2) domain for survival through heme acquisition, an HA2 domain-based vaccine (rHA2) was administered to rats resulting in significantly enhanced IgG levels and some protection against experimental periodontitis. However, one clinical trial reported that periodontal patients demonstrated high IgG titers to the HA domain but not to the catalytic domain, because the catalytic domain is not exposed on the gingipain complex [18]. 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 suggesting the need for inclusion of the catalytic subunit in vaccine design. Interestingly, a mouse immunization study, utilizing a synthetic peptide representing the N-terminus of the RgpA catalytic domain, RgpA45, coupled to an oligolysine, reported an increased level of IgG with protective capacity against *P. gingivalis*. Additional studies performed in murine models incorporating peptides of the catalytic domains or DNA vaccine encoding the subunit have strengthened the importance of inclusion of the catalytic subunit in the vaccine regimen by demonstrating the protective function of the anti-catalytic domain antibodies against *P. gingivalis* infection [19].

The fimbriae of *P. gingivalis*, which consist of one major

JPIS Journal of Periodontal & Implant Science

fimbriae and two minor fimbriae of 67 kDa and 72 kDa, respectively, are virulence factors in the pathogenesis of periodontal disease. When rats were parenteraly immunized with purified 43-kDa fimbrial protein, the resultant fimbrial A-specific antibodies in serum and saliva gave a satisfactory level of protection against *P. gingivalis*-induced alveolar bone loss [20].

Intranasal administration of *P. gingivalis* fimbrial antigen with recombinant cholera toxin B subunit also induced a significant immune response (fimbrial-specific secretory IgA-sIgA) in mice, which could reduce *P. gingivalis*-mediated alveolar bone loss. It is also possible that this mucosal immunization resulted in peripheral tolerance and hence a reduced inflammatory response and alveolar bone loss. However, it has further 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 [21]. The feasibility of this 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.

A conjugate vaccine incorporating both fimbriae and *P. gingivalis* CPS, has been introduced in a study that led to the production of a high IgG response and which was effective in protecting against *P. gingivalis* infection [22]. However, in this study it was not clear whether the protection came from the CPS or fimbriae. CPS, by virtue of its encapsulation and antigenic shift, constitutes a robust strategy for *P. gingivalis* survival against opsonophagocytic activity. Due to its poor T-cell stimulating ability, however, CPS of other Gram-negative bacteria is usually conjugated to a protein antigen (for stimulating helper T-cells) in many vaccine trials in infectious diseases, such as pneumonia and meningitis. More recently, *P. gingivalis* CPS alone has nevertheless, been used as an immunogen, and it has been reported to result in an elevated production of serum IgG and IgM that provided protection against *P. gingivalis*-induced bone loss [23].

PASSIVE IMMUNIZATION AGAINST *PORPHYROMONAS GINGIVALIS*

In terms of an anti-infective scheme, monoclonal antibodies targeting these antigenic molecules of *P. gingivalis* could potentially be adopted as a sophisticated mode of immunotherapy.

Outer membrane proteins (OMPs) are important coaggregation factors and as such are major colonization factors of *P. gingivalis* [24-26]. Since IgG specific for the 40 kDa-OMP inhibited coaggregation of *P. gingivalis* vesicles and *S. gordonii*, it could conceivably be used to prevent *P. gingivalis* infection [27]. In support of this, a panel of mouse monoclonal antibodies (mAb) against purified r40-kDa OMP specifically inhibited the coaggregation of *A. naeslundii* with several strains of *P. gingivalis* [28]. Furthermore, an IgG2 human mAb (HAB-OMP1) has been shown to significantly inhibit the coaggregation activity of *P. gingivalis* vesicles with *A. naeslundii* [29]. 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 [30].

Erythrocyte-derived protoheme is known to be one of the absolute requirements for the persistent growth of *P. gingivalis* [31]. It is the hemagglutinins of *P. gingivalis* that facilitate its attachment to the erythrocyte cell surface, allowing it to access protoheme. Hence, applying an mAb 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, local passive immunization with rabbit antiserum against *P. gingivalis* hemagglutinin has in fact resulted in a reduced colonization by exogenous *P. gingivalis* in the subgingival area over a 3-week period [32]. In addition, localized administration of a *P. gingivalis*-specific mAb (MAb61BG1.3) at severely infected subgingival sites has been shown to significantly reduce subsequent *P. gingivalis* recolonization for up to 9 months in periodontal patients [33]. It was subsequently shown that the mechanism by which MAb61BG1.3 inhibited the adhesion of *P. gingivalis* to the receptors on erythrocytes was due to its ability to block the hemagglutinating protease [34].

As an advanced step forward in this approach, a single-chain variable fragment (scFv) mAb that recognized the 43-and 49-kDa proteins of *P. gingivalis* vesicles was prepared [35]. This scFv mAb was found to inhibit vesicle-associated hemagglutinating activity in a dose-dependent manner. Also, a monoclonal antibody using *P. gingivalis* vesicles as the immunogen (MAb-Pg-vc) was shown to inhibit vesicle-associated hemagglutinating activity when incubated with rabbit erythrocytes [36].

Recently, 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 (59). The human mAb 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 [37]. Interestingly, in a novel introduction of XenoMouse technology, Shibata et al. [38] constructed an IgG2 Xeno-monoclonal antibody against the recombinant 130-kDa hemagglutinin domain of *P. gingivalis* and demon-

strated a significant inhibition of hemagglutination by *P. gingivalis* and its vesicles [38].

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

IMMUNIZATION AGAINST *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*

A. actinomycetemcomitans is considered another important pathogen in human periodontal disease, especially in the localized form of aggressive periodontitis.

Harano et al. [39] prepared an antiserum against a synthetic fimbrial peptide of *A. actinomycetemcomitans* and found that it blocked the adhesion of the organism to saliva-coated hydroxyapatite beads, to buccal epithelial cells, and to a fibroblast cell line. 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 [40].

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

REGULATION OF THE IMMUNE RESPONSE TO THE POLYMICROBIAL BIOFILM: A VACCINE PERSPECTIVE

The subgingival microbial community demonstrates the distinctive ecologic features characteristic of a polymicrobial biofilm world with both synergistic and antagonistic communications among the complex microbial organisms. When the polymicrobial biofilm mass becomes mature and thicker with continuous colonization by the early, intermediate, and late colonizers, it demonstrates both quorum sensing [42] and genetic communication through pathogenicity islands [43].

The host immune response to this biofilm is modulated by the array of different microbial challenges including both specific and cross-reactive antigens. This phenomenon then results in the recruitment of diverse antigen-specific B- and T-lymphocytes, which may in turn demonstrate a wide spectrum of poly-reactivity [44-47].

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 [48]. At the same time, Choi et al. [49] 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 [50,51].

This observation may explain in part why the opsonophagocytic function of anti-*P. gingivalis*-specific antibodies from periodontal patients is impaired [52].

This immune regulating phenomenon observed with co-infecting microorganisms within the subgingival polymicrobial biofilm community should be taken into consideration when researchers design any periodontal vaccine. The fine tuning of helper T-cell polarization secreting an array of characteristic cytokines would obviously influence the ultimate outcome of any vaccine trial.

Immunization of *M. fascicularis* with *P. gingivalis* produced antibodies reactive not only with homologous antigens but also with those of *B. forsythus* (now called *T. forsythia*), a putative Gram-negative periodontopathic bacterium sharing antigens within the subgingival microbial community [53].

VACCINE DESIGN VIA FINE-TUNING OF ANTIGEN-SPECIFIC T-CELLS IN PERIODONTAL DISEASE

Polarization of helper T-cells depends, 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 [54].

Periodontal disease severity is counterbalanced by the fine-tuning of the so-called Th1/Th2 lymphocyte axis and the array of cytokine profiles 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 [55]. 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 [56].

In order to study immune modulation mechanisms, Yamashi-

JPIS Journal of Periodontal & Implant Science

ta et al. [57] adoptively transferred cloned *A. actinomycetemcomitans*-specific T-helper cells into a rat model followed by infection with *A. actinomycetemcomitans*. Rats adoptively transferred with the *A. actinomycetemcomitans*-specific T-cell clone demonstrated a significantly lower amount of bone loss when compared with the control group [57]. In a following study, they further showed the beneficial role of the Th2 clone in terms of antibody production and protection from periodontal bone loss [58].

In contrast, it is well established that chronic periodontitis is a B cell/plasma cell lesion while gingivitis is identical to delayed type hypersensitivity and as such is a T cell/macrophage lesion. In this context therefore, Gemmell and Seymour [59] 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. It could also pave the way to the development of an adequate protection strategy against pathogenic agents. At this stage, however, it would appear that it is the balance between Th1 and Th2 cytokines that plays an important role in maintaining alveolar bone homeostasis [59,60].

Therefore, more refined mechanisms may have to be investigated using adoptive transfer of T-cell subsets specific to the more defined antigens interest for a vaccine before the specific vaccines can be fully developed. 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 [61].

DEVELOPMENT STRATEGIES FOR A VACCINE AGAINST PERIODONTITIS AS A POLYMICROBIAL INFECTION

Most periodontal immunization studies have targeted a single pathogenic species. However, a number of the potential candidate antigenic determinants may share a sequence homology with other periodontopathic bacteria. These antigens may include phosphorylcholine [62], CPS [63], and heat-shock protein (HSP) [64,65]. 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 [22]. 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.

The notion that periodontal disease is a polymicrobial infection prompted a study in which rats were immunized with *P. gingivalis* HSP60. Alveolar bone loss was experimentally induced by infection with multiple periodontopathogenic bacteria. Significantly high levels of anti-*P. gingivalis* HSP IgG antibody were elicited and there was a substantial reduction in alveolar bone loss induced by either *P. gingivalis* or by multiple bacterial infections [66]. This study postulated that *P. gingivalis* HSP60 could potentially be developed as a vaccine to inhibit periodontal disease induced by multiple pathogenic bacteria [67]. These results may well pave a new way in the development of periodontal vaccines targeting the mixed microbial component. Moreover, conjugating the cross-reactive HSP60 with CPSs may also be a potential versatile vaccine in the future. 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 [45,68].

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 [69]. Furthermore, it has been suggested that the microbial components responsible for periodontal infection may trigger the development of autoimmune disease. Most recently, 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 [70-73].

Recently, Choi et al. [74-78] 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 mam-

malian HSP60. The poly-reactive monoclonal antibody recognized peptide number 19 (TLVVNRLRGSLKICAVKAPG) of 37 synthetic peptides spanning the whole molecule of *P. gingivalis* HSP60 [46,79]. This novel finding could provide a clue to identifying a possible candidate peptide epitope that could be further develop into periodontal disease-systemic autoimmune diseases (i.e. atherosclerosis, diabetes mellitus, or rheumatoid arthritis) vaccine. Such a vaccine might be useful in multifactorial diseases such as atherosclerosis and diabetes, where human HSP may be a critical target molecule in autoimmunity triggered by exogenous bacterial HSP. *P. gingivalis* HSP peptide number 19 was recognized by all the sera from atherosclerosis patients, strongly supporting the role of molecular mimicry in the periodontal-atherosclerosis link [46,79]. The basic tenet of the hypothesis is that peptide number 19 may stimulate specific CD4+, CD25+, and FoxP3+ regulatory T-cells, which may in turn suppress the development of autoimmune diseases. This hypothesis is being investigated; however, care must be taken to ensure that any vaccine based on this concept does not itself trigger an autoimmune response. Interestingly, as mentioned above, patients whose sera recognized both *P. gingivalis* HSP peptide number 19 and human HSP peptide #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 [68]. The fact that all atherosclerosis patients also exhibited antibodies to peptide number 19, however, suggests that it may be also involved in the pathogenesis of atherosclerosis.

DEVELOPING THE ADVANCED TOOLS FOR ENHANCING VACCINE EFFICACY

Recently, a variety of strategies to enhance the immunogenicity of antigenic components of B- or T-lymphocytes have been adopted in vaccine trials against periodontal disease. These include, but not limited to, immunization of dendritic cells pulsed with antigens, the use of improved adjuvant formulas (e.g. the use of alum as an alternative to HSP-based adjuvant), the use of recombinant plant monoclonal antibodies (plantibodies) [80,81], and the use of transgenic microorganisms as antigen vectors [82,83]. These attempts leave challenging areas to be pursued further in the quest for a more sophisticated design that may guarantee the efficacy and safety of prolonged immune memory.

LESSONS FROM THE PAST FOR THE FUTURE

As yet, there are no periodontal vaccine trials that have been successful in satisfying all requirements; to prevent the colonization of multiple pathogen biofilm in the subgingival area, to elicit a high level of effector molecules such as immunoglobulin 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.

As an innovative strategy, vaccines using cross-reactive immunodominant epitopes as antigenic molecules in an attempt to stimulate antigen-specific regulatory T-cells (Tregs, CD4+, CD25+, FoxP3+), secreting IL-10 and TGF-β, may provide new clues for periodontal disease prevention, through the induction of either immune tolerance or an effector function.

Periodontal disease as a multifactorial and polymicrobial disease requires a sophisticated vaccine design regimen targeting multiple pathogenic species. Vaccine regimens including the commonly shared antigens by selected periodontopathogenic species would be considered an innovative strategy.

Traditional periodontal vaccine trials aim to stimulate the immune system to produce increased levels of immunoglobulin of desired specificity. To accomplish this end, a conjugate vaccine (i.e. protein-CPS conjugate), dendritic-cell based immunotherapy, and subunit DNA vaccine encoding the desired immunogenic epitope have been devised.

Animal models for vaccine trials may pose discrepancies with human models in major histocompatibility complex-restriction of antigens presented by antigen presenting, thus obscuring the immunodominant epitope(s). A humanized mouse system has been proposed that has been reconstituted with human PBLs. This system needs to meet the requirement of least leakiness of a mouse immune system. More recently, a genetically engineered mouse system, such as the NOD.CB17-prkdc^{scid}/J mouse, has been introduced for the study of infectious and autoimmune diseases in humans. This model may also prove useful for the study of periodontal disease and putative periodontal vaccines.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor Katsuji Okuda, Tokyo Dental College, Japan, for his constructive comments. The manuscript has been supported in part by grants from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea A080391, KOSEF RO1-2008-000-20044-0, and KRF E00130.

JPIS Journal of Periodontal & Implant Science

REFERENCES

1. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134-44.
2. Slots J. Systemic antibiotics in periodontics. J Periodontol 2004;75:1553-65.
3. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. Oral Microbiol Immunol 1996;11:289-93.
4. Contreras A, Umeda M, Chen C, Bakker I, Morrison JL, Slots J. Relationship between herpesviruses and adult periodontitis and periodontopathic bacteria. J Periodontol 1999;70:478-84.
5. Kubar A, Saygun I, Yapar M, Ozdemir A, Slots J. Real-time PCR quantification of cytomegalovirus in aggressive periodontitis lesions using TaqMan technology. J Periodontal Res 2004;39:81-6.
6. Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL. Association between human herpesviruses and the severity of periodontitis. J Periodontol 2004;75:1479-85.
7. Dzink JL, Socransky SS, Haffajee AD. The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. J Clin Periodontol 1988;15:316-23.
8. Sundqvist G. Pathogenicity and virulence of black-pigmented gram-negative anaerobes. FEMS Immunol Med Microbiol 1993;6:125-37.
9. Persson GR, Engel D, Whitney C, Darveau R, Weinberg A, Brunsvold M, et al. Immunization against Porphyromonas gingivalis inhibits progression of experimental periodontitis in nonhuman primates. Infect Immun 1994;62:1026-31.
10. Houston LS, Lukehart SA, Persson GR, Page RC. Function of anti-Porphyromonas gingivalis immunoglobulin classes in immunized Macaca fascicularis. Oral Microbiol Immunol 1999;14:86-91.
11. Page RC. Vaccination and periodontitis: myth or reality. J Int Acad Periodontol 2000;2:31-43.
12. Gibson FC 3rd, Gonzalez DA, Wong J, Genco CA. Porphyromonas gingivalis-specific immunoglobulin G prevents P. gingivalis-elicited oral bone loss in a murine model. Infect Immun 2004;72:2408-11.
13. Travis J, Pike R, Imamura T, Potempa J. Porphyromonas gingivalis proteinases as virulence factors in the development of periodontitis. J Periodontal Res 1997;32:120-5.
14. Kadowaki T, Yoneda M, Okamoto K, Maeda K, Yamamoto K. Purification and characterization of a novel arginine-specific cysteine proteinase (argingipain) involved in the pathogenesis of periodontal disease from the culture supernatant of Porphyromonas gingivalis. J Biol Chem 1994;269:21371-8.
15. Imamura T. The role of gingipains in the pathogenesis of periodontal disease. J Periodontol 2003;74:111-8.
16. 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;69:686-97.
17. Gibson FC 3rd, Genco CA. Prevention of Porphyromonas gingivalis-induced oral bone loss following immunization with gingipain R1. Infect Immun 2001;69:7959-63.
18. Inagaki S, Ishihara K, Yasaki Y, Yamada S, Okuda K. Antibody responses of periodontitis patients to gingipains of Porphyromonas gingivalis. J Periodontol 2003;74:1432-9.
19. Kuboniwa M, Amano A, Shizukuishi S, Nakagawa I, Hamada S. Specific antibodies to Porphyromonas gingivalis Lys-gingipain by DNA vaccination inhibit bacterial binding to hemoglobin and protect mice from infection. Infect Immun 2001;69:2972-9.
20. Evans RT, Klausen B, Sojar HT, Bedi GS, Sfintescu C, Ramamurthy NS, et al. Immunization with Porphyromonas (Bacteroides) gingivalis fimbriae protects against periodontal destruction. Infect Immun 1992;60:2926-35.
21. 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;16:144-52.
22. 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;66:391-3.
23. Gonzalez D, Tzianabos AO, Genco CA, Gibson FC 3rd. Immunization with Porphyromonas gingivalis capsular polysaccharide prevents P. gingivalis-elicited oral bone loss in a murine model. Infect Immun 2003;71:2283-7.
24. Takazoe I, Nakamura T, Okuda K. Colonization of the subgingival area by Bacteroides gingivalis. J Dent Res 1984;63:422-6.
25. Slots J, Gibbons RJ. Attachment of Bacteroides melaninogenicus subsp. asaccharolyticus to oral surfaces and its possible role in colonization of the mouth and of periodontal pockets. Infect Immun 1978;19:254-64.
26. Mouton C, Bouchard D, Deslauriers M, Lamonde L. Immunochemical identification and preliminary characterization of a nonfimbrial hemagglutinating adhesin of Bacteroides gingivalis. Infect Immun 1989;57:566-73.
27. 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;23:2513-21.
28. 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;28:675-80.
29. 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;65:3966-9.
30. 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;19:4135-42.
31. Chu L, Bramanti TE, Ebersole JL, Holt SC. Hemolytic activity in the periodontopathogen Porphyromonas gingivalis: kinetics of enzyme release and localization. Infect Immun 1991;59:1932-40.
32. Okuda K, Kato T, Naito Y, Takazoe I, Kikuchi Y, Nakamura T, et al. Protective efficacy of active and passive immunizations against experimental infection with Bacteroides gingivalis in ligated hamsters. J Dent Res 1988;67:807-11.
33. Booth V, Ashley FP, Lehner T. Passive immunization with monoclonal antibodies against Porphyromonas gingivalis in patients with periodontitis. Infect Immun 1996;64:422-7.
34. Booth V, Lehner T. Characterization of the Porphyromonas gingivalis antigen recognized by a monoclonal antibody which prevents colonization by the organism. J Periodontal Res 1997;32:54-60.
35. Shibata Y, Kurihara K, Takiguchi H, Abiko Y. Construction of a functional single-chain variable fragment antibody against hemagglutinin from Porphyromonas gingivalis. Infect Immun 1998;66:2207-12.
36. Hosogi Y, Hayakawa M, Abiko Y. Monoclonal antibody against Porphyromonas gingivalis hemagglutinin inhibits hemolytic activity. Eur J Oral Sci 2001;109:109-13.
37. Kaizuka K, Hosogi Y, Hayakawa M, Shibata Y, Abiko Y. Human monoclonal antibody inhibits Porphyromonas gingivalis hemagglutinin activity. J Periodontol 2003;74:38-43.
38. 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;23:3850-6.
39. Harano K, Yamanaka A, Okuda K. An antiserum to a synthetic fimbrial peptide of Actinobacillus actinomycetemcomitans blocked adhesion of the microorganism. FEMS Microbiol Lett 1995;130:279-85.
40. 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;11:220-5.
41. 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.
42. Shao H, Demuth DR. Quorum sensing regulation of biofilm growth and gene expression by oral bacteria and periodontal pathogens. Periodontol 2000 2010;52:53-67.
43. Kuboniwa M, Lamont RJ. Subgingival biofilm formation. Periodontol 2000 2010;52:38-52.
44. Lee J, Suh J, Choi J. B-1 cell-derived monoclonal antibodies and costimulatory molecules. J Surg Res 2009;154:293-8.
45. Choi JI, Kim SJ, Lee JY, Lee JY. Production and characterization of cross-reactive anti-Porphyromonas gingivalis heat shock protein 60 monoclonal antibody. J Korean Acad Periodontol 2008;38:565-78.
46. Ha JA, Choi JI. Monoclonal antibody to Porphyromonas gingivalis heat-shock protein (Abstract 2631). 88th General Session & Exhibition of the IADR; 2010 Jul 14-17; Barcelona, Spain. Barcelona: International Association for Dental Research; 2010.
47. Notkins AL. Polyreactive antibodies and polyreactive antigen-binding B (PAB) Cells. Curr Top Microbiol Immunol 2000;252:241-9.
48. 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;19:247-51.
49. 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 Immunol 2000;15:181-7.
50. Choi J, Borrello MA, Smith E, Cutler CW, Sojar H, Zauderer M. Prior exposure of mice to Fusobacterium nucleatum modulates host response to Porphyromonas gingivalis. Oral Microbiol Immunol 2001;16:338-44.
51. Choi JI, Kim US, Kim SJ, Son WS, Park HR. Fusobacterium nucleatum impairs serum binding to Porphyromonas gingivalis biofilm. Oral Microbiol Immunol 2003;18:92-4.
52. Sjostrom K, Darveau R, Page R, Whitney C, Engel D. Opsonic antibody activity against Actinobacillus actinomycetemcomitans in patients with rapidly progressive periodontitis. Infect Immun 1992;60:4819-25.
53. Vasel D, Sims TJ, Bainbridge B, Houston L, Darveau R, Page RC. Shared antigens of Porphyromonas gingivalis and Bac-

JPIS Journal of Periodontal & Implant Science

teroides forsythus. Oral Microbiol Immunol 1996;11:226-35.
54. Seymour GJ, Taylor JJ. Shouts and whispers: an introduction to immunoregulation in periodontal disease. Periodontol 2000 2004;35:9-13.
55. 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;39:432-41.
56. 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;43:328-33.
57. Yamashita K, Eastcott JW, Taubman MA, Smith DJ, Cox DS. Effect of adoptive transfer of cloned Actinobacillus actinomycetemcomitans-specific T helper cells on periodontal disease. Infect Immun 1991;59:1529-34.
58. Eastcott JW, Yamashita K, Taubman MA, Harada Y, Smith DJ. Adoptive transfer of cloned T helper cells ameliorates periodontal disease in nude rats. Oral Microbiol Immunol 1994;9:284-9.
59. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. Periodontol 2000 2004;35:21-41.
60. Alayan J, Ivanovski S, Farah CS. Alveolar bone loss in T helper 1/T helper 2 cytokine-deficient mice. J Periodontal Res 2007;42:97-103.
61. Belkaid Y. Regulatory T cells and infection: a dangerous necessity. Nat Rev Immunol 2007;7:875-88.
62. 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.
63. Laine ML, Appelmelk BJ, van Winkelhoff AJ. Prevalence and distribution of six capsular serotypes of Porphyromonas gingivalis in periodontitis patients. J Dent Res 1997;76:1840-4.
64. 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 [corrected]. Oral Microbiol Immunol 1998;13:55-8.
65. Maeda H, Miyamoto M, Hongyo H, Nagai A, Kurihara H, Murayama Y. Heat shock protein 60 (GroEL) from Porphyromonas gingivalis: molecular cloning and sequence analysis of its gene and purification of the recombinant protein. FEMS Microbiol Lett 1994;119:129-35.
66. 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 Periodontal Res 2006;41:10-4.
67. Choi JI, Choi KS, Yi NN, Kim US, Choi JS, Kim SJ. Recognition and phagocytosis of multiple periodontopathogenic bacteria by anti-Porphyromonas gingivalis heat-shock protein 60 antisera. Oral Microbiol Immunol 2005;20:51-5.
68. 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;40:61-8.
69. 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;80:1021-32.
70. Van Eden W, Wick G, Albani S, Cohen I. Stress, heat shock proteins, and autoimmunity: how immune responses to heat shock proteins are to be used for the control of chronic inflammatory diseases. Ann N Y Acad Sci 2007;1113:217-37.
71. Van Eden W, Van der Zee R, Prakken B. Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 2005;5:318-30.
72. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006;6:508-19.
73. Rajaiah R, Moudgil KD. Heat-shock proteins can promote as well as regulate autoimmunity. Autoimmun Rev 2009;8:388-93.
74. Choi JI, Chung SW, Kang HS, Rhim BY, Kim SJ. Establishment of Porphyromonas gingivalis heat-shock-protein-specific T-cell lines from atherosclerosis patients. J Dent Res 2002;81:344-8.
75. 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;83:936-40.
76. Choi J, Chung SW, Kim SJ. Establishment of Porphyromonas gingivalis-specific T-cell lines from atherosclerosis patients. Oral Microbiol Immunol 2001;16:316-8.
77. Choi JI, Kang HS, Park YM, Kim SJ, Kim US. Identification of T-cell epitopes of Porphyromonas gingivalis heat-shock-protein 60 in periodontitis. Oral Microbiol Immunol 2004;19:1-5.
78. Bak JG, kim SJ, Choi JI. Epitope specificity of Porphyromonas gingivalis heat shock protein for T-cell and/or B-cell in human atherosclerosis. J Korean Acad Periodontol 2003;33:179-91.
79. Choi JI, Chung SW, Lee SY, Kim KH, Choi BK. Immunoreactivity of poly-specific peptide from Porphyromonas gingivalis heat shock protein (Abstract 2716). 88th General Session & Exhibition of the IADR; 2010 Jul 14-17; Barcelona, Spain. Barcelona: International Association for Dental Research; 2010.
80. Ma JK, Hiatt A, Hein M, Vine ND, Wang F, Stabila P, et al.

Generation and assembly of secretory antibodies in plants. Science 1995;268:716-9.
81. Shin EA, Lee JY, Kim TG, Park YK, Langridge WH. Synthesis and assembly of an adjuvanted Porphyromonas gingivalis fimbrial antigen fusion protein in plants. Protein Expr Purif 2006;47:99-109.
82. Sharma A, Sojar HT, Hruby DE, Kuramitsu HK, Genco RJ. Secretion of Porphyromonas gingivalis fimbrillin polypeptides by recombinant Streptococcus gordonii. Biochem Biophys Res Commun 1997;238:313-6.
83. Sharma A, Honma K, Evans RT, Hruby DE, Genco RJ. Oral immunization with recombinant Streptococcus gordonii expressing porphyromonas gingivalis FimA domains. Infect Immun 2001;69:2928-34.