

Periodontal Vaccine—A Boon In Periodontics

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Abstract: Periodontal disease though is multifactorial it is thought to be caused by group of gram negative anaerobic bacteria resulting in destruction of periodontal ligament and alveolar bone. The current treatment of periodontitis is non specific and is centered on removal of supragingival and subgingival plaque by mechanical debridement and surgical procedures. Due to its high prevalence rate this disease has created an innovative interest to find a solution in the form of vaccine. The search for periodontal vaccine revolves around the antigenic factors of the periodontal pathogens. The objective of periodontal vaccine is to identify the antigens involved in the destructive process of periodontitis against which antibodies would be evoked to exert protection. It also aims to induce mucosal antibody response with little or moderate doses of vaccine. Till date no preventive modality exists for periodontal disease and treatment rendered is palliative. Thus availability of periodontal vaccine would not only prevent and modulate periodontal disease but also enhance the quality of life for whom periodontal treatment cannot be easily obtained.

Keywords: Vaccines, immunization, gingivitis, peptides, plantibodies.

I. Introduction

Vaccines is name applied generally to substance of nature of dead or attenuated living infectious material introduced into body with object of increasing its power to resist or get rid of a disease.^[1] Vaccination is induction of immunity by injecting a dead or attenuated form of pathogen.^[2] Vaccination is the best-known and the most important application of immunological principles to human health.^[3] The first vaccine was named after vaccinia, the cowpox virus. Jenner pioneered its use 200 years ago. It was the first deliberate scientific attempt to prevent an infectious disease (small pox).^[4] With the rapid growth of microbial genome sequencing and bioinformatics analysis tools, we have the potential to examine all the genes and proteins from any human pathogens. This technique has capability to provide us with the new targets for antimicrobial drugs and vaccines. Availability of periodontal vaccine would not only prevent or modulate the course of periodontal disease but also enhance the quality of life of people for whom periodontal treatment cannot be easily obtained.^[1]

II. Need for development of Periodontal vaccine

1. For bacteria which are capable of evading host immune responses and invading the tissue

P. gingivalis produces proteases that degrade serum antibacterial components (antibodies, complement protein) and immune cell derived peptides (eg. Cytokines). *A. actinomycetemcomitans* produce a protein (leukotoxin) that is specially toxic to host immune cells (e.g. neutrophils and monocytes) and also produces factors that can inhibit immune responses.

2. To decrease the incidence of Periodontal disease

Periodontal disease are not isolated lesions but have systemic sequelae. It results in higher systemic levels of inflammatory markers viz C-reactive protein and fibrinogen. These systemic changes predispose the individuals to various conditions viz myocardial infarction, cerebrovascular stroke, pneumonia etc. Another link can be microbial front. There is evidence that *P. gingivalis* antigen heat shock protein is an immunodominant antigen of many microorganisms. HSP-60 has been associated with atherosclerosis and Chlamydia pneumonia infection.

3. Financial

Periodontal treatment puts a financial burden on the individuals suffering from it. Availability of vaccine for preventing or modulating periodontal disease would be of great benefit in both developing and developed countries.^[1]

Type of Vaccination^[5]

Active Immunization: Here an individual immune system is stimulated by administering killed or live attenuated products derived from micro-organisms.

Passive immunization: Here, the antibodies formed in one individual are transferred to another.

DNA vaccination: Here, DNA plasmids encoding genes required for antigen production are transferred

to an individual.^[5]

Characteristics of an effective vaccine^[6]

- Safety
- Protectivity
- The ability to provide sustained protection
- The ability to produce neutralizing antibodies
- Stimulation of protective t-cells.

Practical considerations like

- (a) Cost-effectiveness
- (b) Biological stability
- (c) Access
- (d) Minimum contraindications and side effects

Indication for periodontal immunotherapy^[6]

- Severe periodontal disease with loss of bone around teeth
- Inflammation and association with oral bacterial infection below gum line
- Exacerbated diabetes and CVD
- Where mouth rinses don't work

History of periodontal vaccines^[6]

In the early twentieth century, three periodontal vaccines were employed:

- Pure cultures of streptococcus and other organisms
- Autogenous vaccines
- Stock vaccines

Examples include Vancott's vaccine and Inava endocarp vaccine.

Mechanism of action^[6]

Types of periodontal immunization

Active immunization

- Whole bacterial cells
- Sub unit vaccines
- Synthetic peptides as antigens

Passive immunization

- (a) Murine monoclonal antibody
- (b) Plantibodies

Genetic immunization

- Plasmid vaccines
- Live, viral vector vaccines

Active immunization

Whole cells

The entire cell with its components is inoculated into a host to bring about active immunization

(a) Klausen; 1991^[7] have shown that levels of serum antibodies to both whole cells and partially purified fimbriae from *P. gingivalis* were elevated in rats immunized with *P. gingivalis* cells.

(b) Kesavalu; 1992^[8] observed protection against invasion, but no colonization against *P. gingivalis* in a mouse chamber model by immunization with either killed heterologous invasive or non-invasive *P. gingivalis* strains. The immune response to whole cells or selected envelope component did not completely abrogate lesions, but eliminated mortality.

P. gingivalis, *Tannerella forsythia*, *Treponema denticola* have been consistently and strongly associated with progression of disease, suggesting that these four bacterial species may be the major pathogens of periodontitis in human.^[9]

P. gingivalis has emerged as the leading candidate pathogen in the development of chronic periodontitis. It is a gram-negative, non-spore/forming, nonmotile, assacharolytic, obligate anaerobic coccobacillus.^[10]

The virulence factors of *P. gingivalis* which have been used as subunits for the development of active immunization are:^[11]

- (a) outer membrane protein,
- (b) gingipains,
- (c) fimbriae and
- (d) heat shock protein.

Outer membrane protein

It was seen that transcutaneous injection of 40 kDa of outer membrane protein (OMP) inhibits co-aggregation of outer membrane protein (OMP) inhibits co-aggregation of *P. gingivalis* with *Streptococcus gordonii*.

This also can be used for vaccine development for passive immunization. Polyclonal anti-40 kDa.. OMP antibody exhibited potentially protective, complement-mediated bactericidal effect.¹²

Gingipains^{[13],[14]}

Coined by Travis and Colleague

These are cysteine proteinases which cleave synthetic and natural substrates after arginine or lysine residues and are referred to as arginine gingipain (Rgp) and lysine gingipain (Kgp), respectively.^{[13]-[16]} HRgpA (90kDa) and RgpB(50kDa) products of 2 distinct but related genes *rgpA* and *rgpB* respectively are specific for Arg-Xaa peptide bonds. Kgp,a product *kgp* genes is specific for Lys-Xaa peptide bonds.

HRgpA and Kgp are non-covalent complexes containing separate catalytic and adhesion/hemagglutinin domains while RgpB has only catalytic domain.^[17]

HRgpA and RgpB induce vascular permeability enhancement through activation of kinin pathway and activate the blood coagulation system which respectively potentially associated with GCF production and progression of inflammation leading to alveolar bone loss in periodontitis site.Kgp is most potent fibrinogen degrading enzyme of 3 gingipains in human plasma and involved in bleeding tendency at diseased gingival.

They are expressed on the outer membrane of *P. gingivalis* . Rgp and Kgp are key determinants in the growth and virulence of *P. gingivalis* .^[17]

Gingipains vaccines are mainly DNA vaccines.DNA vaccines induce both humoral and cellular immunity.^[13]

Fimbriae

Fimbriae from *P.gingivalis* play an important role in adhesion to oral tissue and are highly immunogenic. Chan 1995 demonstrated that immunization with purified outer membrane protein reduces the activities of collagenase, gelatinase and cysteine proteases in gingival tissue.^[18]

These are cell surface structure components and serve as a critical antigen. These are the most advanced immunogens.

Functions of fimbriae are the following:

- (a) Adherence to host.
- (b) Invasion of oral epithelial cells and fibroblasts.
- (c) Modulation of inflammation by release of interleukin (IL)-1 α , IL-1 β , tumor necrosis factor (TNF)- α .^[19]

Currently, five *P. gingivalis* fimbrial types (I-V) have been described based on their antigenicity. However, a vaccine based on one fimbrial type may be strain specific and hence ineffective against other *P. gingivalis* strains of different fimbrial types.

GroEL heat shock protein

Heat shock proteins have an important role in inflammatory mechanism, autoimmune disease and atherosclerosis. Homologues of specific stress protein families have been demonstrated to be present in oral bacteria including *Fusobacterium nucleatum*,*Prevotella intermedia*, *Prevotella melaninogenica*, *A. comitans* and *P. gingivalis* .Rats immunized with *P. gingivalis* HSP60 showed decrease in bone loss induced by infection with multiple periodontopathic bacteria.Significant association between HSP90 concentration and microbial colonization has been observed.^[20]

Hemagglutinins

Non-fimbrial adhesion hemagglutinin B (HagB) is a potential vaccine candidate. Hemagglutinin mediates bacterial attachment and penetration into the host cells, as well as agglutinates and lyses erythrocytes to intake heme, an absolute requirement for growth.Mice intragastrically inoculated within a virulent strain of *Salmonella typhimurium* expressing HagB gene mounted both systemic and mucosal antibody response and this response could be boosted indicating that a memory T-cell or B-cell response was induced.Furthermore, rats immunized subcutaneously with recombinant HagB were protected against periodontal bone loss induced by *P. gingivalis* strain ATCC 33277.Human antibody against hemagglutinin should be ideal for practical use in immunotherapy.^[21]

Synthetic peptide

These require synthesis of linear and branched polymers of 3-10 amino acids based on known sequence of microbial antigens.Such peptides are weakly immunogenic by themselves and need to be coupled to large proteins to induce antibody response.

Two ways of developing synthetic peptide vaccine are as follows :

- (i) By deduction of protein sequence of microbial antigens from RNA sequence data.
 - (ii) By testing overlapping peptides and by mutational analysis.
- Genco 1992 found that synthetic peptides based on protein structure of fimbrin inhibit adhesion of Pg to saliva coated hydroxyapatite crystals in vitro. [22]

Mapping the adhesion, T-cell and B-cell epitopes are essential for investigating synthetic vaccines. Since IgG and secretory IgA may play a role in preventing bacterial adhesion to salivary glycoprotein or mucosal receptors, adhesion epitopes are also indispensable to the immune response elicited by synthetic peptide vaccine. Synthetic peptide based on the protein structure of fimbrillin inhibit the adhesion of P.gingivalis to saliva coated hydroxyapatite crystals. [1]

III. Passive immunization

Passive immunization is short lived because host does not respond to immunization and protection lasts only as long as injected antibody persists.

Antigens are injected into vector that produce antibodies. These antibodies when inoculated into host bring about passive immunization. [6]

Passive immunization can be brought about in two ways:

- (i) Murine monoclonal antibodies
- (ii) Plantibodies

(i) Murine monoclonal antibodies

In this, the antibodies are obtained by inoculating the antigens into mice. These antigens are then injected into host that brings about passive immunization. Booth, 1996 developed a murine monoclonal antibody to P.gingivalis that prevented recolonization of deep pockets by this pathogen in periodontitis patients. [23] Monoclonal antibodies have been used for passive immunization against periodontitis. Passive immunization with monoclonal antibody shown to prevent selective colonization by P.gingivalis in humans. It is important to realize that for vaccination to be successful, it should limit the transmission and/or intraoral dissemination of periodopathic bacteria and it would appear advantageous for an effective vaccine to induce immunity at three levels. [1]

1. Local mucosal secretory IgA
2. Local draining lymph nodes
3. Circulating specific T and B-cell responses

(ii) Plantibodies

Molecular biological techniques to express bacterial or viral antigens in plants which could be used as orally administered vaccines. Ma; 2000 characterized a secretory IgG antibody against streptococcus mutans, produced in transgenic plants. [24]

Advantages

- (a) Higher stability
- (b) Higher degree of functionality
- (c) Protection against colonization by S.mutans

Probiotics defined as live microorganisms administered in adequate amounts conferring beneficial health effect on host. Restoring the decreased number of beneficial bacteria via probiotics might be of considerable interest in the treatment of the plaque related periodontal diseases. In 1954 the first attempt was made in Russia on the use of periodontitis. Inoculation of Streptococcus sangius inhibits growth of periopathogens, P.gingivalis, A.comitans and T.forsythia. [25]

IV. Genetic Immunization

Gene therapy is insertion of genes into an individual cells and tissues to treat a disease. The strategy involves genetic engineering or recombinant DNA technology. [1] There are two types:

(i) Plasmid Vaccines

DNA does not have ability to grow whereas plasmids have ability to grow. With this ability of the plasmids, they are fused with DNA of a particular pathogen of interest and inoculated in an animal for production of antibodies. This is then transferred to the host for immunization.

Disadvantages – In some cases it may lead to oncogenesis.

Example :- Salivary gland of mouse, when immunized using plasmid DNA encoding the P.gingivalis fimbrial gene, produces fimbrial protein locally in the salivary gland tissue, resulting in production of specific salivary immunoglobulins IgA, IgG serum IgG antibodies. This secreted IgA could neutralize P.gingivalis and limit its ability to participate. [6]

(ii) Live, viral vector vaccines

Variety of infectious but non-disease causing DNA or RNA viruses or bacteria have been engineered to express the proteins of a disease producing organism. The vector enters the body cells where the proteins are generated and then induce humoral or cellular immune responses. [6]

Methods of DNA Vaccine administration

- (i) Intranasal
- (ii) Intramuscular
- (iii) Gene gun

Advantages of DNA vaccines

- (i) Ease of manipulation
- (ii) Stable by nature
- (iii) Simple
- (iv)

V. Human Periodontal Vaccines

Three types of vaccines are employed for the control of periodontal disease [26] :-

- (i) Pure culture of streptococci and other oral organism.
- (ii) Autogenous vaccine
- (iii) Stock vaccine such as vancott's vaccine or Inava Endocarps Vaccines.

Autogenous Vaccine

Prepared from dental plaque samples of patients with destructive periodontal disease. Plaque samples are removed from diseased site. They are sterilized by heat or by immersion in iodine or formalin solution and reinjected into same patient either locally or systemically.

Stock Vaccine

Prepared from stock microbial stain.

VI. Hurdles in Periodontal Vaccine development

Periodontal disease is a multifactorial disease. Elimination of certain bacteria may not prevent the onset and progression of disease. Problems such as maintaining adequate levels of antibodies for long enough, generating T-cell mediated response, multiple antigenicities of various microorganisms remain to overcome. Incidence of toxic reaction to inactivated whole cell vaccines. [6]

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 [27], CPS [28] and heat shock protein (HSP). [29]-[30] 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.

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 periodontopathic 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 multiple pathogenic bacteria. 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 potential versatile vaccine in the future. Interestingly, patients whose sera recognised both *P.gingivalis* HSP peptide number 19 and cross-reactive human HSP peptide number 19 demonstrated a significantly higher level of alveolar bone, strongly suggesting an immune-modulating role for the cross-reactive peptide number 19 in periodontitis. [31],[32]

VII. Conclusion

Vaccination may be an important adjunctive therapy to mechanical debridement in human to prevent colonization of periopathogens. When present in subgingival plaque as an undisturbed biofilm, specific antibodies restrict the progression of disease by blocking penetration into gingival tissue and neutralizing key virulence factors associated with acquisition of essential nutrients.

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