

Periodontal Vaccine-An Insight

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Abstract: Periodontology is advancing in the field of technological advancements in diagnostic as well as treatment and preventive strategies. Periodontal vaccine is also the brain child of one such innovative thought Vaccination is a process that induces specific immune resistance to a bacterial or viral infectious disease. Vaccines have prevented several infectious diseases for many years, and are still being investigated. Regarding a vaccine against the periodontal disease, the complexity of the periodontopathic bacteria might be a problem in determination of Antigens. Among some 300 species of bacteria involved in subgingival plaque, 5-7 species have been implicated in the etiology of periodontitis but one or two species; *P.gingivalis* or *B. forsythus* might play an important role as primary pathogens. Vaccination accomplished can be active immunization, passive immunization or DNA vaccination, made from the antigenic epitopes in periodontopathic bacteria. This paper intends to provide an insight into the mechanism and bottlenecks in the future research directions.

Keywords: Vaccines, Plantibodies, Gingipains, Porphyromonas Gingivalis, DNA vaccines

I. Introduction

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). But it was done in complete ignorance of viruses (or indeed any kind of microbe) and immunology. It was not until the work of Pasteur 100 years later that the general principle governing vaccination emerged that altered preparations of microbes could be used to generate enhanced immunity against the fully virulent organism. Thus, Pasteur's dried Rabies-infected rabbit spinal cords and heated anthrax bacilli were the true forerunners of today's vaccines, while Jenner's animal-derived (i.e. heterologous) vaccinia virus had no real successors. 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. This strategy may work wonders but it also needs more further research to make it a feasible reality like other vaccines.¹

Type Of Vaccinations

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 individual²

Key features of a successful vaccine

It should be safe to administer

It should induce the right sort of immunity

Vaccine should be effective against the particular infectious agent and prevent the disease

It should be stable and have a long shelf life.

Vaccines should be affordable by the population at which they are aimed

Relevance In Periodontics

A substantial number of bacteria (exceeding 300 species) appear to be involved in subgingival plaque. Among these five to seven species have been implicated in the etiology of periodontitis, but one or two species, *P. gingivalis* or *B. forsythus*, might play an important role as primary pathogen³ Furthermore, regarding antigenic epitopes in periodontopathic bacteria some researches have demonstrated that epitopes were shared among gram negative bacteria, possibly because of the polyclonal B-cell activating properties of lipopolysaccharide. Several investigations regarding the humoral immune response in periodontitis patients have been performed. Chen et al. 1991, reported that 24 out of 36 rapidly progressive periodontitis patients were seronegative to antigens of *P. gingivalis* and both serum antibody titers and avidity against *P. gingivalis* antigens from 24 seronegative patients

increased significantly following only scaling and root planning. Similar observations were made by Sjostrom K et al. 1994, on groups of subjects with rapidly progressive periodontitis tested for antibodies against *A. actinomycetemcomitans*. Ten of 12 patients became seropositive from seronegative following scaling and root planning, and the post-treatment sera enhanced stronger capacity of phagocytosis and killing than the pretreatment ones did. Periodontal therapy, scaling and root planning, could elicit the humoral immune response in seronegative patients, resulting in seroconversion and production of effective antibodies. This might be due to bacteremia provoked by treatment⁴. These observations suggest that the development of vaccine against periodontitis might be possible and that utilization of it could be an effective method for control and prevention of periodontal disease. So the three strategies can be

1. To decrease the incidence of Periodontal disease
2. 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 pneumoniae* infection.
3. For bacteria which are capable of evading host immune responses and invading the tissue
4. *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.
5. 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.

History⁵

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.

Classification

Active immunization

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

Passive immunization

- (a) Murine monoclonal antibody
- (b) Plant antibodies

Genetic immunization

- Plasmid vaccines
- Live, viral vector vaccines

***Porphyromonas Gingivalis*- The Primary Target**

P. gingivalis has emerged as the leading candidate pathogen in the development of chronic periodontitis. It is a gram-negative, non-spore/forming, nonmotile, saccharolytic, obligate anaerobic coccobacillus.⁶ The virulence factors of *P. gingivalis* which have been used as subunits for the development of active immunization are⁷

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

Outer membrane protein

Gingipains^{8,9}

The term was 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. ¹⁰HRgpA (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*.¹¹Gingipains vaccines are mainly DNA vaccines.DNA vaccines induce both humoral and cellular immunity.

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.¹²These 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, tumor necrosis factor (TNF)-alpha

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.^{13,14}

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.¹⁵

Experimental Models

Humans may not be used as experimental subjects in studies in periodontopathic bacteria. Accordingly, the prevention of colonization, adhesion and bacterial invasion has been studied in various animal models. Non human primates & humans are similar in both periodontal structure & microflora composition. However, ligatures must be tied around the teeth to elicit periodontitis in non human primates, because it is difficult to colonize the oral cavity with *P.gingivalis* and establish periodontal lesions. Studies in non-human primate models using ligature-induced experimental periodontitis suggest that antibody responses by active immunization against *Porphyromonas gingivalis* can safely be induced, enhanced, and obtained over time. There are some advantages in using rats for adhesion experiment. Since rats resemble humans in periodontal anatomy and bacterial composition, bone loss can be evaluated.^{16,17} Furthermore, *P.gingivalis* quickly colonizes the rat oral cavity and induces bone loss. The invasion ability of bacteria has been investigated using the subcutaneous abscess model in mice and the subcutaneous chamber model in mice and rabbits. Kesavalu et al.

studied active immunization using whole cells or selected cell envelope components and suggested that the murine model would be useful for investigating the tissue-destructive components of *P.gingivalis*¹⁸

Bottlenecks In Periodontal Vaccines

First of all the complexity of the periodontopathic bacteria might be a problem as a substantial number of bacteria appear to be involved in periodontal disease. So determination of an antigen for the vaccine may pose as a major limitation. Some more of the serious complications may stem from the vaccine or from the patient. Vaccines may be contaminated with unwanted proteins or toxins, or even live viruses. Supposedly killed vaccines may not have been properly killed; attenuated vaccines may revert to the wild type. The patient may be hypersensitive to minute amounts of contaminating proteins, or immuno-compromised, in which case any living vaccine is usually contraindicated. 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, CPS and heat shock protein (HSP).^{19,20} 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.

Future Research Directions

Subunit vaccines have been developed based on viral and bacterial peptides or plasmid vectors. In fact, DNA vaccines that were first described less than five years ago have already progressed to phase I clinical trials in healthy adult humans. They might induce immunity to numerous agents, including periodontopathic bacteria, following confirmation of their safety. DNA vaccines offer several distinct advantages. Firstly, DNA vaccines can be manufactured more easily than vaccines consisting of an attenuated pathogen, an outer or internal protein or a recombinant protein. The second advantage is that since DNA is stable by nature and resistant to extremes of temperature, storage, transport and distribution, it might be highly practical. The third advantage of vaccination with DNA is the simplicity of changing the sequences encoding antigenic proteins by means of mutagenesis and of adding heterologous epitopes by basic molecular genetics. The immunogenicity of the modified protein may be directly assessed following an injection of DNA vaccine. DNA vaccination has been studied in animals. Most of the investigated pathogens have been viruses, for instance bovine herpes virus, hepatitis B virus, hepatitis C virus, herpes simplex virus, human immunodeficiency virus-1, influenza virus and lymphocytic choriomeningitis virus. Further more, some pathogenic bacteria have also been investigated. Lowrie et al. demonstrated that expression of the gene for a single mycobacterial antigen (*Mycobacterium leprae* hsp 65) in adult BALB/c mice caused substantial cell mediated protection against challenge with *Mycobacterium tuberculosis*. Some genes from periodontopathic bacteria have been cloned and these genes could be used as a vaccine to protect against periodontitis^{21,22}

II. Conclusion

Different forms of active and passive immunization methods have been tried. Although, most of these studies have yielded encouraging results, none of these modalities of immunization have been able to be incorporated as a sole or complete vaccine against periodontal disease for use in the human population as yet. Thus, the current status of our understanding in the field of vaccines against periodontal disease is incomplete but extensive research in this direction may hold a promising future in development of periodontal vaccines. We can hope for a day when the periodontal vaccine also becomes a part and parcel of our universal immunisation schedule.

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