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Periodontal Vaccine - A Novel Emerging Therapeutic Modality for Periodontal Diseases

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ABSTRACT

Epidemiological studies reveal that more than two thirds of the world's population suffers from one of the chronic forms of periodontal disease. Due to its high prevalence rate, this disease has created an interest in finding a solution in the form of vaccines. Vaccines are generally prophylactic, i.e. they ameliorate the effects of future infection. It had long been recognized that individuals who recovered from a disease developed subsequent resistance to the same. In late eighteenth century, Edward Jenner developed and established the principle of vaccination using the cross protection conferred by cowpox virus, which is non pathogenic in humans. Vaccination is the best known and the most important application of immunological principles to human health. The focus of this paper is to review the literature on primary role of periodontal vaccine that would eradicate the global periodontal disease burden along with the disease associated morbidity in humans.

Keywords: Active immunization, Epitope, Passive immunization, Periodontitis, Porphyromonas gingivalis.

INTRODUCTION

Periodontitis is a disease of multifactorial origin, "an inflammatory disease of the teeth caused by specific microorganisms or group of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both."[1]

The current concept on the etiology of periodontitis considers three groups of factors that determine whether active periodontitis will occur in patients:

1.) Susceptible host
2.) Presence/ Absence of pathogenic species
3.) Proportion of "beneficial bacteria." The current treatment of periodontitis is nonspecific and is centered on the removal of supragingival and subgingival plaque by mechanical debridement and surgical procedures.

Need for Vaccine

Periodontal disease due to its high prevalence rate, has created an innovative interest to find a solution in the form of vaccine.[2] Vaccination is induction of immunity by injecting a dead or attenuated form of pathogen.[3] The complexity of the periodontopathic bacteria might be a problem in the determination of antigens, thus complicating the development of periodontal vaccine.

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History

Vaccine is the name applied generally to a substance of the nature of dead or attenuated living infectious material introduced into the body with the object of increasing its power to resist or get rid of a disease. (Roderich, 2004)

Vaccines are generally prophylactic, i.e. they ameliorate the effects of future infection.

- British physician Edward Jenner, who in 1796 used the cowpox virus to confer protection against smallpox. (The first vaccine, named after Vaccinia, the cow pox virus was pioneered by Jenner 200 years ago). It was the first deliberate scientific attempt to prevent an infectious disease (small pox).

In 1885, the French microbiologist Louis Pasteur and Emile Roux developed the first vaccine against rabies.

In the early 20th century, three periodontal vaccines were employed:

1) Pure cultures for streptococcus and other microorganism
2) Autogenous Vaccines
3) Stock vaccines.

Examples include:

Vancott's vaccine, Goldenberg's vaccine or Inava endocarp vaccine.

The complexities in the etiopathogenesis of the periodontal diseases have been the prime obstacle in the hunt for vaccine. Till date, no preventive modality exists for periodontal disease and treatment rendered is palliative. 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 pathogen. These techniques have the

capability to provide us with new targets for antimicrobial drugs and vaccines.[4]

Pathogenesis Of Periodontitis

Periodontitis is a disease of multifactorial origin with interaction among host, micro-organisms and environmental factors which includes genetic factors as well.

Over 300 species of micro-organisms have been found to colonize the periodontal tissues, of which the following are considered to be the primary pathogens causing periodontitis:[1,5,6]

- Porphyromonas gingivalis
- Aggregatibacter actinomycetemcomitans
- Tannerela forsythensis

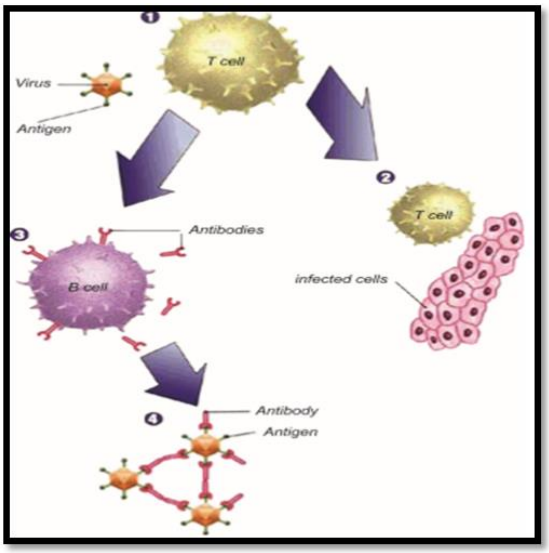

Figure 1: Immune Response

These bacteria produce an array of antigens that stimulate pro- inflammatory cells and leads to the production of a wide variety of cytokines. These antigens may stimulate Th1 or Th2 cells. Antigens are taken up by dendritic cells and presented to CD-8 or CD-4 cells along with MHC antigens.[7]

CD-8 cells → Th 1 response → CMI → Pro inflammatory

CD-4 cells → Th 2 response → Ab response → Protective

The host produces anti-bacterial substances such as defensins, cathelicidins and saposins, which protect the host tissues from bacterial products and forms the first line of defense [Figure 1]. However, sometimes these are inactivated by the bacterial virulence factors. Once bacteria break this barrier, cytokines are produced, which can be both proinflammatory and anti-inflammatory. Production of inappropriate cytokines results in periodontitis.[7]

Various Types of Vaccines

Vaccines may be synthetic or natural, Monovalent or polyvalent. In practice, this means isolating or creating an organism, that is unable to cause full blown disease, but that still retains the antigenic components responsible for inducing the host's immune response.

One way is to kill the organism using formalin; these are called "inactivated" or "killed" vaccines. Second way is to use only the antigenic part of the disease causing organism, for example the capsule, the flagella, or part of the protein cell wall; these vaccines are called "acellular vaccines."[8]

A third way of making a vaccine is to "attenuate" or weaken a live microorganism by aging it or altering its growth conditions. However, these vaccines also carry the greatest risk because they can mutate back to the virulent form at any time, resulting in induction of the disease rather than protection against it.[8]

"Toxoids" are vaccines made from toxins, which are adsorbed onto aluminum salts to decrease their harmful effects and is administered with an "adjuvant" which can have effects on antigen delivery, immune modulatory cytokines, and antigen-presenting cells.[9]

Experimental Models for Vaccine Development

Humans could not be used as experimental subjects in studies of periodontal vaccine development against periodontopathic bacteria. Non-human primates and humans are comparable in both periodontal structure and microflora composition. Nevertheless, ligatures must be tied around the teeth to elicit periodontitis in non-human primates, because it is not easy to colonize the oral cavity with P. gingivalis and establish periodontal lesions.

McArthur et al.[10] suggested that the squirrel monkey could be used as a model for studying the parameters of black-pigmented anaerobic rods colonization in gingival crevices. However, the mechanisms of bacterial retention around ligated teeth are totally different from those of adhesion around the teeth or gingival tissue.

Persson et al.[11] investigated the constituents of subgingival microflora and immune reactions (antibody titers and avidities against P. gingivalis) in experimental Macaca fascicularis periodontitis and concluded that M. fascicularis was a useful model for testing and developing vaccines for periodontitis.

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.[12,13] Furthermore, P. gingivalis quickly colonizes the rat oral cavity and induces bone loss.

Kesavalu et al.[14] 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.

Primary Goals of a Successful Vaccine

- It should be safe to administer

- It should induce the right kind 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 general population.

Types of Periodontal Immunization

Active immunization

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

Passive immunization

- Murine monoclonal antibody
- Plantibodies.

Genetic immunization

- Plasmid vaccines
- Live, viral vector vaccines.

Most experiments on immunization of periodontitis, despite its poly-infectious nature, have been directed towards a very limited number of antigenic components of a single specific pathogen, either P. gingivalis or A. actinomycetemcomitans.

Principal Antigenic Model for Vaccine Development

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, Treponema denticola, and T. forsythia, A. actinomycetemcomitans, P. intermedia, C. rectus, and Fusobacterium nucleatum, which are predominantly cultivated in sites demonstrating disease activity

P. gingivalis and its antigenic components: P. gingivalis, a Gram-negative non-motile pleomorphic rod and obligate anaerobe is an aggressive and opportunistic periodontal pathogen producing a series of virulence factors. [Figure 2]

Virulence factor	Antigenic structure	Mode of action
Lipopolysaccharide	Polysaccharide chain-O specific antigen, core, polysaccharide, lipid A	Endotoxic activity, stimulates host, inflammatory response, significant, immunological activity
Capsule	Polysaccharide, heteropolymer-six, serotypes (KI-K6)	Antiphagocytic activity
Fimbriae	6 fimA genotypes (type I-V, Ib) fim A type IV-most virulent	Adhesion and invasion to epithelial cells
Extracellular proteolytic enzymes	Cysteine endopeptidases	Proteolytic activity

Figure 2: Antigenic components of P. Gingivalis

➢ Active Immunization Against P. Gingivalis Components

1) Whole Cells: Here, the entire cell with its components is inoculated into a host to bring about active immunization.

- Klausen; 1991,[15] 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 and that the activities of collagenase and cysteine proteinases in gingival and periodontal tissues were decreased.
- Kesavalu; 1992,[16] 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.

Active immunization with whole cells might induce exaggerated inflammatory responses in the host. It was found that bone density was significantly decreased in ligated teeth with nonhuman primates immunized with whole cell antigens of P. gingivalis.

2) Outer Components: In this type, a part of the bacterial cell is used for immunization. Either the outer component or the fimbriae is used.Fimbriae from P. gingivalis play an important role in adhesion to oral tissues and are highly immunogenic.[17]

- Evans; 1992 reported that immunization with highly purified P. gingivalis fimbrial preparations as well as whole cells and soluble antigens of P. gingivalis 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.
- Bird; 1995 showed that immunization of experimental animals with an outer membrane preparation isolated from P. gingivalis induces elevated levels of specific antibody and provides protection against the progression of periodontal disease.
- Chen; 1995 demonstrated that immunization with a purified outer membrane protein reduces the activities of collagenase, gelatinase and cysteine proteases in gingival tissues. However, it did not prevent periodontal bone loss.

3) Gingipains: Gingipains are produced by Porphyromonas gingivalis, a major causative bacterium of adult periodontitis.

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. [Figure 3]

Rgp and Kgp are key determinants in the growth and virulence of P. gingivalis.

Gingipains are of two types:

a) Gingipain –R : which hydrolyse Arg-Xaa bond
b) Gingipain- K: which hydrolyse Lys-Xaa bond

GENE	GINGIPAIN	Enzymes
rgpA gene	gingipain R with hemagglutinin/adhesion domains	1) RgpA(cat) 2) mt-RgpA 3) HRgpA
rgpB gene	Gingipain R without adhesion domains	1) RgpB 2) mt-RgpB
kgp	gingipain K	1) kgp

Figure 3: Gingipains

a) RgpA(cat) is a form of the catalytic domain alone and is made by either aberrant proteolytic processing of the initial protein or by an interrupted transcription process
b) mt-RgpA(cat): the catalytic domain is modified with lipopolysaccharides.
c) HRgpA stable complex of the catalytic domain and a hemagglutinin/adhesion domain(s)

Therefore, it is likely that virulence of P . gingivalis can be attenuated by inactivation of Rgp and Kgp with proteinase inhibitors of antibodies specific to Rgp and Kgp.

4) Synthetic Peptides: These require synthesis of linear and branched polymers of 3-10 amino acids based on the known sequences 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 vaccines are as follows:

- By deduction of the protein sequence of microbial antigens from RNA sequence data.
- By testing overlapping peptides and by mutational analysis.

Advantages of synthetic peptide are:

- Safe
- Cheap
- Easy to store and handle
- Ideally suited for specific targeting, which is not possible with classical vaccines.

Genco; 1992 found that synthetic peptides based on the protein structure of fimbrillin inhibit the adhesion of Pg to saliva-coated hydroxyapetite crystals in vitro.[18]

5) Fimbriae: Fimbriae from P. gingivalis are cell surface structure component that plays an important role in adhesion to oral tissue and are highly immunogenic.[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.

6) 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]

7) Hemagglutinin: 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 avirulent 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.

➢ **Passive immunization against P. gingivalis**

This approach employs preformed antibodies administered to "at risk" individuals or to individuals during "at risk" intervals to interfere with microbial pathogenic processes. Here, the antigens are injected into a vector that produces antibodies. These antibodies, when inoculated into a host, bring about passive immunization.

➢ **It can be done in 2 ways:[21]**

- Murine monoclonal antibodies
- Plantibodies.

Ma et al. characterized a secretory IgG antibody produced in transgenic plants.[22]

This antibody was more stable and exhibited a higher functional affinity than the native antibody and provided protection against Streptococcus mutans colonization in humans.[22]

Okuda et al.[23] reported that repeated passive immunization with rabbit antiserum to P. gingivalis hemagglutinin into the oral cavities of the hamsters reduced colonization by exogenous P. gingivalis in the periodontal region. Furthermore, passive immunization with monoclonal antibodies against P. gingivalis effect selectively prevents recolonization by this organism in humans.

➢ **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. [24]

There are two Types:

1) Plasmid vaccines.
2) Live, viral vector vaccines.

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.

Live, viral vector vaccines: Variety of infectious but non-disease causing DNA or RNA viruses or bacteria has 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.[25]

Methods of DNA Vaccine Administration:[26]

1. Intranasal.
2. Intramuscular.
3. Gene gun.

Advantages of DNA Vaccines:

1. Ease of manipulation.
2. Stable by nature.
3. Simple

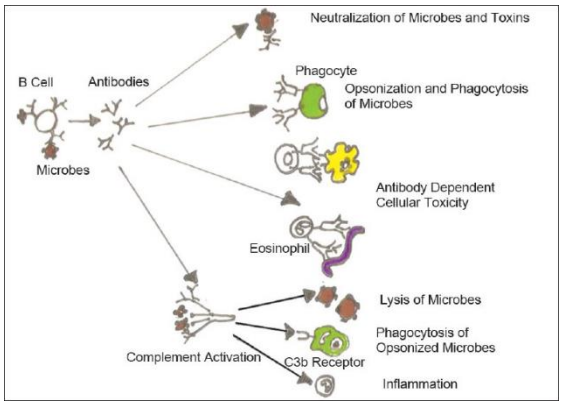

Figure 4: Mechanism of Action of Vaccine

The following results have been achieved by immunization, which indicate a positive preventive response:

- ✓ Potentiation of humoral immune response.
- ✓ Increased mucosal immunity, viz. increased levels of IgA and IgG2.
- ✓ Decreased levels of P. gingivalis and other species in the subgingival flora by inhibiting invasion into tissues and colonization of the periodontal tissues.
- ✓ Decreased bone loss was observed in the immunized group as compared to the group not immunized and this has been associated with decreased levels of prostaglandin E2 (PGE2) in the gingival crevicular fluid (GCF).[27]

Human Periodontal Vaccine

Three types of vaccines were employed for the control of periodontal diseases.[28]

These include the vaccines prepared from:

- pure cultures of streptococci and other oral organisms,
- autogenous vaccines and
- stock vaccines such as
- Van Cott's vaccine
- Goldenberg's vaccine or Inava Endocarp vaccine.

Autogenous vaccine

These are prepared from dental plaque samples of patients with destructive periodontal diseases.
Plaque samples are removed from the diseased site. They are sterilized by heat or by immersion in iodine or formalin solution and re-injected into the same patient either locally at the site or systemically.

Why success is still elusive for humans for periodontal vaccine?

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:

1) Complexity and uncertainty of the different forms of periodontal diseases.
2) To accurately differentiate between primary colonizers and secondary invaders.
3) The relative difficulty in growing and identifying 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.
4) 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.
5) Variations in disease state and chronicity of the diseases.
6) Difficulty in clinically detecting and quantitating active periodontal disease.
7) 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.
8) The nonfatal nature of the disease.[29]

CONCLUSION

DNA vaccines that were described <5 years ago have already progressed to Phase I clinical trial in healthy humans. Although 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 the reasons include complexity and uncertainty of the different forms of periodontal diseases; difficulty in accurately differentiating between primary colonizers and secondary invaders; relative difficulty in growing

and identifying 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.Thus, the current status of our understanding in the field of vaccines against periodontal disease is not complete, but extensive research in this direction may hold a promising future in the development of periodontal vaccine.

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