PERIODONTAL VACCINES: ARTILLARY TO CONQUER MICROBES

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ABSTRACT

The periodontal disease is an inflammatory disease of tooth-supporting tissues. Its initial form, gingivitis which often remains symptomless, can progress into periodontitis leading to bad breath, oral discomfort and tooth loss. Its polymicrobial etiology is one of the major reasons for tooth loss. Main pathogens involved are gram negative anaerobic bacteria which include Porphyromomas gingivalis, Aggregatibacter actinomycetemcomitans. Present treatment options available only helps in arresting the disease to progress. It can neither cure the disease completely nor stop its recurrence. The dental plaque bacteria only starts the disease, it is the host specific inflammatory response which acts as main driven factor for disease progression. Hence main aim should be to alter the host specific response as well as to control the bacteria to progress.

Vaccines may act as potent adjunct to mechanical debridement for the treatment and prevention of periodontal disease. The polymicrobial nature of periodontitis and its immune-pathogenic complexity hinders the development of periodontal vaccines. A successful periodontal vaccine must provide protective immunity in the oral cavity.

Key words- periodontitis, immunity, vaccines.

INTRODUCTION AND BASICS OF VACCINATION

With the recent advancement in cellular and molecular biology new techniques have developed for vaccines against various infectious diseases. Vaccination is the development of immunity or resistance to infection, after a secondary response that is adequate to consider the individual immune to a subsequent infection.[1]Main step in vaccine development is to identify an antigenic component from an organism that can provide immune protection and mostly the target is on the antigens of specific bacteria and pathogens.

Types of vaccination

1. **Active Immunisation-** In this an individual immune system is stimulated by administrating killed

or live attenuated products derived from micro-organisms.[2]

2. **Passive Immunisation-** It is provided when a person is given antibodies to a disease rather than producing them through his or her own immune system.
3. **DNA Vaccination-** DNA plasmids encoding genes required for antigen production are transferred to an individual.

Essential features of an effective vaccine

- Protectivity
- Safety
- It should provide continuous protection.
- It should encourage protective t-cell
- It should produce neutralizing antibodies.
- Should be cost-effective.
- Easy accessibility.

Specific immune response

Two types of receptors are required to generate specific immune responses, the t-cell antigen receptor and the b-cell antigen receptor.

Four phases are involved in the generation of specific immunity[3]

1. Clonal selection – Selection of lymphocytes that bear receptors recognizing the specific antigen.
2. Clonal expansion – Proliferation of those lymphocytes.
3. Clonal contraction – Death of effector lymphocytes.
4. Memory – Maintenance of an expanded clone of cells that bear the specific receptors recognizing the antigen.

The individual is said to be immune until enough number of lymphocytes are present to provide protection against a specific pathogen.

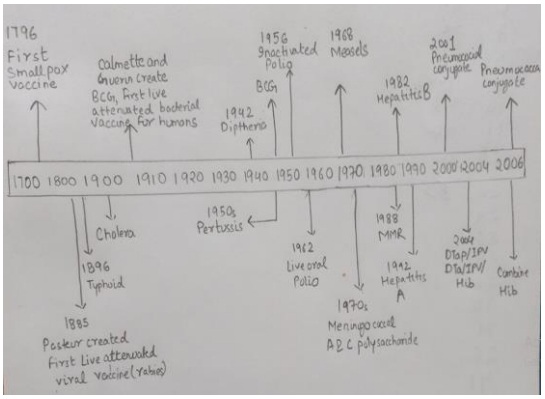
History of Periodontal Vaccines

Three periodontal vaccines were discovered in the early twentieth century-

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

Examples include Vancott's vaccine and Inava endocarp vaccine.

The most important reason for the failure was the inability to conduct appropriate controlled clinical trials and experiments.



NEED FOR THE DEVELOPMENT OF PERIODONTAL VACCINE

The main goal of periodontal vaccine is to get rid of the periodontal disease burden. Positive result of vaccine must be to improve the oral health and to decrease the need for prosthesis in the oral cavity. Recent studies link periodontitis with systemic conditions like diabetes mellitus, atherosclerosis, rheumatoid arthritis and pre term low birth weight. Therefore treatment of periodontitis is the primary step to control these systemic conditions. Along with this, the financial burden of the patient can also be reduced.[4]

Indications for immunotherapy in periodontitis

- Grave periodontal disease with bone loss around teeth.
- In diabetes and CVD patients.

- Inflammation associated with oral bacterial infection below gum line
- In cases where mouth rinses doesn't work.

More than 300 species of micro-organisms have been found to colonize the periodontium, primary pathogens causing periodontitis are-Porphyromonas gingivalis, Agregatibacter actinomycetemcomitans, Tannerela forsythensis. The bacteria produce antigens that stimulate pro-inflammatory cells which leads to the production of a variety of cytokines which stimulate Th1 or Th2 cells.[5]

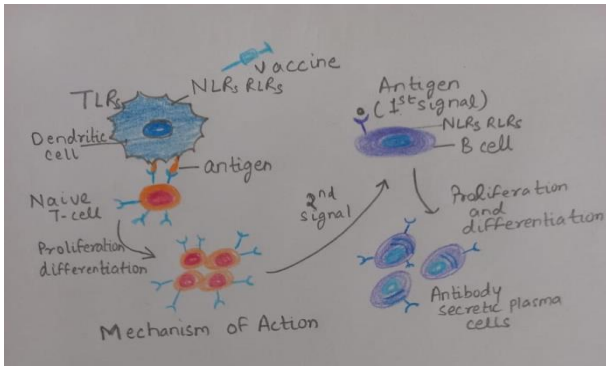
Antigens are taken up by dendritic cells and present to CD-8 or CD-4 cells along with MHC antigens.

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

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

The host forms the first line of defense by producing anti-bacterial substance such as cathelicidins saposins and defensins. But sometimes they get ineffective because of virulence factor of the bacteria. Release of inappropriate cytokines by the bacteria leads to periodontitis.[6]

PERIODONTAL IMMUNISATION-TYPES AND MECHANISM OF ACTION



- 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

Active Immunisation

Whole cells- the entire cell with its components is inoculated into a host to produce active immunization. Klausen in 1991[7] showed that levels of serum antibodies in both whole cells and partially purified fimbriae from P. gingivalis were raised in rats immunized with P. gingivalis cells.

Kesavalu in 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.

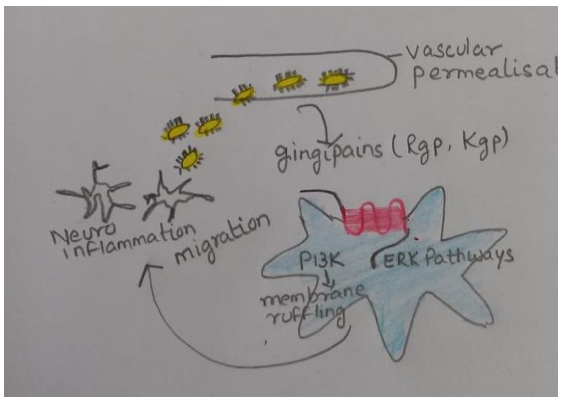
In this type of immunisation, enhanced inflammatory response is seen.

P.gingivalis is the primary pathogen for causing chronic periodontitis. It is a gram-negative, nonmotile, assacharolytic, obligate anaerobic coccobacillus[9]. . In vitro studies have showed that P. gingivalis has the ability to penetrate epithelial cells of gingiva, which protects against humoral immunity factors. P. gingivalis engages with several aspects of the immune system. Its virulence factors are used as subunits for the development of active immunization which includes-

1. **Outer membrane protein-** On observation it was noticed that transcutaneous injection of 40 kDa of outer membrane protein (OMP) prevents coaggregation of P. gingivalis with Streptococcus gondonii. Can also be used for vaccine development for passive immunization. OMP antibody

contains potentially protective, complement-mediated bactericidal effect.[10]

2. **Gingipains-** Describes as cysteine proteinases grouped into gingipains R(Rgp) which cleave synthetic and natural substrates after arginine and gingipain K(Kgp) which cleave lysine residues. Both have a haemagglutinin domain which is essential for adherence to erythrocytes.[11] HRgpA and Kgp are a non-convalent complex that includes separate catalytic and adhesion/ hemaggulutin domains while RgpB has only catalytic domain. HRgpA and RgpB promotes vascular permeability which leads to activation of kinin pathway, activating the blood coagulation system associated with GCF production and progression of inflammation which causes alveolar bone loss in periodontitis. The most potent fibrinogen degrading enzyme of gingipains in human plasma and also is involved in bleeding tendency at diseased gingiva is Kgp. Expressed on the outer membrane of P. gingivalis. Rgp and Kgp are primary determinants in the growth and virulence of P. Gingivalis. Gingipains vaccines are mainly DNA vaccine which leads to both humoral and cellular immunity.



3. **Fimbriae-** Fimbriae from P.gingivalis are highly immunogenic and play a major role in adhesion to oral tissue.[12] They are structure components of cell surface acts as a critical antigen and most advanced immunogens.

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 put forward that fimbrial protein might serve as a model of effective vaccines against periodontitis.

Chan in 1995 explained that immunization with purified omp decreases the activities of collagenase, gelatinase and cysteine proteases in gingiva. Role of fimbriae is to adhere to host, to invade oral epithelial cells and fibroblasts and to modulate inflammation by release of interleukins , tumor necrosis factor (TNF). At present, five P. gingivalis fimbrial types (I-V) have been described based on their antigenicity. A vaccine based on one fimbrial type may be strain specific which might be ineffective against other P. gingivalis strains of different fimbrial types.

4. **Heat shock protein-** Play an important role in inflammatory mechanism, autoimmune disease and atherosclerosis.

Synthetic Peptides- They need production of linear and branched polymers of 3-10 amino acids which depends on known sequence. They are weakly immunogenic by and need to be coupled to large proteins to initiate antibody response. There are two ways to develop synthetic peptide vaccines: By deduction of the protein sequence of microbial antigens from RNA sequence

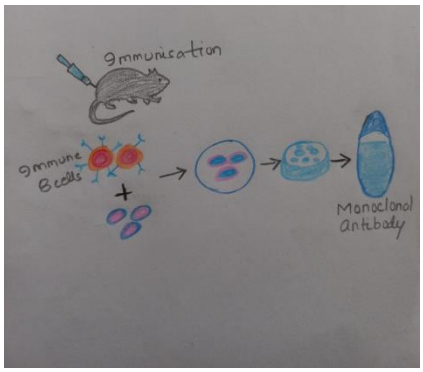
data and By testing overlapping peptides and by mutational analysis.

Advantages of synthetic peptide are -Easy to store and handle, cheap, safe, ideally suited for specific targeting. In 1992, Genco discovered that synthetic peptides based on the protein structure of fimbrillin inhibit the adhesion of Pg to saliva-coated hydroxyapatite crystals in vitro.[13]

Passive Immunisation

Its time period is short because the host does not respond to the immunization and protection lasts only as long as the injected antibody remains. The antigens are injected into a vector that releases antibodies. These antibodies, when inoculated into a host leads to passive immunization. Passive immunization can be brought about in two ways:- Murine monoclonal antibodies and Platibodies.

Murine Monoclonal Antibodies- Here the antibodies are obtained by inoculating the antigens into mice which are then injected into the host causing passive immunization. Booth et al (1996) produced a murine monoclonal antibody to P.gingivalis which inhibited the recolonization of pathogen in the deep pockets in periodontitis patients.



Plantibodies- New approach for vaccination strategies is molecular biological techniques to express bacterial or viral antigens in plants, which could be used as orally administered vaccines. Ma in 2000, identified a secretory IgG antibody against Streptococcus mutans produced in transgenic plants. Advantages- More stability, more functionality and protection against colonization by S mutans.

Genetic Immunisation

In the early 1990's, scientists had begun to look for new approaches for the production of vaccines which differs in structure. The method involves genetic engineering or recombinant DNA technology. They are of two types:

• Plasmid vaccines - Plasmids can grow where DNA cannot grow. Therefore, plasmids are fused with the DNA of a particular pathogen of interest and inoculated in an animal for the production of antibodies which is then transferred to the host for immunization. Disadvantages of plasmid vaccines are that it may lead to oncogenesis in some cases.

• Live, viral vector vaccines- 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 immunity.[14]

DNA vaccine administration includes intranasal, intramuscular and gene gun.

Advantages of DNA vaccines- Can be manufactured more easily and stable by nature.

A.actinomycetemcomitans as a target- It is another important pathogen, especially in localised form of aggressive periodontitis. Harano et al(1995) prepared an anti-serum against it and found that it blocked the adhesion of the organism to the saliva coated hydroxyapatite beads and to the fibroblast line.

BARRIER IN PERIODONTAL VACCINE DEVELOPMENT

Since periodontal disease is a multifactorial disease, elimination of certain bacteria may

not prevent the onset and progression of the disease. Complications such as to maintain adequate levels of antibodies for long enough, generating T-cell mediated response, multiple antigenicities of various microorganisms remain to overcome.

Numerous in vitro studies in animal models have proved the efficacy of these vaccines but to develop same results in human and their application in clinical scenario is the daunting next step in periodontal vaccination. Periodontal vaccines supplement scaling and root planning by promoting pathogen-specific removal of bacteria, inhibiting certain virulence factors and shifting the immune reaction from destructive over inflammation to control immunity. P. gingivalis is a key candidate for periodontal vaccines, due to its unequal large influence on the microbial community and its role in down regulation of the host immune response. Successful vaccination against the involved virulence factors may prevent the down regulation of the host's immune response and may add to oral environment. P. gingivalis is only one of many bacteria implicated in periodontitis, specific immunity to this keystone pathogen has been associated with protection against disease in animal models such as mice, rats and non-human primates.

CONCLUSION

The current treatment of periodontitis is nonspecific and is centered on the removal of plaque by mechanical debridement, sometimes surgical procedures is also involved. This ongoing therapy is costly, painful and has a variable prognosis due in part to poor patient compliance.

REFRENCES

1. Kudyar N, Dani N, Mahale S. Periodontal vaccine: A dream or reality. J Indian Soc Periodontol. 2011 Apr;15(2):115-20. doi: 10.4103/0972-124X.84378. PMID: 21976833; PMCID: PMC3183660.
2. Reid R, Roberts F. Textbook of pathology illustrated. 6th Ed. Churchill Livingstone; 2005.
3. Seymour GJ, Taylor JJ. Shouts and whispers: An introduction to immunoregulation in periodontal disease. Periodontol 2000. 2004;35:9-13. doi: 10.1111/j.0906-6713.2004.003555.x. PMID: 15107054.
4. George, V.T., George, A.K., John, S., & Thomas, A. (2015). Periodontal vaccine: A therapeutic modality on the horizon? *The Saudi Journal for Dental Research, 6,* 73-78.
5. Takei N, Carranza K. Text book of clinical Periodontology. 9th Ed. Saunders; 2005
6. Slots J, Ting M. Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in human periodontal disease: occurrence and treatment. Periodontol 2000. 1999 Jun;20:82-121. doi: 10.1111/j.1600-0757.1999.tb00159.x. PMID: 10522224.
7. Pattanshetty et al., Periodontal vaccine a review.2018:4(1);48-55
8. Kesavalu L, Ebersole JL, Machen RL, Holt SC. Porphyromonas gingivalis virulence in mice: induction of immunity to bacterial components. Infect Immun. 1992 Apr;60(4):1455-64. doi: 10.1128/iai.60.4.1455-1464.1992. PMID: 1312516; PMCID: PMC257018.
9. Micael CL and Peter LM. Oral microbiology and the immune response. In: Richard IJ. Robert BA, Martilyn LS, Donald J and Le Blanc,

editors, Oral Microbiology and st Immunology.1 ed.2006.Pg.201-2

10. Katoh M, Saito S, Takiguchi H and Abiko Y. Bactericidal activity of monoclonal antibody against a recombinant 40-kDa outer membrane protein of Porphyromonas gingivalis. J periodontal 2000;71:368-75.
11. Nakagawa T, Saito A, Hosaka Y, Ishihara K. Gingipains as candidate antigens for Porphyromonas gingivalis vaccine. Keio J Med. 2003 Sep;52(3):158-62. doi: 10.2302/kjm.52.158. PMID: 14529148.
12. Cullen PA, Haake DA, Adler B. Outer membrane proteins of pathogenic spirochetes. FEMS Microbiol Rev. 2004 Jun;28(3):291-318. doi: 10.1016/j.femsre.2003.10.004. PMID: 15449605; PMCID: PMC2666356.
13. Evans RT, Klausen B, Sojar HT, Bedi GS, Sfintescu C, Ramamurthy NS, Golub LM, Genco RJ. Immunization with Porphyromonas (Bacteroides) gingivalis fimbriae protects against periodontal destruction. Infect Immun. 1992 Jul;60(7):2926-35. doi: 10.1128/iai.60.7.2926-2935.1992. PMID: 1351883; PMCID: PMC257256.
14. Barry MA, Johnston SA. Biological features of genetic immunization. Vaccine. 1997 Jun;15(8):788-91. doi: 10.1016/s0264-410x(96)00265-4. PMID: 9234514.