Periodontal Vaccines

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Abstract
Periodontitis is an infectious disease caused by predominantly gram-negative, anaerobic bacteria like P. gingivalis, A. actinomycetemcomitans, T. denticola and T. forsythus etc. Various immunization approaches both as active and passive immunization, against periodontal pathogens have been explored either using the whole microorganism or their specific virulence factors. Non-human primate and other study models have demonstrated raised production of specific antibody titers against various antigens without any recognizable systemic side-effects. But, the current status of our understanding in the field of vaccines against periodontal disease is incomplete. Ongoing research & collaborative efforts can result in development of functional periodontal vaccine for human use in future.

Keywords: Periodontal vaccine, Active immunization, P. gingivalis, A. actinomycetemcomitans, Gingipains

Introduction
The current concept of etiopathogenesis of periodontal disease includes a multifactorial model in which the essential components for the disease causation includes host-associated factors, genetic predisposition, immune system dysfunction and environmental factors, such as the presence of virulent periodontal pathogens (bacteria or viruses) in the form of dental biofilm. Hence, any intervention to arrest or prevent the progression of periodontal disease would include combination approaches, including that of host immune modulation and pathogen-specific approaches. Periodontal pathogens associated with periodontitis predominantly are gram-negative, anaerobic bacteria namely P. gingivalis, A. actinomycetemcomitans, T. denticola and T. forsythus etc. Thus, various immunization approaches both as active and passive immunization, against periodontal pathogens have been explored either using and the whole organism or specific virulence factors.

Antibodies are produced by plasma cells differentiated from B cells. B lymphocytes are classified into two major subsets, B-1 and B-2 (conventional). B-1 cells are the primary source of natural antibody, and the antibody is polyreactive, weakly autoreactive, and reactive with many common pathogen-associated carbohydrate antigens such as lipopolysaccharide and phosphoryl choline. B-2 cells produce high-affinity antibodies against pathogenic bacteria. Effective vaccination stimulates B-2 cells in the lymph nodes to produce high avidity IgG antibodies. In the vaccine development, affinity maturation of B-1 cells should be monitored and carefully avoided to prevent autoimmune reactions and B-2 cell should be stimulated to produce specific antibodies.

Developing Periodontal Vaccine: A Difficult Target
Main limitation in the vaccine preparation is the fact that periodontal disease is multifactorial and polymicrobial in origin. Thus, a vaccine targeting only the most probable pathogenic organism may have to be used. Apart from this, efficacy in each individual may not be same due to the variations in the serotypes or genotypes of the organisms among different individuals. Animals differ qualitatively from humans, with respect to the oral microbial ecosystem, the histological components of the periodontal lesions, the nature of immune responses and control over immunoglobin class and subclass responses. So, results of animal studies may not be directly generalized to humans. Most of the tested approaches afforded short-term protection thus challenge exists in maintaining immune memory to prevent reinfection at a later date and also to trigger T-cell dependent immune responses. Toxic reactions against vaccines based on killed bacteria may occur.

Active Immunization against Porphyromonas gingivalis
For active immunization against periodontal disease various target organisms for vaccine preparation have been tried. P. gingivalis and A. actinomycetemcomitans are of prime importance owing to their omnipresent role in the pathogenesis of periodontal disease.

Porphyromonas gingivalis whole cell as a target antigen
This was one of the first approaches tried in various animal models. In preliminary studies, Persson et al reported that active immunization of nonhuman primate, Macaca fascicularis, with killed P. gingivalis whole cell conjugated with syntex adjuvant formulation inhibits the progression of periodontal tissue destruction. Later, Houston LS et al and page et al reported raised specific serum IgG and IgA titers with significant opsonic capacity But the major drawback was only humoral immune response was elicited that lasted for a short period. No cell-mediated immune response was triggered that could provide immune memory and thus provide long-term protection.

Gingipains as target antigens
Gingipains is the specific term used to describe cysteine proteases that impart major pathogenic capability to P. gingivalis and can be grouped into:

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1. **Gingipains R (RgpA and RgpB)**: cleaves proteins at arginine residues
2. **Gingipain K (porphyrin 2, Kgp)**: cleaves proteins at lysine residue.

Two types of domains are present in gingipains:
1. **Hemagglutinin domain**: present in RgpA and Kgp (but not RgpB)
2. **Catalytic domain**: present in RgpA, RgpB and Kgp
   A study in the nonhuman primate, *Macaca fascicularis*, using a purified *P. gingivalis* cysteine protease (porphypain-2) demonstrated significantly elevated specific IgG antibody by 194-fold, which gave protection against *P. gingivalis* - induced bone loss. But immunization with this protease did not suppress the emergence of *P. gingivalis*. Reason may be poor opsonic properties of antibodies or these antibodies targeted that antigen that was not required for *P. gingivalis* colonization.

**HA2 domain as target antigen**
Furthermore, *P. gingivalis* is known to require hemagglutinin (HA2) domain on gingipains for heme acquisition. Rats immunized with rHA2 immunogen developed significantly higher IgG response levels and a relatively lower Th2/Th1-driven response, which gave some clinical protection from periodontitis.

**Catalytic domain as target antigen**
As the levels of antibodies against the catalytic subunits of RgpA and RgpB did not increase in patients with periodontitis, it was suggested that the inability to produce sufficient antibodies to the catalytic subunit of gingipains may be an etiological factor for chronic periodontitis. A study on mice using N-terminus of the RgpA catalytic domain, RgpA45, coupled to an oligolysine produced an increased level of IgG, which gave protection against *P. gingivalis*.

**Fimbriae as target antigens**
The fimbriae of *P. gingivalis* are significant virulence factor in the pathogenesis of periodontal disease. A structural subunit of the *P. gingivalis* major fimbriae, fimbriillin, promote adherence of the bacteria to host surfaces and also induce an immune response and thus may serve as a critical target antigen.

In a study on rats, when rats were parenterally immunized with highly purified 43-kDa fimbriillin (Fim) protein, it induced Fim A-specific antibodies in serum and saliva and gave 100% protection against *P. gingivalis* - induced alveolar bone loss. On the contrary, a study demonstrated that rabbits immunized with 43-kDa fimbriillin polymer of *P. gingivalis* did not show evidence of any protection against all the strains of *P. gingivalis*. It is suggested that opsonic target sites are not shared across serotypes or five types of *P. gingivalis* fimbriae.

**Capsular polysaccharide as a target antigen**
It was shown that by immunization with *P. gingivalis* capsular polysaccharide led to production of a higher IgG response that gave protection against *P. gingivalis* infection.

**Passive Immunization against Porphyromonas gingivalis**
Adherence of bacteria to host tissues is a prerequisite for colonization and also one of the virulence factors of bacteria. Developing monoclonal antibodies against the colonization factor of *P. gingivalis* could also be a potential target for immunotherapy. The two major colonization factors of *P. gingivalis* are coaggregation factor (outer membrane proteins OMPs) & hemagglutinins.

As 40k-OMP specific IgG was noted to inhibit coaggregation of *P. gingivalis* vesicles and S. gordonii, thus can be an important tool for periodontitis vaccine development. Further, purified r40-kDa OMP antibodies specifically inhibited coaggregation of *A. naeslundii* with several strains of *P. gingivalis*. On the other hand, hemagglutinins facilitate attachment to the erythrocyte cell surface causing hemolysis and thus meeting the requirement for protoheme. It was demonstrated that local passive immunization with rabbit antiserum against *P. gingivalis* hemagglutinin reduced colonization by exogenous *P. gingivalis* in the periodontal area over a 3-week period.

**Targeting Aggregatibacter actinomycetemcomitans**
After *P. gingivalis*, *A. actinomycetemcomitans* is considered an important pathogen in human periodontal disease, especially in aggressive periodontitis. Development of vaccine against this pathogen has also been tried using its different antigens. A synthetic oligopeptide was prepared based on the amino acid sequence of *A. actinomycetemcomitans* fimbriae which was found to be effective in rabbit model, ensuring inhibition of adhesion and its subsequent colonization. Apart from this, Subcutaneous and intranasal immunization of mice with capsular serotype b-specific polysaccharide antigen (SPA) has given positive results. Mice immunized with antisurface associated material from *A. actinomycetemcomitans* exhibited a rise in protective antibody levels acting as an opsonin.

**Preventing co-aggregation by targeting early colonizers**
Co-aggregation between *Fusobacterium nucleatum* and *P. gingivalis* is well known step in the development of dental biofilm. In human plaque, *Fusobacterium nucleatum* colonizes prior to *P. gingivalis*. In a recent report, it was demonstrated that when mice were immunized with *F. nucleatum* there was a significantly increased IgG2a response to *P. gingivalis*. Further, various non-oral and oral microorganisms are known to have epitopes of certain periodontal pathogens or can be modified via plasmid transfer to express antigenic epitopes of known periodontal pathogens. This approach can result in stimulation of the host immune response, where in synthesis of antigen specific antibodies can be induced in the host without any actual infection of the host, conferring active immunization to the organism possessing the epitopes in question.
Recombinant Plant Monoclonal Antibodies (Plantibodies)

Apart from various microorganisms, plants are being increasingly used for the production of recombinant immunotherapeutic agents. Recently, the possibility of edible plants synthesizing biologically active *P. gingivalis* fimbrial antigen, for application as an oral vaccine, was tested. A cDNA fragment of *P. gingivalis* major fimbrial protein (fimA) was cloned into a plant expression vector. When this chimeric plasmid was transferred into potato (*Solanum tuberosum*) cells, the ctb-fimA cDNA fragment was detectable in its genome. This suggests the potential use of plants in synthesizing adjuvant fimbrial protein for the development of adjuvant mucosal vaccines against *P. gingivalis*. Further studies must be needed to test the efficacy of plantibodies in eliminating periodontopathic bacteria.

Conclusion

Various 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.

References


How to cite this Article: Daisy H, Hadge P, Khopade S, Sayyed J, Sable S. Periodontal vaccines. J Dent Allied Sci 2013;2(1);21-23.
Source of Support: Nil. Conflict of Interest: None Declared.