VACCINE FOR PERIODONTITIS - SCOPE FOR HUMAN USE (BASED ON EXPERIMENTAL STUDIES)

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Abstract

Adherence of bacteria to host tissues is a prerequisite for colonization and one of the important steps in the process of periodontal diseases. Bacterial co-aggregation factors and hemagglutinins likely play major roles in colonization in the subgingival area. Emerging evidence suggests that inhibition of these virulence factors may protect the host against periodontal diseases. Active and passive immunization approaches have been developed for immunotherapy of these diseases. Recent advances in mucosal immunology and the introduction of novel strategies for inducing mucosal immune responses now raise the possibility that effective and safe vaccines can be constructed. In this regard, some successful results have been reported in animal experimental models. In this review, salient advances in immunization against periodontal diseases are summarized.

Key Words: mAbs-monoclonal antibodies, LPS-lipopolysaccharides, GCF-gingival crevicular fluid, S-IgA-salivary immunoglobulin A

Introduction

Periodontitis can be defined as an infection-driven chronic inflammatory disease that affects the integrity of tooth-supporting tissues. Several oral bacteria within the sub-gingival dental plaque bio-film have been implicated in the initiation and progression of the disease. These include the "red complex" bacteria, Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, as well as other organisms, such as Aggregatibacter actinomy-cetemcomitans, Prevotella intermedia, and Eikenella corrodens.These bacteria have been implicated in the initiation of a specific host response that is reflected by alterations in the local cellular infiltrate in the tissues, by a systemic humoral immune response manifested by enhanced levels of serum antibody, and by the local antibody response in the periodontium and GCF (Ebersole et al., 1982b, 1984; Gmür, 1985)[1,2]. Also periodontal therapy appears to have a marked effect on humoral immune response to antigens of periodontopathic bacteria. Treatment by scaling and root planing or by surgery significantly enhances serum antibody levels and avidities to antigens of P. gingivalis and induces seroconversion in seronegative patients (Sjöstrom et al., 1994)[3].

Immunization studies in rodents

By definition, immunization is the deliberate stimulation of an adaptive immune response. Immunization, also called vaccination or inoculation, is a method of stimulating resistance in the human body to specific diseases using microorganisms that have been modified or killed. These treated microorganisms do not cause diseases, but rather trigger the body's immune system to build a defence mechanism that continuously guards against the disease.

Active immunization strategies have been developed in immunotherapy against periodontal disease, and several reports in this area have described their effectiveness and promise in experimental animal models. Rodent models, mostly rats and mice but also rabbits and hamsters, have been used extensively in periodontitis immunization and host response studies. Rats are similar to humans with respect to periodontal structure as well as pathogenesis of periodontal disease and in the cellular composition of periodontal lesions. In addition, rats have been established as reliable models for the assessment of periodontal bone loss (Klausen et al., 1989)[4].

Investigations Involving P. Gingivalis

Some studies have concentrated on the colonization and subsequent disease related to infection by P. gingivalis. Although it is difficult to establish P. gingivalis in rats for more than a few weeks, several studies have shown that this infection time is sufficient to induce detectable periodontal bone loss without ligation. (Evans et al., 1992a,b,c)[5].

Antigen Testing And Immune Protection

Whole bacteria: Marked protection from P. gingivalis induced periodontal bone loss was found in monoinfected rats after immunization with whole cells of P. gingivalis (Evans et al., 1992c)[6]. However, in a recent study, it was shown that injection of P. gingivalis in rats resulted in increase the inflammatory and destructive Th1 responses which occur in part through up-regulating the innate immune response and enhanced osteoclastogenesis and fibroblast apoptosis[20].

Hemagglutinin, LPS and outer-membrane vesicles: Several antigen extracts and purified antigens from P.gingivalis have also been applied in immunization studies. Immunization with purified hemagglutinin of P. gingivalis appeared as effective as immunization with whole cells in reducing the recovery of P.gingivalis in ligated teeth in hamsters (Okuda et al., 1988). In another

mouse abscess study, immunization with LPS slightly decreased the size of lesions and reduced the lethality to 60% of the control level. Immunization with a preparation of outer-membrane vesicles of P. gingivalis resulted in reduced lethality and in a slight reduction in abscess size (Kesavalu et al., 1992)[7].

Fimbrial structures: P. gingivalis fimbriae are highly immunogenic and biochemically well characterized. Rats immunized with purified fimbriae and with the purified fimbrillin subunit were protected from P. gingivalisinduced periodontal destruction as efficiently as rats immunized with whole P. gingivalis and gingival collagenase[19].

Cysteine proteases: Recently the cysteine proteases, arginine porphypain [gingipain] (Rgp A) and lysine-porphypain [gingipain] (Kgp), from P. gingivalis have received considerable attention as potential antigens in antiP. gingivalis vaccines. These enzymes are potent virulence factors, and they account for more than 85% of the proteolytic activity of this species. Furthermore, these enzymes are present in all naturally occurring strains studied. P. gingivalis induced alveolar bone loss in rats can be inhibited by immunization with either killed P. gingivalis or a combination of RgpA-Kgp in incomplete Freund's adjuvant (IFA) (Rajapakse et al., 2002)[9].

Immunization Studies In Nonhuman Primates

Studies with intact bacteria: Nonhuman primates offer many advantages to investigators developing periodontal vaccines. The anatomic structure of the periodontium, the periodontal microflora, host immune responses, and host defense mechanisms closely resemble those of humans. Several immunization studies have been performed with nonhuman primates. Person and coworkers used M. fascicularis with ligature-induced periodontitis and a vaccine of formalin-killed P. gingivalis (monkey isolate) and Syntex adjuvant formulation M (SAF-M). High titers of antiP. gingivalis antibody were induced. Immunization significantly reduced the levels but did not eliminate P. gingivalis from the flora. Control animals manifested twice the amount of alveolar bone loss as the immunized animals at both 30 and 36 weeks[10]. Thus, immunization in this primate model inhibits the onset and progression of periodontitis, as assessed by alveolar bone status.

Studies with purified antigen: A study was conducted involving the use of cysteine protease (RgpA/Kgp, porphypain) purified from P. gingivalis as antigen and SAF-M adjuvant (Page et al., 2004). Immunization resulted in significantly reduced levels of P. gingivalis in plaque samples. Inhibition of alveolar bone loss in the immunized animals was at least as great as that observed when the whole-cell vaccine was used, and inhibition was surprisingly uniform among animals[11]. Levels of prostaglandin E2 in GCF and presumably in the tissues were significantly reduced in the immunized animals, and site-specific amounts of bone loss were positively associated with the extent of prostaglandin inhibition[18].

Scope For A Vaccine That Interferes With Periodontal Infection

The observations described earlier and data obtained from recent rodent studies support the idea that development of a vaccine for control and prevention of periodontitis may be feasible. Species associated with periodontal disease may express shared antigenic epitopes. Most of the putative periodontal pathogens are gram-negative and therefore have LPS. Antigenic epitopes in LPS, especially in lipid A and to a lesser extent in core carbohydrates, are highly conserved and may be shared among gram-negative bacteria (Di Padova et al., 1993)[12]. With the use of known sequences and recombinant DNA technology, an appropriate antigenic sequence (or series of sequences) may be constructed to induce immunity to several of the important periodontal pathogens.

Table 1. Characteristics of an Ideal Antigen for a Putative Periodontal Disease Vaccine

- Of major importance in the molecular pathogenesis of periodontal disease associated with a particular microorganism
- Present in large quantities on the bacterial cell surface.
- Probably has some type of associated virulence properties (e.g., adhesin, hemagglutinin, protease)
- Obtainable in a highly purified (recombinant if possible) form in large quantities
- Induces an enduring high level of biologically effective antibody, preferably of the IgG1 and IgG3 isotypes, or an appropriate cell response, preferably of the Th2 type
- Does not elicit Th1 or destructive host response (particularly in gingival and periodontal tissues)
- Present in all serotypes and ribotypes of the species
- Strongly enhances opsonization, phagocytosis, and killing of the species in question
- No toxic side effects
- Stable in a vaccine formulation

Summary Of Rat Protection Mechanisms : On the basis of rodent studies, potential mechanisms of protective immunity against periodontal pathogens include the following.

Mucosal immunity : In higher species, mucosal immunity consists almost exclusively of S-IgA, but in rodents some IgG in saliva, as well as S-IgA, may play a

role. These antibodies mediate their effects by inhibiting initial colonization, hence limiting infection by periodontal pathogens to the oral cavity and adjacent tissues.

Humoral Immunity of Mucosal Tissues: The antibodies in the serum are found in the gingival tissue and may be effective in enhancing phagocytosis by neutrophils in the gingiva, gingival crevice, or periodontal pocket. In addition, humoral antibodies could exert their protective effects by neutralizing toxins (leukocyte-lethal leukotoxin of Actinobacillus), neutralizing bacteria derived host cell lytic enzymes (proteases produced by P. gingivalis) or by inhibiting factors that trigger host cells (LPS).

Cellular Immunity : Several cellular mechanisms involving enhancement of host-derived destructive-type cells producing bone resorptive cytokines have been described and theoretically attributed to CMI and Thl-type cells. One can propose that in developing a vaccine an attempt should be made to maximize the potential for mucosal immunity, since this could be intrinsically the safest method of immunization. Specific induction of mucosal immunity mediated by S-IgA in the absence of, or with low levels of, cellular immunity could provide protection.

Selection Of Antigens For A Periodontal Vaccine

Microbial focus on P. gingivalis: Epidemiological evidence associates P. gingivitis with the onset, progression, and recurrence of periodontitis in humans and animals and its absence with periodontal health and stability. P.gingivalis may be unique among the putative pathogens in that it appears to have "stealth" properties. P. gingivalis can block expression of E-selectin by vascular endothelium and production of IL-8 by gingival epithelial cells and thereby block a first step in host defense (Darveau et al., 1998)[13].This property may permit P. gingivalis and other periodontopathic bacteria in the immediate environment to establish a foothold and grow in the virtual absence of host defense.

On the basis of the earlier observations, there seems little doubt that a strong case exists for focusing on P. gingivalis for vaccine development. Since a vaccine containing intact P. gingivalis provided protection in the animal model described earlier, this model provides a convenient way to identify the antigen/s most likely to be successful in a periodontitis vaccine. One can use the preimmune and postimmune sera from the monkeys that were protected to identify which P. gingivalis antigen(s) induce the highest titers of biologically effective antibody.

Although the role of P. gingivalis LPS in the pathogenesis of periodontitis is well documented, its use as a potential vaccine antigen is ruled out by its innate toxicity. There are at least five fimbrial types of P. gingivalis, five serotypes, and seven types based on cell-surface polysaccharide K antigens.The antigenic molecular determinants of these various types do not cross-react, or they cross-react poorly (Sims et al., 2001)[14] and therefore are not good candidates for a vaccine antigen.

Cysteine proteases appear to have potential to meet some criteria needed for a successful anti-periodontitis vaccine antigen. In addition to the properties listed in Table 1, sera of protected monkeys have high titers of IgG antibody specific for these molecules, and use of cysteine proteases (prophypain) in the M. fascicularis model induced protection (assessed as attenuation of alveolar bone loss) at least equivalent to that induced by a whole-cell vaccine. These proteins and sequences derived from them hold promise as antigens for antiP. gingivalis vaccines[12].

Vaccination studies in primates

Intact Bacteria: Reasonably high levels of serum IgG antibodies reactive with the immunizing species were observed. In all studies, the antibody levels observed were not completely enduring. For example, levels returning to a baseline level by week 51 and in mean titers at the end of the experiment (week 36) were less than half-peak values[12]. Levels that are not long-lasting may pose problems for the development of a vaccine for use in humans. Other possibilities may include a replicating antigen with the potential to produce a more extended response.

Purified Antigens: Others noted that immunization with P. gingivalis resulted not only in reductions in the immunizing organism but also in reductions in P. intermedia levels (Ebersole et al., 1991). The diminution of P. intermedia could have resulted from the presence of antigenic epitopes shared with P. gingivalis, from changes in the subgingival environment, or from altered interspecies interactions that are known to occur[15].

Biological Effects of Immunization: The nonhuman primate studies demonstrate that immunization can reduce levels of targeted bacterial species (P. gingivalis) in the subgingival microflora. However, immunization did not result in clearance of the immunizing species from the subgingival flora in any study. A study by Clark et al. supports the idea but does not prove that immunization may have the potential to suppress recolonization or reemergence of pathogenic species that have been previously eliminated or greatly reduced[16].

Passive Immunization

Immunization may be achieved passively by the administration of pre-formed immunoreactive serum or antibodies. Application of a specific antibody that

neutralizes bacterial adhesion to gingival tissues could provide a practical and satisfactory treatment approach. In an experimental model, patients with periodontitis, who harbored P. gingivalis in their subgingival plaque, were treated with scaling, root planing, and metronidazole to suppress any detectable P. gingivalis. Subsequently, mAbs against P. gingivalis was applied to the periodontal pocket. This treatment significantly reduced the numbers of P. gingivalis at sites showing the most severe periodontitis for up to nine months after mAb application[17].

In humans, this regimen could be used to investigate whether a specific micro-organism is involved in the pathogenesis of periodontitis, by repeated application of the corresponding mAbs at frequent intervals. It should be noted that the use of a single mAb for immunotherapy against periodontal disease to implicate a single pathogen is not straightforward, since the lack of crossreactivity against other potential periodontopathogens must be established. Selective inhibition of the colonization by other periodontopathic bacteria by application of each mAb needs to be established.

Conclusion

Studies performed with experimental animals support the hypothesis that the host immune system has significant potential for intervention or interference in periodontal infection. Taken as a whole, immunization studies demonstrate that immunization can reduce pathogenic subgingival flora, even in the presence of ligatures, and high levels of specific antibody titers can alter the progression of periodontal tissue destruction. Further studies involving systematic evaluation of both mucosal and systemic vaccines are necessary.

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Fig. 1: Porphyromonas vaccine for dogs (commercially available in U.S.A)