REVIEW

Gingipains as candidate antigens for *Porphyromonas gingivalis* vaccine

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Abstract. *Porphyromonas gingivalis* (*P. gingivalis*), a gram-negative anaerobe, is involved in the pathogenesis of periodontal disease, and is found frequently in the subgingival flora in patients with periodontitis. This organism possesses a variety of virulence factors including lipopolysaccharide, capsular material, fimbriae and proteases (enzymes). Among the *P. gingivalis* antigens, enzymes such as Arginine-specific gingipains (RgpA, RgpB) and lysine-specific gingipain (Kgp) have been studied for their ability to induce biologically significant antibodies. This review summarizes recent information on the gingipains and their possible application in the development of an anti-*P. gingivalis* vaccine. (Keio J Med 52 (3): 158–162, September 2003)

Key words: *Porphyromonas gingivalis*, gingipain, vaccine, periodontal disease

Introduction

Two major form of periodontal disease are common in man and other animals, gingivitis and periodontitis. Seventy to eighty percent of the adult population suffer from this disease. Both forms are characterized by inflammation of the gingival tissue in response to the presence of microbial plaque on the teeth. Gingivitis can be distinguished from the various types of periodontitis by absence of surrounding tissue destruction. Gingival inflammation can be eliminated in gingivitis by plaque removal and improved dental hygiene. In the case of periodontitis, chronic inflammation induced by plaque is accompanied by the formation of characteristic gingival lesions. When the periodontal ligament and bone supporting affected teeth are partially destroyed, the gingival epithelium becomes detatched from the teeth, and pathologic periodontal pockets are formed. The disease is caused by a group of predominantly gram-negative anaerobic bacterial species in dental plaque. Principal among these are *Porphyromonas gingivalis* (*P. gingivalis*) and *Bacteroides forsythus* in case of adult periodontitis and *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis. This review mainly focuses on *P. gingivalis*.

Pathogenicity of *P. gingivalis*

P. gingivalis, a gram-negative anaerobe, is involved in the pathogenesis of periodontal disease, found more frequently and as a higher population of the subgingival flora in patients with periodontitis than in control subjects.[1,2] This microorganism easily survives in a hostile environment by successfully evading host antimicrobial defenses, utilizing variety of virulence factors like fimbriae, a polysaccharide capsule and lipopolysaccharide, hemagglutinin and hemolyzing activity, release of toxic products of metabolism, outer membrane vesicles and numerous enzymes.[3–6] The fimbriae of this microorganism not only play a role in colonization of the microorganism[7] but also activate cytokine production like lipopolysaccharide.[8,9] The activation of host defense and induction of the inflammatory cytokines such as interleukin 1 and tumor necrotizing factor α play an important role in bone absorption.[10]

Significant interest has been shown in the "trypsin-like activity" to clarify the pathogenesis of this microorganism. These enzyme downregulate polymorphonuclear neutrophils, degrade extracellular protein and bioactive peptides such as C5, prekallikrein and kininogen. *P. gingivalis* has several cysteine proteases

called as gingipains. Gingipains are classified into two groups based on substrate specificity. Gingipains R (RgpA and RgpB) cleaves proteins after arginine residue and are encoded by two similar genes, RgpA and RgpB, while gingipain K (Kgp) cleaves proteins after lysine residue. RgpA together with RgpB, account for all trypsin-like activity of *P. gingivalis*. Kgp plays an important role in Fe acquisition by binding to hemoglobin. These properties strongly affect the virulence of this microorganism.[11]

Immune Response to *P. gingivalis*

Humoral responses to *P. gingivalis* have been found in high frequency, and our data have shown that the serum IgG antibody levels to *P. gingivalis* correlated with the presence of *P. gingivalis* in periodontal pockets, but not with other bacteria tested.[1,12]

However, the IgG titers to *P. gingivalis* were merely related to the 'number' of microflora present. Therefore, to assess the biological significance of the antibody, we investigated IgG avidity to *P. gingivalis*.[13] The study showed that patient sera with high IgG titers demonstrated low values for avidity, suggesting that IgG responses in patients with adult periodontitis play a limited role in colonization inhibition or elimination of *P. gingivalis*. The data also showed that periodontally healthy individuals have highly functional antibodies, which may protect them against *P. gingivalis* colonization. Also our data indicated periodontitis patients mount a humoral immune response to the antigens of *P. gingivalis* following therapy, and these antibodies may be protective. Recently, several reports indicate that infection with periodontopathic bacteria is associated with systemic diseases such as endocarditis, cardiovascular disease, and aspiration pneumonia.[14–17] Although the precise role of a specific IgG antibody in the etiology of the disease is still uncertain, vaccine directed against antigens of the infecting bacteria may be effective in preventing and arresting the progresses of these diseases, and there has been considerable interest in vaccine development for periodontitis.

Another aspect for the requirement of vaccine development for periodontal disease is as follows. Efforts to prevent or arrest the progress of periodontitis have traditionally been directed in a major part toward removal and control of deposit of microorganism on the surface of teeth and elimination of infectious microorganisms. Treatment requires specialty care which is expensive and of limited availability. It can be painful and frequently unsuccessful, resulting in recurrence of or a contribution to the destructive process. More effective approaches for the prevention and control of periodontitis are needed.

Using the ligature-induced periodontitis model in Macaca fascicularis, Persson et al.[18] have shown that immunization using a vaccine containing whole killed *P. gingivalis* as antigen significantly inhibits the onset and progress of disease as measured by radiographic assessment of alveolar bone loss. Booth et al.[19] reported that the monoclonal antibodies against *P. gingivalis* whole cells prevent the re-colonization of *P. gingivalis* into the gingival crevice. The results indicated that antibodies against certain parts of the *P. gingivalis* surface antigen play a protective role. The next step in vaccine development is the identification and testing of a purified *P. gingivalis* component that can induce protection against infection by this microorganism.

Gingipains as Target Antigens for Vaccine

In searching for the appropriate antigen, we and others have examined a group of cell surface carbohydrates designated as K-antigens,[20,21] lipopolysaccharides,[22,23] and various proteins including fimbriae,[24,25] the 53-KDa and 67K-Da cell surface proteins,[26] hemagglutinin[27] and cysteine proteases referred to as gingipains.[28–32] Of the *P. gingivalis* components studied to date, the gingipains have shown the highest potential for use as vaccine antigens. Gingipains are present in large quantities on the cell surface of *P. gingivalis*, and they can significantly contribute to the virulence exhibited by this species.

The mature form of RgpA possesses both a catalytic domain and a hemagglutinin domain, while RgpB possesses only a catalytic domain. The hemagglutinin domain plays a role in the adherence of this microorganism to erythrocytes of this microorganism. There is a high degree of homology between the catalytic domains of RgpA and RgpB at both the DNA and protein levels, while the hemagglutinin domain of RgpA is similar to the *P. gingivalis* hemagglutinin domain of Kgp. *In vitro* studies have shown that gingipains are able to degrade collagen and fibronectin, inactivate protease inhibitors, degrade immunoglobulins, and facilitate iron acquisition. Furthermore, they are able to destroy host coagulation cascade proteins, degrade complement, and digest various cytokines.[31]

Arginine-specific Gingipains (RgpA) and lysine-specific gingipain (Kgp), enzymes produced by *P. gingivalis*, may be candidates for an anti-*P. gingivalis* vaccine. We initiated a study to determine whether RgpA and Kgp have opsonic target sites and whether these sites are available and accessible on intact *P. gingivalis* cells.[32] Rabbits were immunized and IgG fractions were isolated from preimmune and immune sera. Functional characteristics of the antibodies were assessed by determining antibody titers by ELISA, generating Western blots, and measuring of the anti-

body enhancement of *P. gingivalis* opsonization, phagocytosis and killing by polymorphonuclear leukocytes of intact cells of *P. gingivalis* representative of the four serotypes. Both RgpA and Kgp induced high titers of IgG antibody. Anti-RgpA and anti-Kgp antibodies bound to both RgpA and Kgp demonstrating a large proportion of shared antigenic epitopes. The two antibodies bound equally well to all four *P. gingivalis* serotypes compared to preimmune IgG. The immunoblot patterns of binding of the two antibodies to RgpA and Kgp and to sonicates of the four *P. gingivalis* serotypes were virtually identical (Fig. 1). Both proteins induced antibodies that significantly enhanced opsonization as assessed by chemiluminescence (Fig. 2). Both antibodies significantly enhanced PMN-mediated bacterial killing of the four *P. gingivalis* serotypes (Fig. 3). These data indicate these two proteins appear to be potential candidate antigens for an anti-*P. gingivalis* vaccine.

We have similar data on RgpB, which possesses only a catalytic domain. The data indicate that RgpB also appear to be potential candidate antigen for anti-*P. gingivalis* vaccine (unpublished data).

It is not clear yet which gingipains (RgpA, RgpB or Kgp) would be the best candidate as a periodontal vac-
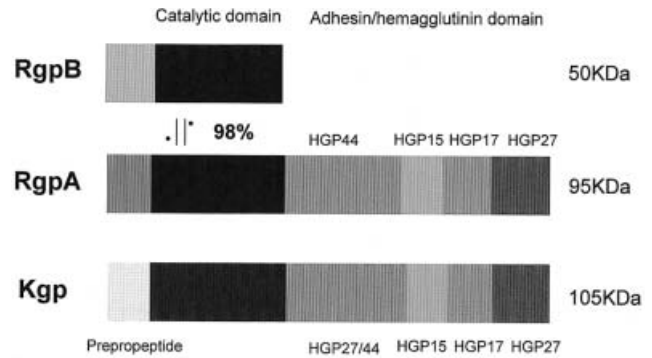

Fig. 1 Schematic comparison of gingipains.

cine. Recent studies have mostly used the mouse or rat model. Booth *et al.* reported that passive immunization using an anti-*P. gingivalis* monoclonal antibody prevented the re-colonization of *P. gingivalis* in human volunteer[19]. Their additional report indicated that the monoclonal antibody recognized the hemagglutinin domain of RgpA.[33,34] Gibson *et al.*[35] examined the abilities of RgpA and RgpB to elicit protection against *P. gingivalis*-mediated oral bone loss in a murine oral
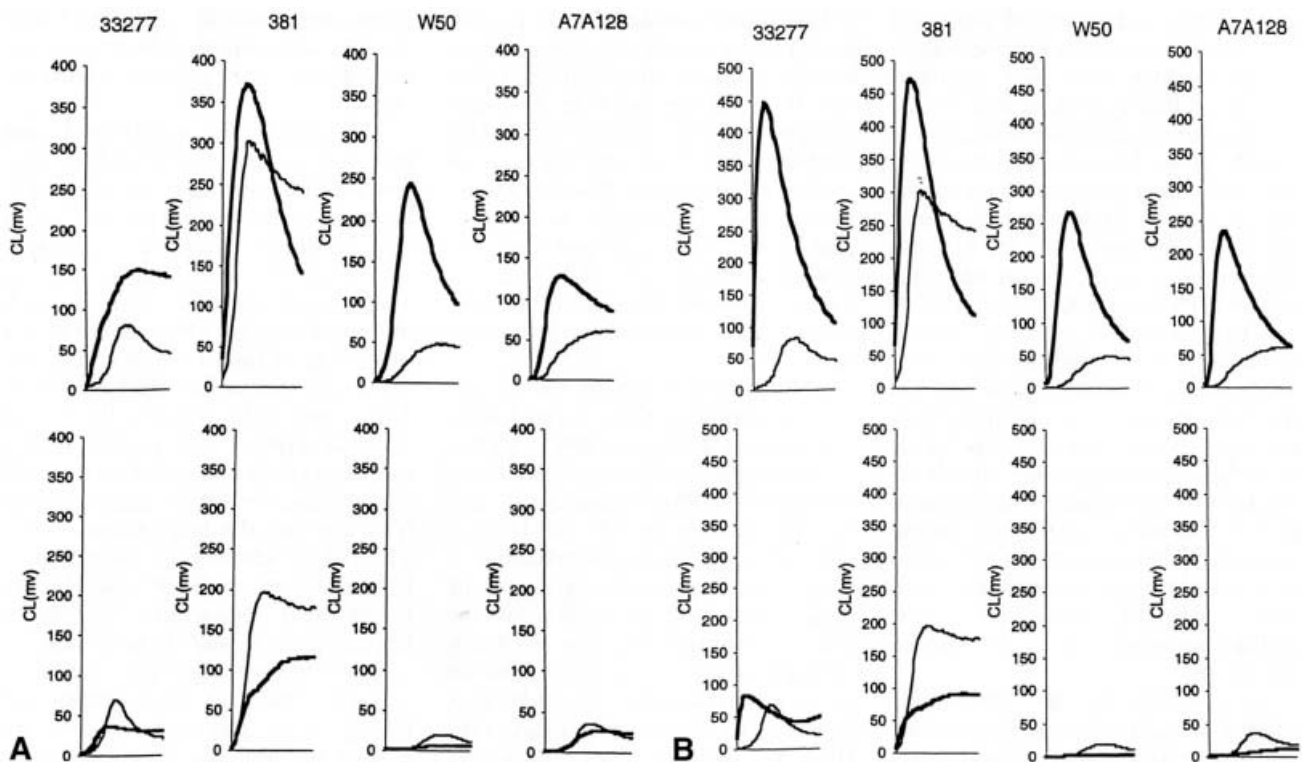
Fig. 2 Opsonic activity measured as chemiluminescence (CL) and reported as millivolts for anti-HRgpA IgG (A) and anti-Kgp IgG (B) antibodies to *P. gingivalis* strains 33277, 381, W50 and A7A1-28. The uppermost set of curves was from incubations containing active complement and lower most contained heat-inactivated complement. The bold lines are the results for incubations containing immune IgGs and fine lines for incubations containing preimmune IgGs. (Reproduce from Nakagawa T, *et al*: Oral microbiology and Immunology 2001; 16: 202–211, Copyright © (2001), with permission from Blackwell Munksgaard)
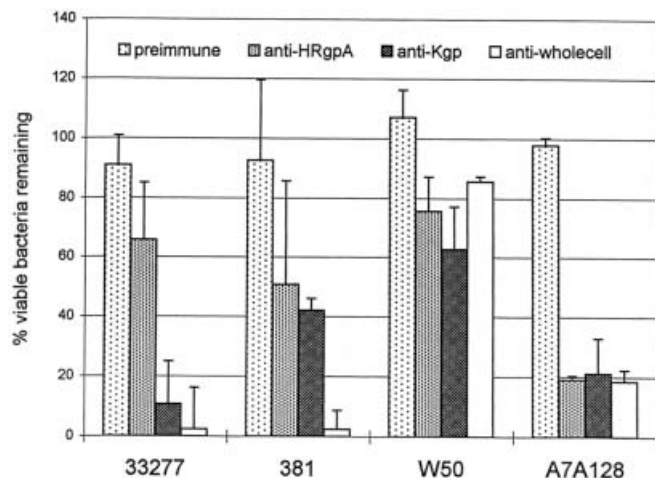
Fig. 3 PMN-mediated phagocytosis and killing of each of the four serotype strains of *P. gingivalis*. In the presence of preimmune IgG (negative control), anti-whole cell IgG (positive control) and anti-HRgpA IgG and anti-Kgp IgG reported as the percentage, with standard error, of viable bacteria remaining at the end of the incubation period. (Reproduce from Nakagawa T, *et al*: Oral microbiology and Immunology 2001; 16: 202–211, Copyright © (2001), with permission from Blackwell Munksgaard)

challenge model. Mice immunized subcutaneously with heat killed RgpA or RgpB possessed elevated levels of *P. gingivalis*-specific immunoglobulin G; however only the animals immunized with RgpA were protected from maxillary bone loss. They concluded that immunization with RgpA stimulates the production of hemagglutinin domain-specific antibodies, which contribute to the prevention of *P. gingivalis*-mediated periodontal disease. This report suggests that antibody responses against hemagglutinin domain contribute to the prevention of *P. gingivalis*-mediated periodontal disease. Yonezawa *et al.*[36] reported immunization of mice with the RgpA DNA vaccine resulted in the production of antibodies. The vaccine strongly induced antibodies to the hemagglutinin domain and weakly to the catalytic domain. A protective effect against *P. gingivalis* virulence was observed in the mouse lesion model. However, Inagaki *et al.*[37] reported that the sera of patients with periodontitis showed high IgG titers against the hemagglutinin domain but not to the catalytic domain. They suggested that the antibodies against the hemagglutinin domain did not have protective immunity. Taken together, some part of the antibody against the hemagglutinin domain antibody seem to be protective and others do not. Further investigation is required to clarify the epitope that induces protective antibodies.

On the other hand, the importance of the antibody against the catalytic domain has stated in several reports. Inagaki *et al.*[37] reported that the levels of antibodies to the catalytic domain are the same between healthy subjects and periodontitis patients. They suggested the antibodies to the catalytic domain are important in the prevention of *P. gingivalis* infection. Genco *et al.*[38] reported that an antibody induced by the peptide of the gingipain R catalytic domain showed a protective effect against *P. gingivalis* infection in the mouse model. O'Brien-Simpson *et al.*[39] also reported that the peptide designed from the catalytic domain induced a protective effect against *P. gingivalis* infection in the mouse model. Kuboniwa *et al.*[40] reported that an antibody against the Kgp catalytic domain DNA vaccine reacted to Rgp and that the vaccine induced a protective effect against a *P. gingivalis* challenge in the murine model. These reports suggested the importance of the anti-catalytic domain antibodies of gingipains, and that induction of the antibodies against the catalytic domain is protective against *P. gingivalis* infection. Further analysis is required to clarify the low antibody titers in periodontal patients and the protective effect of antibodies against the catalytic domain.

The reports also suggested some epitope in catalytic and hemagglutinin domain elicited a protective effect against *P. gingivalis* infection. *P. gingivalis* is known to invade epithelial cells. A recent report suggested that the hemagglutinin domain was implicated in the nuclear targeting of this protease.[41] The antibodies against gingipain are important in the invasion. The evaluated vaccines are mainly peptide and DNA vaccines. They induce different types of immunoresponses. DNA vaccine induced both cellular immunity and humoral immunity. On the other hand, a peptide vaccine induces humoral immunity. Further analysis is required to evaluate at which stage these immunoresponses prevent *P. gingivalis* infection. Evaluation of the epitopes, immunization rates and the effective adjuvant is in progress in our laboratories.

In summary, recent data indicate that the gingipains (RgpA and Kgp) of *P. gingivalis* are potential candidates for a vaccine that could be used for prevention of *P. gingivalis*-mediated periodontal disease.

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