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Porphyromonas gingivalis: Its virulence and vaccine

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ABSTRACT

Background: The microbial florae in adult periodontitis lesions are comprised of anaerobic rods with *Porphyromonas gingivalis* as one of the major components (Slots 1976; Slots 1979; and Tanner *et al*., 1979). *P. gingivalis* is a black-pigmented gram-negative anaerobic rod and a secondary colonizer of dental plaque requiring antecedent organisms. The presence of this organism either alone or as a mixed infection with other bacteria and with the absence of beneficial species appears to be essential for disease activity. It is a predominant member of the subgingival microbiota in disease. It possesses and "excretes" numerous potentially toxic virulence factors. Aim of this study is to perform a systematic review of studies on *P. gingivalis* and its virulence factors with a special focus on its vaccine. **Materials and Methods:** An electronic and manual search based on agreed search phrases between the primary investigator and a secondary investigator was performed for the literature review till January 2014. The articles that were identified by this systematic review (total of 190) were analyzed in detail, which included the study of inference and conclusion. **Conclusions:** Within the limits of this systematic review, it can be concluded that *P. gingivalis* induce immune inflammatory response in periodontitis subjects. Therapeutic vaccines need to be developed and studied for their efficacy in controlling periodontitis.

Key words: Gingipains, host cells, *P. gingivalis*, virulence factors

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INTRODUCTION

Porphyromonas gingivalis, a black-pigmented gram-negative anaerobic rod, has been implicated as a major pathogen of chronic periodontitis. Recent studies using deoxyribonucleic acid (DNA) hybridization also indicated the increased prevalence of *P. gingivalis* as well as other 'red complex species' (*P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) in the subjects with chronic periodontitis.[1] It is also evident that the colonization of the putative pathogenic bacteria in subgingival plaque is not sufficient for the initiation/onset of periodontitis as most periodontopathic bacteria including *P. gingivalis* may also be present at healthy sites (around 11.2 times less in healthy sites than periodontitis).[2] Thus, the onset and progress of chronic periodontitis is based on the balance between the pathogenesis of the periodontopathic microorganisms and the host-defense against them [Figure 1].

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The complex interaction to the host response fundamentally responsible for chronic periodontitis cannot be reproduced *in vitro*. The studies with animal models that *P. gingivalis* can induce experimental periodontitis with alveolar bone losses clearly indicate that *P.gingivalis* is a major causative pathogen of chronic periodontitis.[3] Its pathogenic factors could be potentially involved solely or cooperatively in every step of the onset and progression of the disease. The virulence factors of *P. gingivalis* including fimbriae, hemagglutinin, capsule, lipopolysaccharide (LPS), outer membrane vesicles, organic metabolites such as butyric acid, and various enzymes such as Arg- and Lys-gingipains, collagenase, gelatinase, and hyaluronidase, could contribute to the induction of chronic periodontitis in diverse ways.[3]

Virulence factors

Virulence factors are described as molecules that result in the establishment and maintenance of a species associated with or within the confines of a host.[4] Virulence factors are classically believed to harm the host, but they can also function in the establishment of a symbiotic or parasitic relationship between the bacterial species and the host.

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Virulence factors of *P. gingivalis*

- Involved in colonization and attachment:
  - Fimbriae
  - Hemagglutinins
  - Outer membrane proteins and vesicles
  - Gingipains
- Involved in evading (modulating) host responses:
  - Capsule
  - LPS
  - Ig and complement proteases, otherantiphagocytic products
  - Fimbriae
- Involved in damaging host tissues and spreading:
  - Proteinases (Arg- and Lys-gingipains)
  - Collagenase
  - Fibrinolytic, keratinolytic, and other hydrolytic enzymes.

INVOLVED IN COLONIZATION AND ATTACHMENT

Bacterial fimbriae

Structure and situation: Fimbriae or pili are proteinaceous, filamentous appendages that protrude outwards from the bacterial cell surface.[5] With only one or two exceptions, all of the *P. gingivalis* strains so far examined contain fimbriae arranged in a peritrichous fashion over the surface of the cell. Ultrastructural examination has revealed the presence of peritrichious fimbriae, 0.3-3.0 μm long and 5 nm wide, on most strains of *P. gingivalis*.[6]

Types: The first fimbriae are called major, long, or *FimA* fimbriae, and the second ones are referred to as minor, short, or *Mfa1* fimbriae.[7] The presence of more than one type of fimbriae on *P. gingivalis* has recently become apparent depending upon the genotype (I-V and Ib).

Fimbriae play a crucial role in virulence by stimulating bacterial attachment to host cells or tissues. Fimbriae appear to be a major adherence-mediating determinant of *P. gingivalis*. Immunization with purified fimbriae confers protection against periodontal destruction in a gnotobiotic rat model[8] [Figure 2].

The initial step of *P. gingivalis* attachment to the oral tissue is fimbriae-mediated. Ogawa *et al*., recently investigated the contribution of various regions of the fimbriae to binding to the human gingival fibroblast cell line.[9] Purified, intact, and radiolabeled fimbriae bound firmly to the surface of the fibroblasts. The synthetic peptides, when either added first to the fibroblast cells or concomitantly with the intact fimbriae, inhibited binding in a dose-dependent fashion.

In addition to mediating adherence, fimbriae have a variety of other properties (such as chemotactic properties and cytokine induction). The fimbriae werealso highly immunogenic, eliciting both an antibodyand cell-mediated response in serum and saliva.[10]

Hemagglutinin

Structure and situation: Hemagglutinin proteins are important virulence factors for a number of bacterial species. These enzymes are either exposed at the surface (in the outer membrane) of the bacterium where they are able to come into contact with host cells and tissues or within the periplasmic space from where they are capable of being transported to the cell surface.[10]

Types: *P. gingivalis* produces at least five hemagglutinating molecules. Three *hag* genes encoding hemagglutinins have been cloned.

When expressed on the bacterial cell surface, hemagglutinins may promote colonization by mediating the binding of bacteria to receptors (usually oligosaccharides) on human cells. *P. gingivalis* binding to erythrocytes with the help of

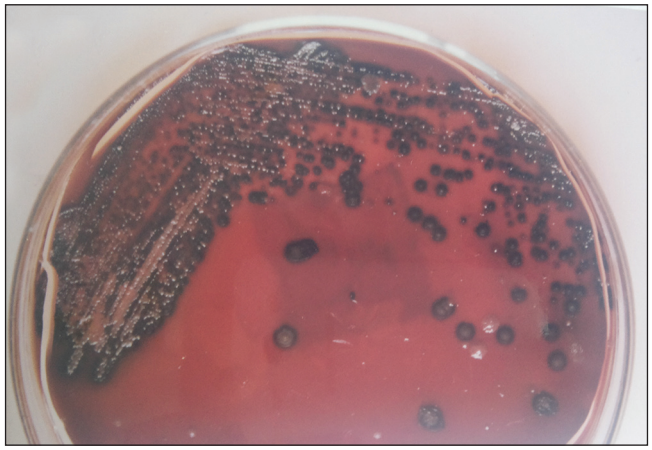

Figure 1: Petridish showing colonies of Porphyromonas gingivalis

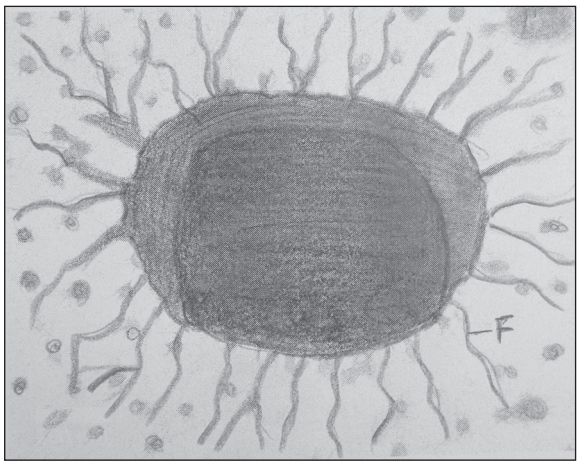

Figure 2: Numerous thin fibrils or fimbriae (F) emerge from the surface of the cells

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hemagglutinin may also serve a nutritional function as it utilizes heme for growth.

It is clear that basically all hemagglutinin activity is related to hemagglutinin-adhesin domains of RgpA, Kgp, and HagA.[11] Arecent study by Lepine *et al*., revealed 9-10 different restriction polymorphism profiles using *hagC* and *hagA* as probes.[12] These results suggest that several copies of this hemagglutinin gene are located on the chromosome of *P. gingivalis*.

Duncan *et al*., have demonstrated that the *P. gingivalis* hemagglutinins may also participate inthe binding of the bacterium to host cells otherthan red blood cells.[13]

Outer membrane proteins/vesicles

Structure and situation: They are released from the outer membrane proper during growth and are referred to as outer membrane vesicles. Trapped within these closed sacs are numerous enzymes that occur in the periplasmic region of the intact cell. These include phospholipase C, proesterases, alkaline phosphatase, hemolysins, and autolysins.[10] The majority of the cells' Arg-gingipain cysteine protease was localized in the outer membrane vesicles.

Vesicles are able to fuse with the outer membrane of other bacterial species, into which virulence factors are released, resulting in an impairment of target cells. Outermembrane vesicles from *P. gingivalis* enhance interleukin-12 induced interferon-c production by T cells, which may augment immunopathology noted in periodontitis.[14] This activity was also noted with the outer membrane from the microorganism, as well as with LPS.

INVOLVED IN EVADING HOST RESPONSE

Capsule

Structure and situation: Bacterial capsules have been considered major virulence factors on the bacterial cell surface.[10] It is formed by a polysaccharide heteropolymer on the outer membrane of the bacterial cell (Woo *et al*., 1979).[15] Mansheim and Kasperdetermined that the capsule of *P. gingivalis* 381 contained galactose, glucose, and glucosamine;[16] whereas, Okuda *et al*., confirmed the presence of these sugars along with rhamnose, glucose, galactose, mannose, and methylpentose.[17]

Types: Six serotypes (K1-K6) and K negative isolates have been identified based on capsular K-antigens.[18]

The presence of a capsule in *P. gingivalis* has been considered an important antiphagocytic virulence factor. The highly encapsulated *P. gingivalis* strains exhibit decreased autoagglutination, lower buoyant densities, and are more hydrophilic than the less encapsulated strains.[19,20] Increased encapsulation is also correlated with increased resistance to phagocytosis, serum resistance, and decreased induction of polymorphonuclear leukocyte chemiluminescence. The decreased tendency for the highly encapsulated strains to be phagocytized has been proposed to be due to the increased hydrophilicity of the strains and their decreased ability to activate the alternative complement pathway.

LPS and lipid A component

Structure and situation: LPS is the major macromolecule found on the outer surface of gram-negative bacteria. LPS is typically composed of three domains: Lipid A, a short core oligosaccharide, and an O-antigen that may be a long polysaccharide. Lipid A is the innermost component of LPS. It is conserved in structure and forms the outer leaflet of the outer membrane [Figure 3].

LPS is critical to the bacterium for maintaining its structural integrity, and for establishing a selective permeability barrier that limits entry of hydrophobic molecules and toxic chemicals such as detergents and antibiotics.[21] LPS is also required for the proper folding and insertion of many outer membrane proteins [Figure 4].

Lipid A, also known as endotoxin, is the bioactive region of LPS that is recognized by the innate immune system. *P. gingivalis* agonist lipid A structures induced the expression of human β-defensin-1, -2, and -3, while *P. gingivalis* antagonist lipid A species downregulated their expression.[22]

INVOLVED IN DAMAGING HOST TISSUES AND SPREADING

Proteinases (gingipains)

Structure and situation: The Arg- and Lys-proteinases are cysteine proteinases and have been given the common name,

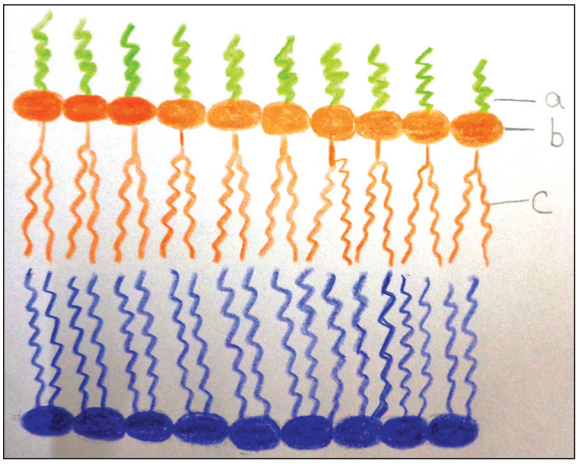

Figure 3: Lipopolysaccharide (LPS).a = O antigen, b = core oligosaccharide, c = lipid A

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gingipains. These enzymes are either exposed at the surface (in the outer membrane) of the bacterium where they are able to come into contact with host cells and tissues or within the periplasmic space capable of being transported to the cell surface, and in outer membrane vesicles, which are sloughed from the outer membrane during growth.[10]

Types: Gingipains, including arginine-specific gingipains (Arg-gingipain-A, RgpA, and Arg gingipain-B, RgpB) and lysine-specific gingipain (Lys-gingipain, Kgp), are encoded by three different genes referred to as *rgpA*, *rgpB,* and *kgp* [Figure 5].[23]

Function of gingipains

- Adherence and colonization: The gingipains are themselves, potent non-fimbrial adhesins avidly binding several extracellular matrix proteins such as fibrinogen, fibronectin, laminin, and collagen type V.[24] They also apparently mediate a tight adherence to epithelial cells and gingival fibroblasts with Kgp being implicated as providing most of the binding.
- Gingipains in hemoglobin binding and heme acquisition: Gingipains exert a sequential action on which Rgps converts oxyhemoglobin to methemoglobin, which render the hemoglobin more susceptible to degradation by Kgp.[25] The occurrence of gingipains in large complexes is a very cleaver design to facilitate hemoglobin degradation and the capture of the released heme is accomplished with high affinity by hemagglutinin-adhesin-2. Gingipains may function as hemophore-like proteins; shuttling captured heme to a hemoglobin receptor (HmuR) in the outer membrane.
- Production of nutritious peptides: Gingipains as the most "aggressive" endopeptidases degrade serum and tissue-derived proteins. The gingipain generated protein fragments are finally subjected to the action of di- and tripeptidyl peptidases to release di- and tripeptides to be transported into the cell and used in *P.gingivalis* carbon and energy metabolism.[26]
- Degradation of antibacterial peptides: In densely populated biofilm, gingipains as well as proteases released by other periodontopathogens can proteolytically inactivate cationic antimicrobial peptides to enable the survival of other bacterial species which are highly sensitive to them.[27] Degradation of cationic antimicrobial peptides also inactivates cationic antimicrobial peptides' ability to neutralize LPSs, which may lead to exacerbated, sustained production of proinflammatory cytokines.
- Exploiting complement: *P. gingivalis* is resistant to killing by the human complement system. In a large part, this resistance is dependent on proteolytic activity of gingipains degrading different components of complement.[28] In addition, gingipains also contribute to proteolysis independent protection of *P. gingivalis* against

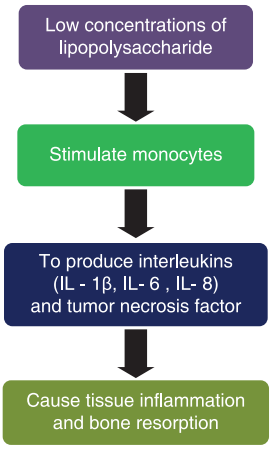

Figure 4: Function of LPS

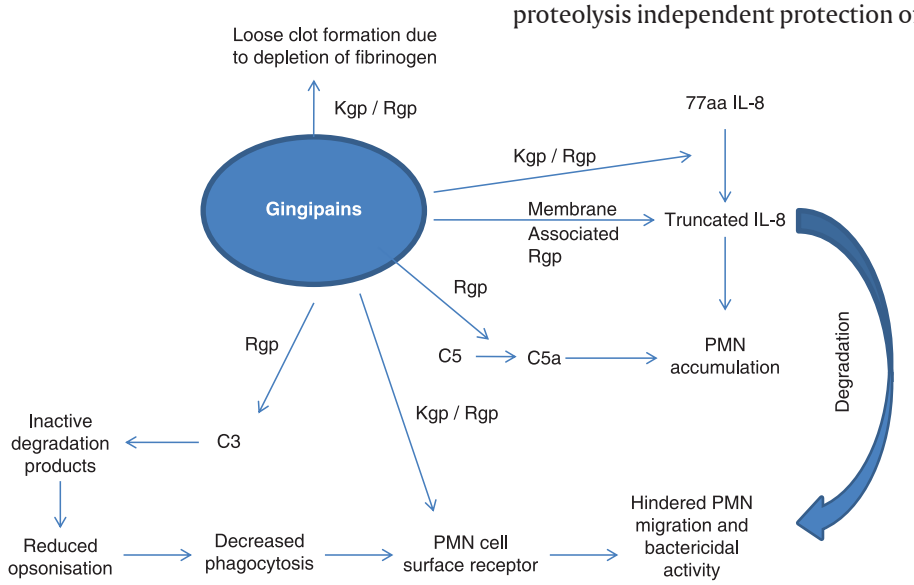

Figure 5: Effects of P. gingivalisproteinases

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Table 1: Studies on immunization with Porphyromonas gingivalis-specific antigens

Antigen type	Modification	Administration	Model	Immunization results
Capsule	Polysaccharide–fimbriae protein conjugate (Choi *et al*., 1998)	ip.	Mouse	Higher serum IgG and highest protection against *P. gingivalis* induced infection compared with CPS or fimbriae alone
	Whole CPS (Gonzalez *et al*., 2003)	sc.	Mouse	CPS-specific IgG and IgM titers elevated. Protection against *P. gingivalis* elicited alveolar bone loss
LPS	LPS/dose-response study (Elkins *et al*., 1987)	ip.	Mouse	Unresponsiveness is antigen-specific and could be induced by a single injection of LPS
	LPS (Chen *et al*., 1990)	ip.	Mouse	No induction of serum IgG, antigen-reactive lymphocytes and protection against *P. gingivalis* induced infection
Fimbriae	Fimbriae accompanied by fimbriae-specific antibodies (Evans *et al*., 1992)	sc.	Rat	Protection against periodontal destruction
	DNA vaccine: Plasmid pcDNA3/fimA (Kawabata *et al*., 1999)	Injection into salivary gland	Mouse	Enhanced fimbriae-specific IgA and IgG in saliva and IgG in serum
	Fimbriae in combination with CT (Nagasawa *et al*., 1999)	Oral	Mouse	In addition to serum IgM, IgG and IgA antibodies, salivary IgA specific for fimbriae was significantly increased
	Fimbriae of a single fimbria variation (Fan *et al*., 2001)	sc., im.	Rabbit	Induced polyclonal antibody only binds closely to related strains of the same fimbrial biovar
	DNA vaccine: *Streptococcus gordonii* vectors (Sharma *et al*., 2001)	Oral	Rat	Enhanced FimA-specific serum IgG, IgA and salivary IgA
	DNA vaccine: Plasmid pIRES-fimA, coexpression plasmid pIRES-fimA:IL-15 (Guo *et al*., 2006)	im, in.	Mouse	in. and im. administration both induced FimA-specific IgG in serum. Only in. administration was able to enhance FimA-specific IgA in saliva, which was further increased by including IL-15 as an adjuvant
	Fimbriae coadministration with rCTB (Takahashi *et al*., 2007)	in.	Mouse	Stimulation of both systemic and mucosal immune responses and reduced *P. gingivalis* induced alveolar bone loss
OMPs	OmpA-like protein: PG32/33 (Ross *et al*., 2001)	sc.	Rat, mouse	Significantly reduced the lesion size and therefore induced protection against *P. gingivalis*
	40-kDa OMP and CT (Namikoshi *et al*.,2003 and Maeba *et al*., 2005)	tc., in.	Mouse	Induced serum IgG, IgA and IgG in saliva
	Anti-r40-kDa OMP hMAb (Hamada *et al*., 2007)	Oral	Rat	Protects against*P. gingivalis*-induced alveolar bone loss
	40-kDa OMP (Koizumi *et al*., 2008)	tc.	Rat	Induced serum IgG, IgA and IgG in saliva. Protection against challenge with *P. gingivalis* 381
	40-kDa OMP with mCTA/LTB (Momoi *et al*., 2008)	in.	Mouse	Induced high levels of 40-kDa-specific serum IgG and IgA and IgA in saliva. Reduction of *P. gingivalis*-induced alveolar bone loss
	40-kDa OMP with pFL (Zhang *et al*., 2009)	sl.	Mouse	Induced high levels of 40-kDa-specific serum IgG and IgA and IgA in saliva. Reduction of *P. gingivalis*-induced alveolar bone loss
Gingipains	RgpA, RgpB, multiple antigenic peptide conjugate Rgp (Genco *et al*., 1998)	ip.	Mouse	*P. gingivalis*-specific serum IgG against functionally defined peptide fragments derived from the catalytic and hemagglutinin/adhesion domains of RgpA
	RgpA-Kgp protease–adhesin complex (O'Brien *et al*., 2000)	sc.	Mouse	Protects against challenge with invasive and noninvasive *P. gingivalis* strains
	RgpA, RgpB, H-K whole cells (Gibson and Genco2001)	sc.	Rat, mouse	All vaccines induced specific serum IgG, but only RgpA induces protection against *P. gingivalis* induced alveolar bone loss
	DNAvaccine: plasmids pSeq2A/*kgp* and pSeq2B/*rgp*cd (Kuboniwa *et al*., 2001)	im.	Mouse	Preventive inflammatory responses, serum IgG, and prolonged survival rate
	rgpA DNA vaccine (Yonezawa *et al*., 2001)	id.	Mouse	Reduced lethality against infection by a lethal dose of *P. gingivalis*
	Active site and ABM peptides from RgpA-Kgp complex conjugated to diphtheria toxoid (O'Brien *et al*., 2005)	sc.	Mouse	Protection against *P. gingivalis*-induced alveolar bone loss
	A1 adhesin domain, rRgpAcat (Frazer *et al*., 2006)	sc.	Rat, mouse	Adhesin domains significant attenuate *P. gingivalis* infection, while rRgpAcat does not
	rgpA DNA vaccine (Miyachi *et al*., 2007)	in., id.	Mouse	in. administration results in higher serum IgG, additional IgA in saliva and reduction of alveolar bone loss, when compared with id. administration
	Purified cysteine protease (Page *et al*., 2007)	sc.	Macaque	Induction of serum IgG and reduction of *P. gingivalis* in subgingival plaque. Onset and progression of alveolar bone loss was inhibited
Hemagglutinin	rHagB (Katz *et al*.,1999)	sc.	Rat	No HagB-specific salivary IgA, slight serum IgM, while serum IgG was actively enhanced. Reduction of bone loss
	rHagB alone or with MPL (Yang *et al*., 2002)	in.	Mouse	Significant increase of HagB-specific salivary IgA and serum IgG compared with rHagB alone

(continued)

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Table 1: (Continued)

Antigen type	Modification	Administration	Model	Immunization results
	130-kD HMGD (Shibata *et al*., 2005)	id.	Mouse	hMAb significantly inhibits hemagglutination by *P. gingivalis* and its vesicles

ABM = Adhesin-binding motif peptides; BSA = bovine serum albumin; CPS = capsule polysaccharide; CT(B) = cholera toxin (B subunit); H-K = heat-killed; (h)MAb = (human) monoclonal antibody; HMGD = hemagglutinin domain; id. = intradermal; ig. = intragastric; im. = intramuscular; in. =intranasal; ip. = intraperitoneal; Kgp = lysine gingipain; LPS = lipopolysaccharide; mCTA/LTB = mutant A subunit cholera toxin/B subunit heat-labile toxin; MPL = monophosporyl lipid A; OMP = outer membrane protein; pFL = plasmid containing the Flt3 ligand; pIRES = plasmid containing an internal ribosome entry site; rCTB = recombinant cholera toxin B; Rgp(A/B) = arginine gingipain (A/B); (r)Hag = (recombinant) hemagglutinin; sc. = subcutaneous; sl. = sublingual; tc. = transcutaneous [8,38-66]

complement-mediated lysis. This is achieved through the capture of the human complement inhibitor C4b-binding protein, thus, hindering deposition of the membrane attack complex on the *P. gingivalis* surface.[26]

- Direct degradation of extracellular matrix proteins: Gingipains efficiently degrade several extracellular matrix proteins *in vitro* gingipains can accomplish a lot more harm indirectly by disturbing the protease-protease inhibitor balance. In the case of human gingival fibroblasts, it was shown that matrix metalloprotease-1 expression was stimulated by Rgp activity.[29] Latent matrix metalloproteases can be directly activated by gingipains.

Collagenase

Structure and situation: Collagenase is perhaps the most important of the *P. gingivalis* proteolytic enzymes. These enzymes are either exposed at the surface (in the outer membrane) of the bacterium where they are able to come into contact with host cells and tissues, within the periplasmic space capable of being transported to the cell surface or in outer membrane vesicles.[10]

If expressed *in vivo,* it is a major destructive enzyme (virulence factor) associated with the soft tissue destruction characteristic of human periodontitis.[30] Mayrand and Grenier were able to dissect the collagenolytic activity into at least two activities: A specific collagenase activity and nonspecific proteinase activity.[31] These thiol-dependent collagenolytic enzymes had a molecular weight of 70 kDa and were purified from the spent culture supernatant, and their inhibition with serum components was studied.

In a study, Hoover and Felton[32] and Li *et al*.,[33] used specific *P. gingivalis* collagenase-deficient mutants generated by nitrosoguanidine mutagenesis and showed that the mutants possessed significantly decreased interaction (that is, adherence) to *A. viscosus* compared with its wild type parent. Takahashi *et al*., were able to isolate a prtCgene from *P. gingivalis* strain 53977, which expressed collagenase activity.[34]

Aminopeptidases

Structure and situation: These enzymes are either exposed at the surface (in the outer membrane) of the bacterium where they are able to come into contact with host cells and tissues or within the periplasmic space capable of being transported to the cell surface, and in outer membrane vesicles.

P. gingivalis is the only member of periodontopathic microbiota that exhibits strong dipeptidyl arylaminopeptidase activity.[35] Abiko *et al*., purified dipeptidylaminopeptidase from the spent growth supernatant of *P. gingivalis* and exposed it to type 1 collagen, cleaving a glycylpropyl dipeptide from the collagen protein.[36] Grenier and McBride were successful in localizing their aminopeptidase activity to the surface of *P. gingivalis*. Immunoelectron microscopy localized the enzyme in the periplasmic space.[37]

Vaccine against *P. gingivalis*

Vaccination is a process that induces specific immune resistance to a bacterial or viral infection. A common finding in patients with periodontitis is the presence of *P. gingivalis*-specific antibodies in serum and gingival crevicular fluid. Immunization with several *P. gingivalis*-specific antigens has been shown to enhance the immune response against *P. gingivalis*, as demonstrated by the induction of specific antibodies and reduction of *P. gingivalis*-induced alveolar bone loss in animal models. The production of antibodies generally indicates the activation of our major host defense mechanism; these antibodies are insufficient to clear *P. gingivalis* infection. Although complete protection through immunization has not yet been achieved, new knowledge about specific *P. gingivalis* antigens holds promising possibilities for the future [Table 1].

CONCLUSION

In general, the major antigens of *P. gingivalis* induce an overall inflammatory immune response, as demonstrated *in vitro* for a wide variety of cell types and also *in vivo*; in experimental animal models. These data correlate with findings from studies with periodontitis patients. New research has highlighted earlier apparent contradictions in the literature demonstrating cytokine stimulation and degradation as well as cellular activation and apoptosis. These apparent contradictions can be explained by *P. gingivalis* antigen concentration effects, and when this is taken into account, the localized dysregulation of the immune response, that is, commonly reported can also be explained. Finally, despite the strong and active inflammatory immune response generated by *P. gingivalis* antigens, more research is needed to study the

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use of these same antigens as vaccine candidates, which, if used appropriately, may have utility as an adjunctive therapy in ameliorating chronic periodontitis.

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