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Minireview

Porphyromonas gingivalis proteinases in periodontitis, a review*

Jan Potempa[a,c] and James Travis[b]

[a]*Department of Microbiology and Immunology, Institute of Molecular Biology, Jagiellonian University, Al. A. Mickiewicza 3, 31-120 Cracow, Poland and* [b]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602, U.S.A.*

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Porphyromonas gingivalis **has been closely associated with the initiation and progression of some forms of periodontal diseases and its proteolytic enzymes have been implicated in invasion, tissue destruction and evasion of host antibacterial defenses. Recently, the primary focus of research has been on cysteine proteinases, referred to as gingipain R and gingipain K which are produced in large quantities and are directly involved in pathological events during development and progression of periodontitis, contributing to clinical hallmarks of the disease including: flow of gingival crevicular fluid, neutrophil accumulation and bleeding on probing. Gingipain R exists as 110-, 95-, 70- to 90- and 50-kDa proteins, the first two being a complex of the 50-kDa catalytic subunit with hemagglutinin/adhesins, with or without an added membrane anchorage peptide. The other forms are single-chain enzymes. The predominant form of gingipain K in *P. gingivalis* strains is a complex of a 60-kDa catalytic protein with hemagglutinin/adhesins. Molecular cloning and structural characterization of the gingipain R and gingipain K genes has shown that they code for 1704 and 1722 amino-acid residue preproenzymes, respectively. Although both structures show no similarity within the preprofragment and only limited identity within the catalytic domain (27%) they are essentially identical within the putative hemagglutinin/adhesin domain. Furthermore, on the basis of gene structure it is now apparent that various soluble and membrane bound forms of gingipains are derived through proteolytic processing of the preproenzymes, and it can be assumed that the Arg-X-specific enzyme is responsible for this processing.**

Periodontitis is a major, chronic infectious disease which, if unchecked, can result in connective tissue loss in the periodontium, bone erosion, and tooth detachment. Both a juvenile and adult form of this disease are known to occur, the hallmarks of each being the presence of specific bacterial species in the periodontal pocket of infected individuals [1]. In the juvenile-type the primary organism is *Actinobacillus actinomycetemcomitans*, a facultative anaerobe which thrives in patients who also have compromised neutrophil function [2]. In order to

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[c]To whom correspondence should be addressed.

Abbreviations: HMWK, high molecular weight kininogen; HRGP, high molecular mass gingipain R; KGP, Lys-gingipain (gingipain K); PrtP, porphypain; RGP, Arg-gingipain (gingipain R); VPE, vascular permeability enhancement.

avoid host-defense systems this pathogen secretes a leukotoxin [3] which further weakens the bactericidal potential of both macrophages and neutrophils and results in the degranulation of the latter cells near the inflammatory site. A variety of proteinases are thus released, including matrix metalloproteinases, elastase, cathepsin G, and proteinase 3, which aid in the degradation of host connective tissue. It is likely that in this manner the organism gains easier access to a source of nutrients, although this has not yet been proven. On the other hand, adult onset periodontitis, the commonest form of this disease, is a mixed infection, with several mostly Gram-negative bacterial species being associated with the disease development and progression [4]. Among these bacteria *P. gingivalis* is generally recognized as the major etiologic agent of human adult periodontitis [5]. In contrast to *A. actinomycetemcomitans*, this organism appears to utilize very different mechanisms to degrade host tissues for its growth and proliferation.

PROPERTIES OF *P. GINGIVALIS*

The organism *P. gingivalis* (formerly *Bacteroides gingivalis*) is a Gram-negative, asaccharolytic, anaerobic, non-motile, non-sporing, short rod which grows as brown or black pigmented colonies on blood agar. The pigment is primarily a mixture of hemin and protohemin and is believed to be a storage depot for iron which is an absolute requirement for growth [6]. However, it is also possible that this high level of iron-bound pigment, together with the synthesis and secretion of reducing agents and superoxide dismutase, may act as an antioxidant shield to repel the oxidative burst utilized by phagocytes to kill foreign organisms.

P. gingivalis is very well equipped with a broad array of functional and structural features which enable it to colonize within its ecological niche of either the gingival salcus or periodontal pocket. The bacterium prospers in this hostile environment by successfully evading host antimicrobial defenses, using such putative virulence factors as fimbriae and lectin--type adhesins, a polysaccharide capsule and lipopolysaccharide, hemagglutinating and hemolysing activity, release of toxic products of metabolism, outer membrane vesicles and numerous enzymes [7].

PROTEOLYTIC ACTIVITIES OF *P. GINGIVALIS*

Accounting and nomenclature of proteinases produced by *P. gingivalis*

The demonstration of proteolytic activity in both culture fluids and extracts of various strains of *P. gingivalis* has caused numerous workers to explore a role for these enzymes in the development of periodontitis. As of early 1995, at least 39 apparently distinct proteolytic activities were claimed to have been purified from this organism, most being referred to as "trypsin-like" enzymes (for extensive review see [8]). Some of these were reported to be highly purified proteinases which had the capability of hydrolyzing peptide bonds at Lys-X and Arg-X residues [9, 10], while others were asserted to have, in addition to their trypsin-like activity, the ability to degrade type I collagen [11, 12]. It is now clear, however, that this latter effect was artificial and due to the fact that the cysteine proteinase was not inactivated with a chloromethylketone prior to subjecting collagen/proteinase samples to reduction, boiling, and SDS/PAGE. Therefore, to our knowledge no true collagenase from *P. gingivalis* has, as yet, been isolated, although an activity has been detected [13, 14].

Adding to this confusion were reports which indicated that there could be as many as eight "trypsin-like" enzymes synthesized by *P. gingivalis* [15] and premature rush to name partially purified, structurally uncharacterized proteinases. In this way, besides trivial names such as trypsin-like proteinase [16, 17], arginine-specific protease [18] or lysine-specific proteinase [19], four different acronyms (gingivain [20, 21], gingipain [9], porphypain [22] and argingipain [11]) are used in the literature to describe cysteine proteinases of Arg-X and/or Lys-X specificity.

Contrary to this apparent diversity we were able to purify only three cysteine proteinases from culture media of *P. gingivalis* H66, two with Arg-X specificity and one with Lys-X specificity. Since the first purified enzyme shared some properties with clostripain, including a similar molecular mass, requirements of cal-

cium for stability and a narrow specificity limited to Arg-X peptide bonds, we proposed the acronym gingipain (*P. **gingi**valis* + clostri**pain** = ***gingi*-pain**) [9] and we have used this name with the prefix Arg- (Arg-gingipain, RGP) or Lys- (Lys-gingipain, KGP) to distinguish between Arg-X and Lys-X specific proteinases [10]. Recently, in line with a recommendation made by the Nomenclature Committee of IUBMB, the names gingipain R (EC 3.4.22.37) and gingipain K have been embraced to account for the unique specificity of two major cysteine proteinases of *P. gingivalis* [23]. Extensive analysis, using the Western blot approach, combined with zymography and inhibition studies [24], has shown that, regardless of the *P. gingivalis* strain and method of bacterium cultivation, gingipains R occur as 110-, 95-, 70- to 90-, and 50-kDa proteins, the first two being a complex of the 50 kDa catalytic subunit with hemagglutinins, while the other forms were single-chain enzymes. In contrast, the predominant form of gingipain K was a complex of the 60 kDa catalytic domain with hemagglutinins. Moreover, lower molecular mass forms of both gingipains were also observed indicating that they could arise by excessive proteolysis from larger precursors. The recent cloning and sequencing of *P. gingivalis* genes encoding gingipains and Southern blot analysis of the bacterium genome fully corroborated data obtained on a protein level (see section below).

Beside gingipains and the elusive collagenase, *P. gingivalis* also produces two other distinct serine type proteinases. One enzyme has glycylprolyl peptidase activity and occurs at least in two molecular mass variants [25, 26] associated with the bacterial surface [27]. The second proteinase separated from culture medium was shown to cleave Gln-His, Glu-Ala and Ala-Leu peptide bonds in the insulin chain B but otherwise was only superficially characterized [17]. From an analysis of general proteolytic activity of different strains of *P. gingivalis* using class specific inhibitors it is apparent that both serine proteinases contribute no more than 15% to the total activity elaborated by this bacterium (J. Potempa, unpublished).

Gene-proteinase relationship

To date, a number of *P. gingivalis* genes purportedly encoding proteinases have been cloned and sequenced including *tpr* [28], *prtC* [29], *prtT* [30], *prtH* [31], *agp* [32], *rgp1* [33], *prtR* [34], *cpgR* [35], *prpR1* [36], *prtP* [37] and *kgp* [38]. In contrast to *tpr* and *prtT* which may encode typical cysteine proteinases homologous to papain and streptopain, respectively, the structure of the remaining genes has no significant similarity to any other database sequence. Except for Tpr, PrtT and PrtC, the products of other genes are either identical with gingipain R (PrtH, Agp, PrtR, CpgR and PrpR1) or gingipain K (porphypain, PrtP) encoded by *rgp1* and *kgp*, respectively.

The comparison of the structure of gingipain R encoding genes has been extensively reviewed [23], and there is no doubt that *prtR*, revised *prpR1* and *rgp1* represent variants of a single genetic locus. Although *agp* and *cpgR* encode proteins identical to the catalytic domain of gingipain R, their existence in the *P. gingivalis* genome has been questioned [37], and it is likely that those gene structures are incomplete due to errors in cloning and sequencing. In addition to *rgp1*, a second related locus homologous to the 5 region one-third of *rgp1* was detected in all tested *P. gingivalis* strains [36, 37]. This gene, referred to as *rgpB* [39] or *rgp2* [33], encodes a 50 kDa cysteine proteinase purified in large quantities from strain HG66 (J. Potempa, manuscript submitted). The structure of RGP-2 (RgpB) inferred from cDNA sequence (GeneBank, accession No. D64081) and confirmed by direct amino-acid sequencing reveals that the N-terminal two-third of the molecule, confining active site cysteine residue and putative histidine residues of the catalytic dyad, are nearly identical to RGP-1, but both differ considerably at the C-terminus (Fig. 1).

In contrast to the two genes encoding the arginine specific gingipain, only one gene for the Lys-X specific proteinase, referred to as either gingipain K (KGP) [10] or porphypain (PrtP) [22], has been detected in *P. gingivalis*. Two independent groups have sequenced this gene from two different strains of the bacterium, H66 [38] or W12 [37]. Despite some differences in the primary structure, especially within its 25% of 3 region of the two genes and some discrepancies regarding enzyme specificity, it is most likely that *kgp* and *prtP* represent variants of a single genetic locus.

Both, *rgp*1 and *kgp* encode a multidomain protein of 1704 and 1723 amino-acid residues, re-

spectively, consisting of a prepropeptide (227 amino-acid residues), a catalytic domain (492 and 509 amino-acid residues, respectively) and a hemagglutinin domain. Although the two structures show no similarity within the pre-profragment and only limited identity within the catalytic domain (27%) (Fig. 1), they are essentially identical within the putative hemagglutinin/adhesin domain. The various soluble and membrane bound forms of gingipains are apparently derived through proteolytic processing of the preproenzymes by cleavage at Arg-X peptide bonds [8, 40].

Pathophysiological functions of *P. gingivalis* proteinases

The destructive potential of the proteolytic activity produced by *P. gingivalis* is not in question. From numerous investigations *in vitro* it is apparent that these enzymes can contribute significantly to bacterial virulence through: (i) direct and indirect periodontum tissue degra-

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RGP2    1     YTPVEEKeNG--RMIVIVAKKYEGDIKDFVDWKNQRGLRTEVKVAEDI   46
RGP1    1     YTPVEEKQNG--RMIVIVAKKYEGDIKDFVDWKNQRGLRTEVKVAEDI   46
KGP     1   DVYTDHGDLYNTPVRMLVVAGAKFKEALKPWLTWKAQKGFYLDVHYTDEA   50
PrtP    1   DVYTDHGDLYNTPVRMLVVAGAKFKEALKPWLTWKAQKGFYLDVHYTDEA   50

RGP2   47   ASPVTANAIQQFV-KQEYEKEGNDLTYVLLVGDHKDIPAKITPGIKSDQV   95
RGP1   47   ASPVTANAIQQFV-KQEYEKEGNDLTYVLLVGDHKDIPAKITPGIKSDQV   95
KGP    51   EVGTTNASIKAFIHKKYNDGLAASAAPVFLALVGDTDVISGEKGKKTKKV  100
PrtP   51   EVGTTNASIKAFIHKKYNDGLAASAAPVFLALVGDTDVISGEKGKKTKKV  100

RGP2   96   ---YGQIVGNDHYNEVFIGRFSCESKEDLKTQIDRTIHYERNITTEDK-W  141
RGP1   95   ---YGQIVGNDHYNEVFIGRFSCESKEDLKTQIDRTIHYERNITTEDK-W  141
KGP   101   TDLYYSAVDGDYFPEMYTFRMSASSPEELTNIIDKVLMYEK-ATMPDKSY  149
PrtP  101   TDLYYSAVDGDYFPEMYTFRMSASSPEELTNIIDKVLMYEK-ATMPDKSY  149

RGP2  142   LGQALCIASAEGGPSADNGESDIQHENVIANLLTQYGYTKIIKCYDPGVT  191
RGP1  142   LGQALCIASAEGGPSADNGESDIQHENVIANLLTQYGYTKIIKCYDPGVT  191
KGP   150   LEKALLIAGADSYWNPKIGQQTI-KYAVQYYYNQDHGYTDVYSYPKAPYT  198
PrtP  150   LEKvLLIAGADysWNsqvGQpTI-KYgmQYYYNQeHGYTDVYnYlKAPYT  198

RGP2  192   PKNIIDAFNGGISLVNYTGHGSETAWGTSHFGTTHVKQLTNSNQLPFIFD  241
RGP1  192   PKNIIDAFNGGISLVNYTGHGSETAWGTSHFGTTHVKQLTNSNQLPFIFD  241
KGP   199   GCYSHL--NTGVGFANYTAHGSETSWADPSVTATQVKALTNKNKYFLAIG  246
PrtP  199   GCYSHL--NTGVsFANYTAHGSETaWADPllTtsQlKALTNKdKYFLAIG  246

RGP2  242   VACVNGDFLFSMPCFAEALMRAQKDGKPTGTVAIIASTIdQyWApPMRGQ  291
RGP1  242   VACVNGDFLFSMPCFAEALMRAQKDGKPTGTVAIIASTINQSWASPMRGQ  291
KGP   247   NCCVTAQFDYPQPCFGEVMTRVKEKGAYAYIGSSPNSYWGEDYYWSVGAN  296
PrtP  247   NCCiTAQFDYvQPCFGEViTRVKEKGAYAYIGSSPNSYWGEDYYWSVGAN  296

RGP2  292   DEMN-----EILCEKHPNNIKRTFGGVTMNG-MFAMVEKYKKD-------  328
RGP1  292   DEMN-----EILCEKHPNNIKRTFGGVTMNG-MFAMVEKYKKD-------  328
KGP   297   AVFGVQPTFEGTSMGSYDATFLEDSYNTVNSIMWAGNLAATHAENIGNVT  346
PrtP  297   AVFGVQPTFEGTSMGSYDATFLEDSYNTVNSIMWAGNLAATHAgNIGNiT  346
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Fig. 1. Continued on next page

dation, (ii) attenuation of host defense mechanisms, (iii) dysregulation of the proteolytic cascades of blood coagulation, fibrinolysis and complement activation, (iv) release of iron from iron transporting/sequestering proteins, (v) exposing cryptotopes for bacterial attachment, and (vi) providing essential amino acids and small peptides for bacterial growth. Therefore, it is no surprise that a strong correlation exists between proteinase activity of *P. gingivalis* and its virulence [41–44] but not until the present the involvement of the single proteinase activity could be related to pathogenic potential of *P. gingivalis*. Recently, however, se- veral isogenic mutant strains of *P. gingivalis* deficient in defined proteinases have become available [39, 45, 46] and tested on animal models. Also, the availability of highly purified, strictly defined, individual enzymes for *in vitro* and *in vivo* studies has made it possible to pinpoint their destructive functions. From all these recent studies it becomes clear that gingipains, especially gingipains R, are the major virulence factor of *P. gingivalis* and can play a pivotal role in the pathogenesis of human periodontal diseases.

Proteolytic processing of bacterial preproproteins

In addition to gingipains R and gingipain K, at least two other *P. gingivalis* proteins, including fimbrilin [47] and the 75-kDa major outer membrane protein [48], are synthesized in a precursor form maturation of which requires processing at Arg-X peptide bonds. This implies that an arginine-X proteinase(s) is involved in this process, and it was suggested that gingipain R takes part in the autoprocessing of its initial preproprotein, as well as the proform of KGP [8]. The construction of the isogenic gingipain R deficient mutants with either *rgpA* (*rgp1*) or *rgpB* (*rgp2*) or both *rgpA* and *rgpB* genes disrupted [46] has allowed a closer study of the processing of *P. gingivalis* proproteins. In this way it was shown unequivocally that gingipains R are involved in both maturation and the assembly of fimbriae since the RGP-null mutant possessed very few fimbriae on the cell surface [49]. Similarly, in the

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RGP2  329  ---GEnMLDTWTVFGDPSLLVRTLVPTeMQVTAPAnIsasaqtfeVaCDY  375
RGP1  329  ---GEKMLDTWTVFGDPSLLVRTLVPTKMQVTAPAQINLTDASVNVSCDY  375
                       | || |       |     | ||      ||
KGP   347  HIGAHYYWEAYHVLGDGSVMPYRAMPKTNTYTLPASLPQNQASYSIQASA  396
PrtP  347  HIGAHYYWEAYHVLGDGSVMPYRAMPKTNTYTLPASLPQNQASYSIQASA  396

RGP2  376  NGAIATlSddGdMvGtAiVk-dGkAiIkLnesiadeTnLTLTVVGYNKvT  424
RGP1  376  NGAIATISANGKMFGSAVVE-NGTATINLTGLTNEST-LTLTVVGYNKET  423
               | ||  |   |  |    | || | |    |      |    |
KGP   397  GSYVA-ISKDGVLYGTGVANASGVATVNMTKQITENGNYDVVITRSNYLP  445
PrtP  397  GSYVA-ISKDGVLYGTGVANASGVATVsMTKQITENGNYDVVITRSNYLP  445

RGP2  425  VIKdvkveGtsiadv-andkpyTvavsgkTitvesPaaglTifdmNgrRv  473
RGP1  424  VIKTINTNGEPNPYQPVSNLTATTQGQKVTLKWDAPSTK-TNATTNTARS  472
           ||| |   ||| ||||||||||||||||||||||||| |         |
KGP   446  VIKQIQA-GEPSPYQPVSNLTATTQGQKVTLKWDAPSAKKAEGSREVKRI  494
PrtP  446  VIKQIQv-GEPSPYQPVSNLTATTQGQKVTLKWeAPSAKKAEGSREVKRI  494

RGP2  474  ataknrmvfeaqngvyavriategktytekvivk                 507
RGP1  473  VDGIRELVLLSVSDAPELLR                               492
            ||
KGP   495  GDGLFVTIEPANDVR                                    509
PrtP  495  GDGLFVTIEPANDVR                                    509
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Fig. 1. Alignment of the amino-acid sequence of the catalytic domain of gingipains R (RGP1 and RGP2) and gingipain K from strain HG66 (KGP) and W12 (PrtP).
Residues in RGP2 and PrtP which are different than that in RGP1 and KGP, respectively, are in lower-case letters. Residues identical in RGP1 and KGP structures are denoted by vertical line. Experimentally determined cysteine residue and predicted histidine which apparently form enzymes catalytic dyad are marked with •. Two conservative variations in RGP1 from strain W50 (Val/Ile and Val/Ala) [34, 36] in comparison to HG66 are marked with +.

RGP-null mutant, but not in the single *rgp* mutants, unprocessed, a higher molecular mass form of 75-kDa major outer membrane protein was found. In both cases, proforms were processed by cleavage of a long, N-terminal leader peptide [47, 49]. Although the importance of the 75-kDa antigen in *P. gingivalis* pathogenecity is obscure, fimbriae are considered an important virulence factor involved in binding of this organism to saliva-coated hydroxyapatite [50], coaggregation with other bacteria [51–53] and adherence to cultured gingival fibroblasts and epithelial cells [54]. In this way, gingipains R activity indirectly plays a crucial role in expression of virulence by *P. gingivalis*.

Dysregulation of the kallikrein/kinin pathways

Gingipains R were found to be a very potent vascular permeability enhancement (VPE) factor of *P. gingivalis*, inducing this activity through plasma prekallikrein activation and subsequent bradykinin release. Since this activity was totally suppressed by anti-gingipain R antibodies (anti-RGP2), and the proteinase specific inhibitor, leupeptin, in crude bacterial extracts, it is obvious that two gingipains R are the only enzymes produced by *P. gingivalis* which can trigger bradykinin release through prekallikrein activation [55]. Gingipain K by itself is not able to induce VPE in human plasma, but working in concert with gingipains R the pair can induce VPE by cleaving bradykinin directly from HMWK [56]. Thus, both gingipains are important VPE factors responsible for gingival crevicular fluid production at periodontitis sites infected with *P. gingivalis*. In this way they aid in the provision of a continuous supply of nutrients important for bacterial growth and virulence.

Hemagglutination, adhesion and fibrinogen degradation

Adhesins and hemagglutinins from oral bacteria perform an important function in the colonization of target tissues and proliferation within the oral cavity [57, 58]. The close association between hemagglutinins/adhesins and *P. gingivalis* trypsin-like activity was first described in the late eighties [59] but not until recently was this phenomenon explained at the structural level. Pike *et al.* [10] were the first to show that two different high molecular mass gingipains are, indeed, non-covalent complexes of the proteinase domains with hemagglutinin(s). This assumption was later, unequivocally, confirmed when gingipain genes were cloned and sequenced [32, 34, 36–38], and it is now apparent that gingipains are initially synthesized as preproteins in which the hemagglutinin/adhesin domain is confined to the C-terminal part. After proteolytic processing the complexes are expressed on the bacterial cell surface where they constitute the major hemagglutinin of *P. gingivalis*. This is in keeping with the observation that RGP-null mutants have also lost hemagglutinin activity [46].

Utilizing specific proteinase inhibitors, it was found that hemagglutination by either of the soluble proteinase-adhesin complexes could occur independently of proteinase activity. Significantly, low concentrations of fibrinogen, fibronectin, and laminin inhibited hemagglutination, indicating that adherence to these proteins was a primary property of the adhesin activity component of complexes, and not hemagglutination. Binding studies with gingipain K and high molecular mass gingipain R suggest that interaction with fibrinogen is a major function of the adhesion domain, with dissociation constants for binding to fibrinogen being 4 and 8.5 nM, respectively. Specific association with fibronectin and laminin was also found. All bound proteins were degraded by the functional proteinase domain, with HRGP being more active on laminin and fibronectin and KGP more effective in the digestion of fibrinogen [60]. Cumulatively, these data suggest that HRGP and KGP, acting as proteinase-adhesins, may progressively attach, degrade and detach from target proteins significantly modifying interaction between host cells and *P. gingivalis*. Indeed, it was shown recently that gingipain R can enhance binding of fimbriae to cultured human fibroblasts and matrix proteins [61].

Dysregulation of plasma clot formation

Among the plasma proteins, fibrinogen appears to be the prominent target for gingipain K. *In vitro*, and at nanomolar concentrations, this enzyme digests the fibrinogen A chain within minutes [60], thus rendering it non-clottable. In normal plasma, fibrinogen degradation is reflected by a prolongation of plasma thrombin time, and gingipain K does exercise a strong counteractive effect on plasma clottability at low nanomolar concentrations [62].

Indeed, gingipain K is the most potent fibrinogenase described to date, and its presence and unrestricted activity in periodontal pockets would contribute to a bleeding tendency, especially since it also efficiently degrades the procoagulant portion of high molecular mass kininogen [21]. The bleeding at periodontitis sites is of major importance for *P. gingivalis*, because it provides, through the release of hemoglobin from erythrocytes, the richest source of heme and iron.

Altering function of the complement system

The interactions of *P. gingivalis* with complement system have been studied intensively, and attenuation of the complement-dependent bactericidal activity due to degradation of C3, C4, C5, factor B and factor D has been described [63–66]. Such experiments were, however, performed with whole bacterial cells and only recently it was shown that gingipain R is the major proteinase which affects the complement system. Both C3 and C5 were found susceptible to limited proteolysis by gingipain R. C3 was converted in a stepwise manner to C3a-like and C3b-like fragments with evidence of extensive further degradation of the C3a-like portion of the molecule, and no C3a activity could be measured. In contrast, proteolysis of C5 led to the release of C5a or a C5a-like fragment that was biologically active for neutrophil activation [67]. The release of proinflammatory C5a-like fragments was further augmented if C5 was oxidized prior to proteinase digestion. In this condition, which may likely occur at inflammatory sites, not only was gingipain R more potent, but also gingipain K became efficient in releasing the active fragment from oxidized C5 [68]. These data suggest a recruiting mechanism for attracting neutrophils to the gingival lesion site in periodontal disease which may be responsible for the massive accumulation of neutrophils in inflamed periodontal tissue.

Survival of *P. gingivalis* in a sea of neutrophils

The ability of *P. gingivalis* to induce neutrophil accumulation at sites of infection in the presence of a normal humoral immune response [69], only superficially seems suicidal. *P. gingivalis* is very well equipped to resist phagocytosis [70–73] and beside having structural countermeasures, including a polysaccharide capsule [74] and surface fimbriae [51], proteolytic activity elaborated by this bacterium plays a major role in this resistance. In one line of defense against phagocytosis bacterial proteinases degrade immunoglobulins and C3, avoiding in this way opsonization [75, 76]. While it is almost certain that gingipain R is responsible for C3 destruction [67], a serine proteinase is involved in IgG degradation [75]. The second line of defense depends on proteolytic modification of the neutrophil surface. It was shown that *P. gingivalis* contains at least two enzymes capable of cleaving the C5a receptor (C5aR; CD88), gingipain K and a second nontryptic serine proteinase associated with vesicles that is distinct from either gingipain K or R [77]. Presently, the relationship between serine proteinases cleaving IgG and C5aR is unknown, but the results obtained indicate for the first time that a proteinase other than gingipains may be involved in the evasion of host defenses by *P. gingivalis*.

Destruction of receptors to pro-inflammatory factors such as C3a and C5a by bacterial proteinases may eliminate the ability of these and other mediators to carry out their anti-bacterial actions and, thereby, limit host defense mechanisms in response to the infecting bacteria. In the case of *P. gingivalis* the situation is particularly unusual because once the gingipains generate potent, plasma-derived inflammatory factors that can enhance edema and deliver essential nutrients to the bacteria, these and other proteinases on shed vesicles may destroy the cellular receptors on attracted phagocytic cells. These receptors transmit the signal activation mechanisms in the infiltrating cells that elicit bacterial killing. If, however, these cells encounter shed vesicles at a distance remote from the bacteria, receptors would be cleaved, leaving such phagocytes to degranulate (releasing powerful hydrolytic enzymes) and die, without ever actually attacking the target bacteria. It is this series of events which might explain the ability of *P. gingivalis* and other periodontopathogens to persist and flourish in gingival tissue.

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